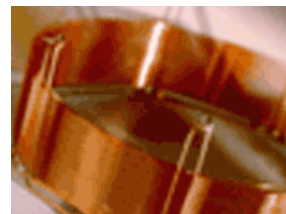


# BOOK OF ABSTRACTS

## 11<sup>th</sup> International Symposium on **RECENT ADVANCES IN FOOD ANALYSIS**

**November 5-8, 2024  
Prague, Czech Republic**

Jana Pulkrabová, Monika Tomaniová, Stefan van Leeuwen, Michele Suman,  
Michel Nielen and Jana Hajšlová  
Editors



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# **RECENT ADVANCES IN FOOD ANALYSIS**

November 5-8, 2024  
Prague • Czech Republic  
Clarion Congress Hotel Prague

Organized by

**Department of Food Analysis and Nutrition,  
University of Chemistry and Technology, Prague (UCT Prague),  
Czech Republic**

**&**

**Wageningen Food Safety Research (WFSR), part of Wageningen  
University & Research, The Netherlands**



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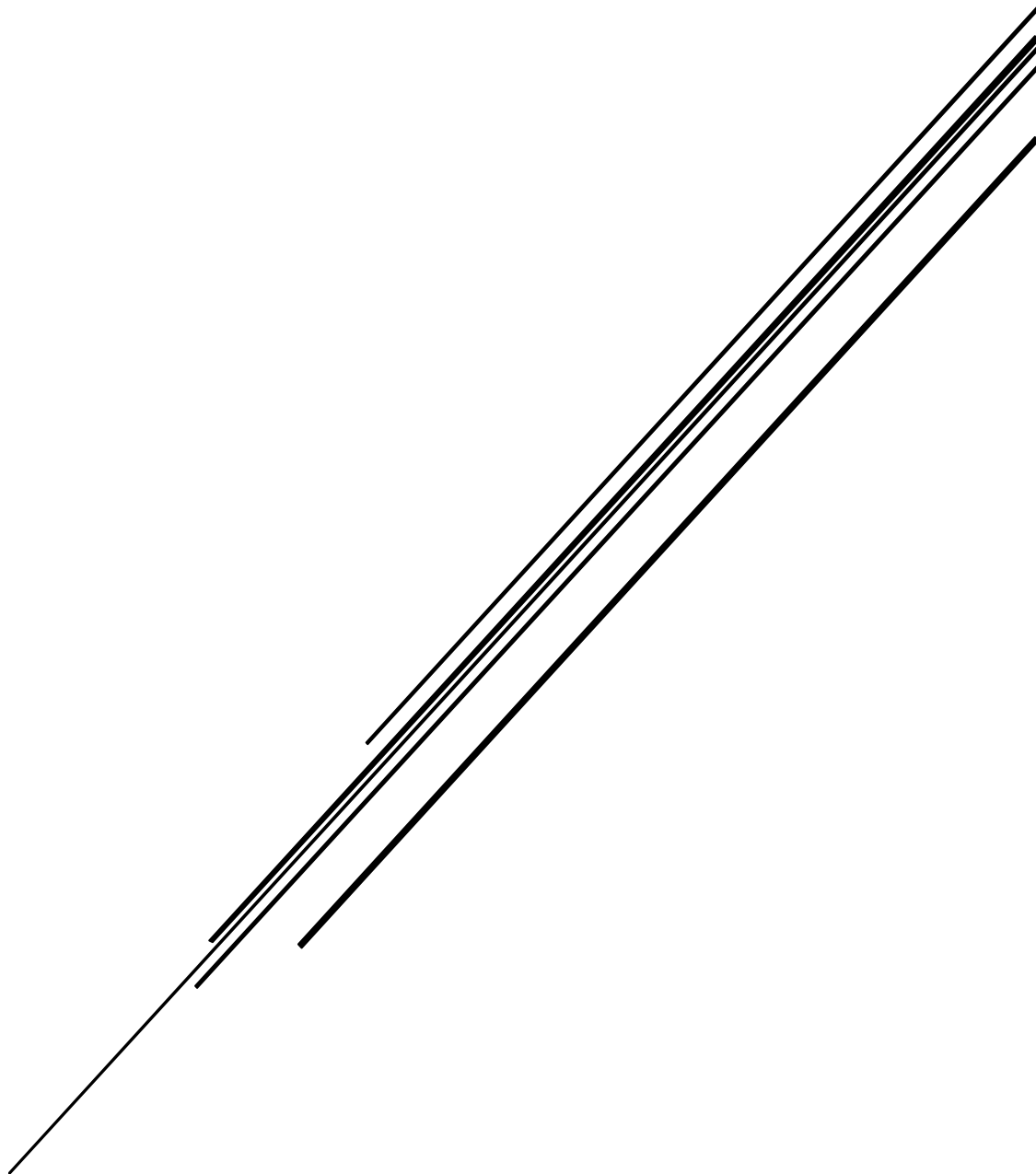
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# CONTENT



# CONTENT

<b>VENDOR SEMINARS .....</b>	<b>50</b>
<b>NOVEMBER 6, 2024 (7:45-8:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>CCS - CATCHING CONTAMINANTS SPEEDILY: THE ROLE OF ION MOBILITY IN RAPID CONTAMINANT DETECTION FOR FOOD AND FEED SAFETY</b>	
CCS - CATCHING CONTAMINANTS SPEEDILY: THE ROLE OF ION MOBILITY IN RAPID CONTAMINANT DETECTION FOR FOOD AND FEED SAFETY	
<i>Nicholas J. Birse</i> .....	51
<b>NOVEMBER 6, 2024 (7:45-8:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>EXPECT THE UNEXPECTED - CLIMATE CHANGE AND (EMERGING) MYCOTOXINS</b>	
EXPECT THE UNEXPECTED - CLIMATE CHANGE AND (EMERGING) MYCOTOXINS	
<i>Julie Brunkhorst, Ronald Niemeijer, Emilee Easter, Jordan Steinberg</i> .....	52
<b>NOVEMBER 6, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>OPTIMIZATION STRATEGIES TO DELIVER PERFORMANT PFAS AND PESTICIDE QUANTITATION IN COMPLEX FOOD MATRICES</b>	
END-TO-END LC/MS WORKFLOWS FOR ANALYSIS OF PFAS IN MEAT, DAIRY PRODUCTS, AND FISH, THROUGH TO INFANT FOODS AND FORMULATIONS	
<i>Day Powell</i> .....	53
EXPLOITING INTELLIGENT SAMPLE REINJECTION WITHIN A LC-HRMS WORKFLOW TO IMPROVE THROUGHPUT AND CONFIDENCE IN PESTICIDE QUANTITATION RESULTS	
<i>Nicola Cimino</i> .....	53
<b>NOVEMBER 6, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>ENHANCING LABORATORY EFFICIENCY WITH BIOTAGE® WORKFLOW SOLUTIONS FOR STREAMLINED SAMPLE PREPARATION FROM A RANGE OF FOOD MATRICES</b>	
ENHANCING LABORATORY EFFICIENCY WITH BIOTAGE® WORKFLOW SOLUTIONS FOR STREAMLINED SAMPLE PREPARATION FROM A RANGE OF FOOD MATRICES	
<i>Monika Vezse</i> .....	54
<b>NOVEMBER 6, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>PARADIGM SHIFTS IN FOOD ANALYSIS: BREAKTHROUGH SOLUTIONS FOR ALLERGIES, AUTHENTICITY AND TRACEABILITY</b>	
MOVING TOWARDS THE NEXT GENERATION OF FOOD ALLERGEN ANALYSIS	
<i>Jens Brockmeyer</i> .....	55
CHROMATOGRAPHY-FREE SCREENING AND QUANTIFICATION OF SAFFRON ADULTERATION WITH SAFFLOWER BY DART-TQ	
<i>Linda Monaci</i> .....	55
SIMULTANEOUS EI AND CI IN SINGLE GC RUNS FOR CONFIDENT UNKNOWN'S IDENTIFICATION IN NON-TARGET ANALYSIS	
<i>Eliska Ceznerova</i> .....	56
<b>NOVEMBER 6, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>RECENT ADVANCES IN LC-MS/MS TECHNIQUES FOR FOOD ANALYSIS</b>	
RECENT ADVANCES IN LC-MS/MS TECHNIQUES FOR FOOD ANALYSIS.....	57
<b>NOVEMBER 6, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>"IN FOOD WE TRUST": A CLOSER LOOK ON AROMA- AND AUTHENTICITY ANALYSIS</b>	
CAN ALCOHOL FREE BEER BE TASTY?	
<i>Erich Leitner</i> .....	58
A NOVEL ION SUPPRESSION-BASED MS METHODOLOGY FOR IDENTIFICATION AND AUTHENTICATION OF LIQUID SAMPLES; WINE AS A CASE STUDY	
<i>Maria Bikaki, Markus Ehrat</i> .....	58

# CONTENT

<b>NOVEMBER 6, 2024 (14:45-15:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>SAMPLEPREP - IMPROVED ANALYSIS OF ORGANIC POLLUTANTS IN FOOD PRODUCTS (PESTICIDE RESIDUES) SAMPLE PREPARATION BY OPTIMISING BULK SAMPLE COMMINUTION ALONGSIDE AUTOMATED RESIDUE EXTRACTION EQUIPMENT</b>	
SAMPLEPREP - IMPROVED ANALYSIS OF ORGANIC POLLUTANTS IN FOOD PRODUCTS (PESTICIDE RESIDUES) SAMPLE PREPARATION BY OPTIMISING BULK SAMPLE COMMINUTION ALONGSIDE AUTOMATED RESIDUE EXTRACTION EQUIPMENT	
<u>Stephan Siniscalco, Eric Smith, Paul Lynch</u> .....	59
<b>NOVEMBER 6, 2024 (14:45-15:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>ADVANCED SOLUTIONS FOR COMPLEX MATRIX MULTI-RESIDUES ANALYSIS</b>	
THE ROLE OF GC×GC IN MOSH & MOAH ANALYSIS	
<u>Giorgia Purcaro, Grègory Bauwens, Aleksandra Gorska, Paula Albendea</u> .....	60
NEW STRATEGIES FOR PESTICIDE RESIDUE ANALYSIS IN CHILLI PEPPERS USING GCXGC-TOFMS	
<u>Michal Stupak, Jingwen Han, Jana Hajslova</u> .....	60
<b>NOVEMBER 6, 2024 (14:45-15:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>OUT OF THE ROUTINE - AUTOMATED ANALYSIS OF CONTAMINANTS LIKE MOSH/MOAH AND MYCOTOXINS IN A CONTRACT LAB</b>	
NEW AUTOMATED WORKFLOW FOR THE ANALYSIS OF MOSH/MOAH IN DIFFERENT FOOD MATRICES...	61
<u>Sebastian Wißmüller</u> .....	61
VALIDATION OF AN ONLINE µSPE SYSTEM FOR THE ANALYSIS OF MYCOTOXINS IN FOOD	
<u>Sebastian Wißmüller</u> .....	61
<b>NOVEMBER 6, 2024 (14:45-15:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>THE LATEST ADVANCEMENTS IN TRACE ELEMENTAL ANALYSIS AND ISOTOPE FINGERPRINTS IN FOOD AUTHENTICITY</b>	
ICP-MS WORKFLOW FOR ENHANCED EFFICIENCY AND PRODUCTIVITY	
<u>Matthew Cassap</u> .....	62
THE NEXT GENERATION LC-IRMS FOR HONEY AUTHENTICITY INVESTIGATION	
<u>Mario Tuthorn</u> .....	62
<b>NOVEMBER 7, 2024 (7:45-8:30)</b>	
<b>VENDOR SEMINAR:</b>	
<b>PRECISION AND INNOVATION: INERT COLUMNS IN MYCOTOXIN AND PESTICIDE ANALYSIS &amp; AUTOMATED ALKALOID ANALYSIS IN HONEY</b>	
HONEY, WE SHRUNK THE ANALYSIS! - A CASE STUDY ON THE AUTOMATION AND MINIATURIZATION OF THE ANALYSIS OF PYRROLIZIDINE ALKALOIDS IN HONEY	
<u>Friedericke Habedank</u> .....	63
UNLOCKING PRECISION: THE POWER OF INERT COLUMNS IN MYCOTOXIN AND PESTICIDE ANALYSIS	
<u>Tina Brandscher</u> .....	63
<b>NOVEMBER 7, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>INNOVATIVE WORKFLOWS FOR THE MULTI-RESIDUE ANALYSIS OF ORGANIC CONTAMINANTS</b>	
"PESTICIDE SMART KIT": A NEW COMPREHENSIVE APPROACH FOR MULTIRESIDUE PESTICIDE ANALYSIS.	64
<u>Valérie Thibert</u>	
AUTOMATED AND HIGH THROUGHPUT PFAS WORKFLOWS	
<u>Aristide Ganci</u> .....	64
<b>NOVEMBER 7, 2024 (13:30-14:15)</b>	
<b>VENDOR SEMINAR:</b>	
<b>TACKLING SEPARATION CHALLENGES: STRATEGIES FOR IDENTIFYING NATURAL TOXIN ISOMERS AND ANALYZING GLYPHOSATE &amp; CO. IN COMPLEX MATRICES</b>	
ION MOBILITY MASS SPECTROMETRY TO ENHANCE THE DETERMINATION OF NATURAL TOXINS IN FOOD SAMPLES	
<u>Laura Carbonell-Rozas</u> .....	65
BRINGING THE DIRECT ANALYSIS OF GLYPHOSATE & CO. IN COMPLEX MATRICES INTO ROUTINE FOOD TESTING LABS	
<u>Claudia Rathmann</u> .....	66



# CONTENT

NOVEMBER 7, 2024 (14:45-15:30)

VENDOR SEMINAR:

INTEGRATED GLUTEN MANAGEMENT - GLUTEN TESTING ALONG THE FOOD PRODUCTION CHAIN ..... 67  
Ronald Niemeijer.....67

## LECTURES..... 68

L1

AUTHENTICITY & TRACEABILITY CLAIMS IN FOOD: HOW INDUSTRY CAN OBJECTIVATE THEM THROUGH ANALYTICAL STRATEGIES

Michele Suman\*<sup>(1)</sup>.....69

L2

AI IN FOOD SAFETY: FROM METHODOLOGY TO APPLICATIONS

Bas Van der Velden\*<sup>(1)</sup>.....70

L3

HIGH THROUGHPUT EFFECT-DIRECTED ANALYSIS FOR IDENTIFICATION OF CHEMICAL MIXTURES IN ENVIRONMENTAL AND HUMAN SAMPLES

Marja Lamoree\*<sup>(1)</sup>.....71

L4

FOOD AND THE CHEMICAL EXPOSOME, WHERE DO WE STAND? SUCCESSES, CHALLENGES, IMPACT ON PUBLIC POLICY

Bruno Le Bizec\*<sup>(1)</sup>, Jean-Philippe Antignac<sup>(1)</sup>, Gaud Dervilly<sup>(1)</sup>.....72

L5

ANALYTICAL CHALLENGES AND DECISION-MAKING PROCESS IN SCREENING FOR HIGHLY-POLAR POTENTIAL EMERGING CONTAMINANTS RELEVANT FOR THE EFSA'S PROCESS OF IDENTIFICATION OF EMERGING RISKS

Vít Kosek\*<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup>.....73

L6

BRIDGING THE GAP FROM MICRO- TO NANO-PLASTIC ANALYSIS IN FOOD

Clementina Vitali<sup>(1)</sup>, Anna K. Undas<sup>(1)</sup>, Hans-Gerd Janssen<sup>(2)</sup>, Michel W.F. Nielen<sup>(1)</sup>, Francesco Simone Ruggeri\*<sup>(3)</sup>.....74

L7

NATURAL TOXINS IN PLANT-BASED FOOD: DOES A SHIFT IN THE DIET COME WITH A SHIFT IN THE EXPOSURE?

Chiara Dall'Asta\*<sup>(1)</sup>, Raquel Torrijos<sup>(2)</sup>, Octavian Mihalache<sup>(1)</sup>.....75

L8

QUALITY, SAFETY, AUTHENTICITY AND TRACEABILITY OF BOTANICALS AND FOOD SUPPLEMENTS: A NEW HOLISTIC CHALLENGE FOR THE FOOD CHEMISTS?

Marco Arlorio\*<sup>(1)</sup>, Jean Daniel Coisson<sup>(1)</sup>, Fabiano Travaglia<sup>(1)</sup>, Monica Locatelli<sup>(1)</sup>, Matteo Bordiga<sup>(1)</sup>.....76

L9

A NOVEL MULTI-TECHNIQUE APPROACH FOR ORGANIC FOOD AUTHENTICITY

Lucie Tsamba\*<sup>(1)</sup>, Freddy Thomas<sup>(1)</sup>, Helene Debetencourt<sup>(1)</sup>, Eric Jamin<sup>(1)</sup>.....77

L10

LC-TWIMS-HRMS TO ADDRESS BOTANICAL FOOD SUPPLEMENTS QUALITY AND AUTHENTICITY

Guillem Campmajo\*<sup>(1)</sup>, Marco Gozzi<sup>(1)</sup>, Renato Bruni<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>.....78

L11

CHICKEN GEOGRAPHICAL ORIGIN VERIFICATION VIA MULTI-ELEMENTAL, ISOTOPIC, SPECTROSCOPIC AND METABOLOMIC FINGERPRINTS

Zora Jandric\*<sup>(1)</sup>, Andreas Zitek<sup>(2)</sup>, Rebecca Hood-Nowotny<sup>(2)</sup>, Lisa Fischer<sup>(2)</sup>, Katharina Schott<sup>(2)</sup>, Sara Kornfeind<sup>(2)</sup>, Andrea Watzinger<sup>(2)</sup>, Christina Troyer<sup>(2)</sup>, Stephan Hann<sup>(3)</sup>.....79

L12

IDENTIFICATION OF MECHANICALLY SEPARATED MEAT IN MEAT PRODUCTS: THE NEW APPROACHES DEVELOPED BY THE "MPSQA" PROJECT IN ITALY

Marco Iammarino\*<sup>(1)</sup>, Oto Miedico<sup>(1)</sup>, Michele Tomaiuolo<sup>(1)</sup>, Giovanna Berardi<sup>(1)</sup>, Monica Casale<sup>(2)</sup>, Paolo Oliveri<sup>(2)</sup>, Cristina Malegori<sup>(2)</sup>, Rogerta Dalipi<sup>(3)</sup>, Emanuele Sangiorgi<sup>(3)</sup>.....80

L13

NOVEL FOODS IN THE EU - REGULATORY FRAMEWORK AND RISK ASSESSMENT. A CLOSE-UP LOOK AT THE ALLERGENICITY RISK ASSESSMENT CHALLENGES: REQUIREMENTS, KNOWLEDGE GAPS AND RESEARCH NEEDS

Marcello Laganaro\*<sup>(1)</sup>, Estefania Noriega Fernandez<sup>(2)</sup>, Antonio Fernandez Dumont<sup>(1)</sup>, Andrea Germini<sup>(1)</sup>.....81

# CONTENT

<b>L14</b>	
<b>A ROBUST MULTI-ALLERGEN PROTEOTYPIC SCREENING METHOD FOR FOOD CONTROL</b>	
<u>Alexander Scherl</u> <sup>(1)</sup> , Alexandre Piletta-Zanin <sup>(2)</sup> , Pierre Zimmerli <sup>(1)</sup> , Didier Ortellì <sup>(1)</sup> , Philippe Eigenmann <sup>(2)</sup> , Patrick Edler <sup>(1)</sup> .....	82
<b>L15</b>	
<b>THE CHANGING FOOD ALLERGEN LANDSCAPE IN EUROPE CALLS FOR HARMONISED FOOD MONITORING</b>	
<u>Nathalie Smits</u> <sup>(1)</sup> , Nicolai Ballin <sup>(2)</sup> , Christine Bruggeman <sup>(1)</sup> , Christiane Fæste <sup>(3)</sup> , Augusto Pastorelli <sup>(4)</sup> , Christof Van Poucke <sup>(5)</sup> , Marleen Voorhuijzen-Harink <sup>(1)</sup> , Yvonne Westphal <sup>(1)</sup> , Michael Walker <sup>(6)</sup> , Matthias Winkel <sup>(7)</sup> , Andries Kooops <sup>(1)</sup> .....	83
<b>L16</b>	
<b>MULTI-ALLERGEN DETECTION BY UHPLC-MS/MS METHOD IN PROCESSED FOODSTUFFS</b>	
<u>Anne-Catherine Huët</u> <sup>(1)</sup> , Jean Henrottin <sup>(2)</sup> , Martin Mailleux <sup>(2)</sup> , Mathieu Dubois <sup>(2)</sup> , Nathalie Gillard <sup>(1)</sup> .....	84
<b>L17</b>	
<b>STANDARDIZATION OF A REFERENCE METHOD FOR MULTIPLE ALLERGEN DETERMINATION IN FOODS, PRECAUTIONARY ALLERGEN LABELLING AND REFERENCE DOSES: THREE ISSUES IN FOOD ALLERGY RESEARCH</b>	
<u>Linda Monaci</u> <sup>(1)</sup> , Elisabetta De Angelis <sup>(1)</sup> , Anna Luparelli <sup>(1)</sup> , Rosa Pilolli <sup>(2)</sup> , Francesca Lambertini <sup>(3)</sup> .....	85
<b>L18</b>	
<b>SAVING TIME, ORGANIC WASTE, AND MONEY: NEW NEEDS IN MULTI-RESIDUE METHODS FOR DETECTING PESTICIDE RESIDUES IN FOOD</b>	
<u>Amadeo R. Fernandez-Alba</u> <sup>(1)</sup> , Francisco Garrido <sup>(2)</sup> .....	86
<b>L19</b>	
<b>DEALING WITH RESIDUE DEFINITIONS ENTAILING ESTERS AND CONJUGATES</b>	
<u>Michelangelo Anastassiades</u> <sup>(1)</sup> .....	87
<b>L20</b>	
<b>EXPERIENCES AND CHALLENGES FROM THE PERSPECTIVE OF THE EURL FOR PESTICIDE RESIDUES IN CEREALS AND FEEDING STUFFS</b>	
<u>Ederina Ninga</u> <sup>(1)</sup> , Elena Hakme <sup>(2)</sup> , Julio Cesar Espana Amortegui <sup>(2)</sup> , <u>Mette Erecius Poulsen</u> <sup>(2)</sup> .....	88
<b>L21</b>	
<b>HARMONISATION OF RESIDUE CONTROL IN EUROPE - EXAMPLES OF TOOLS OF THE EURL FOR RESIDUES OF VETERINARY DRUGS</b>	
<u>Joachim Polzer</u> <sup>(1)</sup> , Ulrike Mülrow-Stollin <sup>(1)</sup> , Ferial Tadjine <sup>(1)</sup> , Katrin Kittler <sup>(1)</sup> , Kai Nieschalke <sup>(1)</sup> , Markus Fifeik <sup>(1)</sup> .....	89
<b>L22</b>	
<b>UPDATE ON THE WORK OF THE EURL/NRL NETWORK FOR HALOGENATED POPS IN FEED AND FOOD</b>	
<u>Alexander Schaechtele</u> <sup>(1)</sup> , Theresa Zwickel <sup>(2)</sup> , Christina Riemenschneider <sup>(1)</sup> , Jakob Hauns <sup>(2)</sup> , Katharina Rund <sup>(1)</sup> .....	90
<b>L23</b>	
<b>PROGRESS MADE AND CHALLENGES ON SAMPLING AND ANALYSIS OF MYCOTOXINS AND PLANT TOXINS IN FOOD AND FEED</b>	
<u>Patrick Mulder</u> <sup>(1)</sup> , Sylvia Kalli <sup>(1)</sup> , Marta Soperl <sup>(1)</sup> , Hans Mol <sup>(1)</sup> , Josipa Grzetic Martens <sup>(1)</sup> .....	91
<b>L24</b>	
<b>NEW FOOD CHALLENGES AND EXPERIENCES FROM THE EUROPEAN UNION REFERENCE LABORATORY FOR PROCESSING CONTAMINANTS</b>	
<u>Arvid Fromberg</u> <sup>(1)</sup> .....	92
<b>L25</b>	
<b>RECENT ANALYTICAL ADVANCES BY THE EURL-MN</b>	
<u>Heidi Amlund</u> <sup>(1)</sup> , Agnieszka Niklas <sup>(1)</sup> , Daniel Clayton-Cuch <sup>(1)</sup> , Doaa Diaaeldin Ahmed M Abouelenein <sup>(1)</sup> , Katrin Löschner <sup>(1)</sup> , Lene Duedahl-Olesen <sup>(1)</sup> , <u>Jens J. Sloth</u> <sup>(1)</sup> .....	93
<b>L26</b>	
<b>EMN FOOD: PRIORITIES AND STRATEGIES FOR DRIVING METROLOGY IN SUPPORT OF FOOD SAFETY AND SUSTAINABILITY</b>	
<u>Chiara Portesi</u> <sup>(1)</sup> , Nives Ogrinc <sup>(2)</sup> , <u>Andrea Mario Rossi</u> <sup>(3)</sup> .....	94
<b>L27</b>	
<b>SAFETY ASSESSMENT OF FOOD CONTACT MATERIALS IN THE CONTEXT OF EUROPEAN REGULATIONS</b>	
<u>Erich Leitner</u> <sup>(1)</sup> .....	95
<b>L28</b>	
<b>INCREASING THE RELIABILITY OF MOSH &amp; MOAH ANALYSIS IN FOOD BY IMPROVED SAMPLE PREPARATION AND HYPHENATED CHROMATOGRAPHIC TECHNIQUES</b>	
<u>Giorgia Purcaro</u> <sup>(1)</sup> , Gregory Bauwens <sup>(1)</sup> , Aleksandra Gorska <sup>(1)</sup> , Paula Albendea <sup>(1)</sup> .....	96

# CONTENT

<b>L29</b>	
<b>INVESTIGATION OF SEMI-QUANTIFICATION ACCURACY AND DETECTOR UNIVERSALITY FOR PACKAGING SAFETY ANALYSIS</b>	
<u>Elsa Omer</u> <sup>*(1)</sup> , Marine Ewers <sup>(1)</sup> , Fabien Clement <sup>(1)</sup> , Yves-Alexis Hammel <sup>(1)</sup> , Oriane Bellahoues <sup>(1)</sup> , Jesus Varela <sup>(1)</sup> , Sander Koster <sup>(1)</sup> .....	97
<b>L30</b>	
<b>CP-MIMS: A NEW FRONTIER FOR THE REAL-TIME MONITORING OF HAZARDOUS CHEMICAL MIGRATION FROM FOOD CONTACT MATERIALS</b>	
<u>Maurizio Piergiovanni</u> <sup>(1)</sup> , Monica Mattarozzi <sup>(1)</sup> , Federica Bianchi <sup>(1)</sup> , Nicolo Riboni <sup>(1)</sup> , Cristian Maffezzoni <sup>(1)</sup> , Veronica Termopoli <sup>(2)</sup> , Viviana Consonni <sup>(2)</sup> , Davide Ballabio <sup>(2)</sup> , Maria Careri <sup>*(1)</sup> .....	98
<b>L31</b>	
<b>INVESTIGATION OF POTENTIAL MIGRATABLES FROM PAPER AND BOARD FOOD CONTACT MATERIALS INTENDED FOR TAKEAWAY</b>	
<u>Mélanie Di Mario</u> <sup>*(1)</sup> , Grégory Bauwens <sup>(2)</sup> , Florian Peltier <sup>(1)</sup> , Pierre-Hugues Stefanuto <sup>(2)</sup> , Séverine Goscinny <sup>(1)</sup> , Giorgia Purcaro <sup>(3)</sup> , Jean-François Focant <sup>(2)</sup> , Els Van Hoeck <sup>(1)</sup> .....	99
<b>L32</b>	
<b>ACRYLAMIDE: 6 YEARS OF REGULATION (EU) 2158/2017 - RESULTS OF AN OFFICIAL CONTROL LABORATORY (OCL)</b>	
<u>Carmen Breitling-Utzmann</u> <sup>*(1)</sup> , Nadja Bauer <sup>(1)</sup> .....	100
<b>L33</b>	
<b>IMPLEMENTING IMAGE DATA ANALYSIS TO PREDICT THE ACRYLAMIDE CONTENT IN CARROT CRISPS PROCESSED BY DIFFERENT FRYING TECHNOLOGIES</b>	
<u>Aristeidis Tsagkaris</u> <sup>*(1)</sup> , Beverly Hradecka <sup>(1)</sup> , Tomas Kourimsky <sup>(1)</sup> , Hana Pirova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	101
<b>L34</b>	
<b>QUANTIFICATION, EXPLORATION AND MITIGATION OF FURAN AND ITS DERIVATIVES IN INFANT FOODS</b>	
Donnelle Sandjong Sayon <sup>(1)</sup> , Aya Fakhri <sup>(1)</sup> , Frederic Mercier <sup>(1)</sup> , Nathalie Kondjoyan <sup>(1)</sup> , Berta Torrents <sup>(2)</sup> , Norbert Fuchsbauer <sup>(3)</sup> , Evangelia Kristalli <sup>(4)</sup> , Rallou Thomopoulos <sup>(5)</sup> , Jeremy Ratel <sup>(6)</sup> , <u>Erwan Engel</u> <sup>*(1)</sup> .....	102
<b>L35</b>	
<b>SEARCH FOR CHLORINATED LIPIDS IN REFINED VEGETABLE OILS</b>	
<u>Tomas Kourimsky</u> <sup>*(1)</sup> , Jakub Tomasko <sup>(1)</sup> , Beverly Hradecka <sup>(1)</sup> , Vojtech Hrbek <sup>(1)</sup> , Jan Kyselka <sup>(2)</sup> , Jana Pulkrabova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	103
<b>L36</b>	
<b>ANALYSIS OF OXIDIZED AND GLYCATED AMINO ACIDS IN FOOD: WHY MASS SPECTROMETRY IS ESSENTIAL</b>	
<u>Michael Hellwig</u> <sup>*(1)</sup> .....	104
<b>L37</b>	
<b>STATISTICAL APPROACHES TO METABOLOMICS DATA HANDLING</b>	
<u>Vit Kosek</u> <sup>*(1)</sup> .....	105
<b>L38</b>	
<b>DATA FUSION AND ARTIFICIAL INTELLIGENCE BASED NOVEL TESTING SYSTEM FOR TEA GEOGRAPHICAL ORIGIN AUTHENTICATION</b>	
<u>Yicong Li</u> <sup>(1)</sup> , Chris Elliott <sup>(2)</sup> , Natasha Logan <sup>(1)</sup> , Awanwee Petchkongkaew <sup>(3)</sup> , Nicholas Birse <sup>(1)</sup> , Terry F. McGrath <sup>(4)</sup> , Di Wu <sup>*(1)</sup> ...	106
<b>L39</b>	
<b>MACHINE LEARNING ALGORITHMS AT THE BRIGHT SIDE OF FTIR-ATR ANALYSIS IN LATHYRUS SATIVUS L.</b>	
<u>Elsa Mecha</u> <sup>*(1)</sup> , Pedro R. Brandao <sup>(2)</sup> , Letice Gonçalves <sup>(3)</sup> , Elsa Brito <sup>(4)</sup> , Andreia Bento da Silva <sup>(5)</sup> , Maria Carlota Vaz Patto <sup>(3)</sup> , Maria Rosario Bronze <sup>(6)</sup> .....	107
<b>L40</b>	
<b>UNLOCKING TOMATO QUALITY ATTRIBUTES IRRIGATED WITH TREATED WASTEWATER USING MACHINE LEARNING</b>	
<u>Anja Vehar</u> <sup>(1)</sup> , Jan Drole <sup>(2)</sup> , Doris Potocnik <sup>(3)</sup> , Ana Kovacic <sup>(3)</sup> , Lidija Strojnik <sup>(3)</sup> , Marta Hudobivnik <sup>(3)</sup> , Marina Pintar <sup>(4)</sup> , Nina Kacjan Marsic <sup>(4)</sup> , David Heath <sup>(1)</sup> , Ester Heath <sup>(1)</sup> , Nives Ogrinc <sup>*(1)</sup> .....	109
<b>L41</b>	
<b>SAFEGUARDING OUR FOOD CHAIN: NOVEL STRATEGIES FOR THE CONTROL AND ANALYSIS OF MYCOTOXINS AND OTHER EMERGING CONTAMINANTS</b>	
<u>Rudolf Krska</u> <sup>(1)</sup> , Stephan Freitag <sup>(2)</sup> , Michele Suman <sup>(3)</sup> , Eleonara Rollo <sup>(3)</sup> , Alexandra Schamann <sup>(4)</sup> , Martin Wagner <sup>(5)</sup> .....	110
<b>L42</b>	
<b>RECENT ADVANCES IN UNDERSTANDING OF MYCOTOXINS IN BEER</b>	
<u>Milena Stranska</u> <sup>*(1)</sup> , Nela Prusova <sup>(1)</sup> , Tereza Dolezalova <sup>(1)</sup> , Adam Behner <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	111
<b>L43</b>	
<b>NAVIGATING COMPLEX MIXTURES: NON-TARGETED APPROACHES IN DETECTING EMERGING NATURAL TOXINS</b>	
<u>Laura Righetti</u> <sup>*(1)</sup> .....	112

# CONTENT

<b>L44</b>	<b>THE ANALYTICAL CHARACTERIZATION OF THE CIGUATERA POISONING AS AN EMERGING RISK IN EUROPE THROUGH THE EFFORTS CARRIED OUT DURING THE TWO EDITIONS OF THE EUROCIGUA PROJECT</b>	
	<i>Ana Gago Martínez</i> <sup>(1)</sup> , David Castro <sup>(1)</sup> , Jorge Giraldez <sup>(1)</sup> , Alejandro Penin <sup>(1)</sup> , Jose Manuel Leao <sup>(1)</sup> .....	113
<b>L45</b>	<b>FATE OF MYCOTOXINS DURING GLUTEN-FREE PASTA PROCESSING: UNTARGETED 13C-LABELLING LC-HRMS BASED APPROACH</b>	
	<i>Eleonora Rollo</i> <sup>(1)</sup> , Alexandra Schamann <sup>(2)</sup> , Alexandra Malachova <sup>(3)</sup> , Michele Suman <sup>(4)</sup> , Rudolf Krska <sup>(5)</sup> .....	114
<b>L46</b>	<b>NMR SPECTROSCOPY - A VERSATILE TOOL IN FOOD CONTROL</b>	
	<i>Carsten Fahl-Hassek</i> <sup>(1)</sup> .....	115
<b>L47</b>	<b>EVALUATION OF LOW-FIELD VERSUS HIGH-FIELD PROTON NMR SPECTROSCOPY FOR THE AUTHENTICITY TESTING OF PEPPER</b>	
	<i>Dalal Werner</i> <sup>(1)</sup> , Horacio Heinzen <sup>(2)</sup> , Marivil Islam <sup>(2)</sup> , Paul Rentz <sup>(1)</sup> , Alina Mihailova <sup>(2)</sup> , Morane Lointier <sup>(1)</sup> , Simon Kelly <sup>(2)</sup> .....	116
<b>L48</b>	<b>1H-NMR AND RAMAN DATA FUSION: A NEW STRATEGY FOR THE DEVELOPMENT OF RELIABLE WINE AUTHENTICATION MODELS</b>	
	<i>Ariana Raluca Hategan</i> <sup>(1)</sup> , Maria David <sup>(1)</sup> , Adrian Pirnau <sup>(2)</sup> , Bogdan Cozar <sup>(2)</sup> , Simona Cinta-Pinzaru <sup>(3)</sup> , Francois Guyon <sup>(4)</sup> , Dana Alina Magdas <sup>(1)</sup> .....	117
<b>L49</b>	<b>INTEGRATED LIBS-RAMAN SYSTEM FOR FOOD AUTHENTICATION AND SAFETY ANALYSIS</b>	
	<i>Bartek Rajwa</i> <sup>(1)</sup> , Sungho Shin <sup>(2)</sup> , Euiwon Bae <sup>(1)</sup> , Kemal Eseller <sup>(1)</sup> , J. Paul Robinson <sup>(1)</sup> .....	118
<b>L50</b>	<b>A HARMONIZED APPROACH FOR FOOD AUTHENTICITY MARKER VALIDATION AND ACCREDITATION</b>	
	<i>Stephane Bayen</i> <sup>(1)</sup> , Chris Elliott <sup>(2)</sup> , Marco Arlorio <sup>(3)</sup> , Nicolai Zederkopff Ballin <sup>(4)</sup> , Nicholas Birse <sup>(2)</sup> , Jens Brockmeyer <sup>(5)</sup> , Shawninder Chahal <sup>(1)</sup> , Lei Tian <sup>(1)</sup> , Jianguo Xia <sup>(1)</sup> , Maria G. Corradini <sup>(6)</sup> , Robert Hanner <sup>(6)</sup> , Stephan Hann <sup>(7)</sup> , Teresa Steininger-Mairinger <sup>(7)</sup> , Kristian Holst Laursen <sup>(8)</sup> , Alina Mihailova <sup>(9)</sup> , Michele Suman <sup>(10)</sup> , Saskia Van Ruth <sup>(11)</sup> .....	119
<b>L51</b>	<b>EXPLOITING THE POTENTIAL OF DART-HRMS FOR RAPID AUTHENTICITY TESTING AND FOOD FRAUD DETECTION: INTRODUCING AN INTEGRATED WORKFLOW IN THE CASE STUDY OF EXTRA VIRGIN OLIVE OIL</b>	
	<i>Dimitrios A. Papelis</i> <sup>(1)</sup> , <i>Sofia K. Drakopoulou</i> <sup>(2)</sup> , Ilona Nordhorn <sup>(3)</sup> , Carsten Baessmann <sup>(3)</sup> , Nikolaos S. Thomaidis <sup>(2)</sup> .....	120
<b>L52</b>	<b>FIGHTING DEFORESTATION THROUGH ADVANCES IN ANALYTICAL CHEMISTRY</b>	
	<i>Yuhne Hong</i> <sup>(1)</sup> , Maria Aparicio Muriana <sup>(1)</sup> , Nicholas Birse <sup>(1)</sup> , <i>Chris Elliott</i> <sup>(2)</sup> .....	121
<b>L53</b>	<b>THE KNOWN AND THE UNKNOWN: THE ANALYTICAL TOOLBOX TO TACKLE THE PFAS CHALLENGE</b>	
	<i>Stefan Van Leeuwen</i> <sup>(1)</sup> .....	122
<b>L54</b>	<b>SIMULTANEOUS ACTIVATION OF 3 LEVERS TO EXTEND THE SPECTRUM OF PFAS STUDIED IN FOOD MATRICES, THE APPROACH THAT MAKES ALL THE DIFFERENCE</b>	
	<i>Cassandre Jeannot</i> <sup>(1)</sup> , Bruno Le Bizec <sup>(2)</sup> , Julien Parinet <sup>(3)</sup> , Gaud Dervilly <sup>(4)</sup> .....	123
<b>L55</b>	<b>PFAS ANALYSIS IN FOOD: A MULTI-TECHNIQUE APPROACH FOR REGULATORY COMPLIANCE AND CONSUMER PROTECTION</b>	
	<i>Xanthippi Theurillat</i> <sup>(1)</sup> .....	124
<b>L56</b>	<b>PFAS IN FRUITS AND VEGETABLES: AN INTERLABORATORY VALIDATION STUDY ON THE ACHIEVABILITY OF EU TARGETED LOQS</b>	
	<i>Consolato Schiavone</i> <sup>(1)</sup> , Chiara Portesi <sup>(1)</sup> , Francesco Romaniello <sup>(1)</sup> , Christina Riemenschneider <sup>(2)</sup> , Katharina Rund <sup>(2)</sup> , Alexander Schaechtele <sup>(2)</sup> .....	125
<b>L57</b>	<b>NON-TARGETED SCREENING FOR PFAS IN COMPLEX FOOD MATRICES: FOCUS ON THE KEYS TO DATA PRIORITIZATION</b>	
	<i>Nicolas Macorps</i> <sup>(1)</sup> , Elysa Oliveira <sup>(1)</sup> , Bruno Le Bizec <sup>(1)</sup> , Gaud Dervilly <sup>(1)</sup> .....	126
<b>L58</b>	<b>MAKING POLYCHLORINATED ALKANE ANALYSIS IN FOOD MORE ACCESSIBLE: EXPLORING LOW-RESOLUTION LC-MS/MS AS A SUITABLE ALTERNATIVE TO LC-HRMS AND GC-HRMS</b>	
	<i>Ingun Perkons</i> <sup>(1)</sup> , Anna Skrastina <sup>(2)</sup> , Laura Lazdina <sup>(2)</sup> .....	127

# CONTENT

<b>L59</b> <b>STUDY OF MINERAL OIL HYDROCARBONS IN DIFFERENT TYPES OF UNPROCESSED MEAT BY LC-GC×GC-FID/MS</b> <i>Paula Albendea</i> <sup>(1)</sup> , <i>Giorgia Purcaro</i> <sup>*(1)</sup> .....	128
<b>L60</b> <b>SHORT- AND MEDIUM-CHAIN CHLORINATED PARAFFINS IN INSECT-BASED FOODS</b> <i>Jakub Tomasko</i> <sup>*(1)</sup> , <i>Michaela Freyova</i> <sup>(2)</sup> , <i>Katerina Sebelova</i> <sup>(1)</sup> , <i>Jana Pulkrabova</i> <sup>(1)</sup> .....	129
<b>L61</b> <b>ANALYTICAL STRATEGIES TO DETERMINE 3-7 RING MOAH IN FOOD AND FOOD CONTACT MATERIALS: DACC-HPLC-GC AND GCXGC</b> <i>Martin Lommatzsch</i> <sup>*(1)</sup> .....	130
<b>L62</b> <b>INTERACTIVE SEMINAR: STEP BY STEP STRATEGIES FOR FAST DEVELOPMENT OF SMART ANALYTICAL METHODS</b> <i>Katerina Mastovska</i> <sup>*(1)</sup> , <i>Hans Mol</i> <sup>(2)</sup> , <i>Michal Stupak</i> <sup>(3)</sup> , <i>Octavian Augustin Mihalache</i> <sup>(4)</sup> .....	131
<b>L63</b> <b>SENSORY EVALUATION AS ESSENTIAL TECHNIQUE FOR THE DETERMINATION OF OFF-FLAVOURS IN FOOD</b> <i>Barbara Siegmund</i> <sup>*(1)</sup> .....	132
<b>L64</b> <b>RESPONSE SURFACE METHODOLOGY (RSM) AND UNTARGETED-TARGETED METABOLOMIC ANALYSIS AS TOOL TO OBTAIN POLYPHENOLS-ENRICHED EXTRACT FROM CITRUS MEDICA L. WITH PROMISING USE IN NUTRACEUTICAL FIELD</b> <i>Vittorio Calucci</i> <sup>(1)</sup> , <i>Ludovica Lela</i> <sup>(1)</sup> , <i>Nadia Benedetto</i> <sup>(1)</sup> , <i>Tereza Jaegerova</i> <sup>(2)</sup> , <i>Luigi Milella</i> <sup>*(1)</sup> , <i>Jana Hajslova</i> <sup>(2)</sup> .....	133
<b>L65</b> <b>USING HRMS TO IDENTIFY POTENTIAL MIGRANTS IN BIOBASED AND BIODEGRADABLE FOOD CONTACT MATERIALS: POLYESTER AND POLYLACTIC ACID BASED MATERIALS AS A STUDY CASE</b> <i>Patricia Vazquez-Loureiro</i> <sup>*(1)</sup> , <i>Ronan Cariou</i> <sup>(2)</sup> , <i>Antía Lestido-Cardama</i> <sup>(3)</sup> , <i>Letricia Barbosa-Pereira</i> <sup>(4)</sup> , <i>Raquel Sendon</i> <sup>(4)</sup> , <i>Juana Bustos</i> <sup>(5)</sup> , <i>Ana Gasco</i> <sup>(5)</sup> , <i>Perfecto Paseiro-Losada</i> <sup>(3)</sup> , <i>Ana Rodriguez Bernaldo de Quiros</i> <sup>(4)</sup> .....	134
<b>L66</b> <b>SIMULTANEOUS ANALYSIS OF 17 ANTIVIRAL SUBSTANCES IN POULTRY MUSCLE BY UHPLC-MS/MS</b> <i>Christelle Robert</i> <sup>*(1)</sup> , <i>Gilles Pierret</i> <sup>(1)</sup> , <i>Nathalie Gillard</i> <sup>(2)</sup> .....	135
<b>L67</b> <b>DEVELOPMENT OF A DNA METABARCODING METHOD FOR PLANT SPECIES IDENTIFICATION IN FOOD</b> <i>Andrea Nicole Aigner</i> <sup>*(1)</sup> , <i>Stefanie Dobrovolny</i> <sup>(1)</sup> , <i>Rupert Hochegger</i> <sup>(1)</sup> , <i>Konrad J. Domig</i> <sup>(2)</sup> , <i>Margit Cichna-Markl</i> <sup>(3)</sup> .....	136
<b>L68</b> <b>PAPER-IMMOBILIZED LIQUID PHASE MICROEXTRACTION FOR DIRECT PAPER SPRAY MASS SPECTROMETRY AND IMMUNO-DETECTION OF ATROPINE IN BABY FOOD, BUCKWHEAT CEREALS, AND EDIBLE OILS AT REGULATORY LEVELS</b> <i>Ids Lemmink</i> <sup>*(1)</sup> , <i>Sipke Bosma</i> <sup>(1)</sup> , <i>Erik Beij</i> <sup>(1)</sup> , <i>Han Zuilhof</i> <sup>(1)</sup> , <i>Toine Bovee</i> <sup>(1)</sup> , <i>Gert IJsbrand Salentijn</i> <sup>(1)</sup> .....	137
<b>L69</b> <b>ELEMENTOMICS, METABOLOMICS, AND CHEMOMETRIC APPROACHES AS TOOLS IN EXPLORING BLACK PEPPER IDENTITY</b> <i>Cynthia Adaku Chilaka</i> <sup>*(1)</sup> , <i>Nicholas Birse</i> <sup>(1)</sup> , <i>Chris Elliott</i> <sup>(1)</sup> .....	138
<b>L70</b> <b>EMERGING CONTAMINANTS EXPOSED: COMBINING EFFECT-BASED TESTING AND ANALYTICAL CHEMISTRY FOR FOOD AND FEED SAFETY</b> <i>Arjen Gerssen</i> <sup>*(1)</sup> , <i>Milou Van de Schans</i> <sup>(1)</sup> , <i>Toine Bovee</i> <sup>(1)</sup> , <i>Caroline Dirks</i> <sup>(1)</sup> .....	139
<b>L71</b> <b>EXPLORING MASS SPECTROMETER LIMITS: UHPLC-MS/MS METHOD FOR DETERMINATION OF 1,000 TOXINS IN 10 MINUTES</b> <i>Lidija Kenjeric</i> <sup>(1)</sup> , <i>Alexandra Malachova</i> <sup>(2)</sup> , <i>Rudolf Krska</i> <sup>(3)</sup> , <i>Michael Sulyok</i> <sup>*(3)</sup> .....	140
<b>L72</b> <b>INVESTIGATION OF DIFFERENT SEPARATION STRATEGIES IN COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR CHARACTERIZATION OF SECONDARY METABOLITES IN COMPLEX FOOD MATRICES</b> <i>Roberto Lagana Vinci</i> <sup>(1)</sup> , <i>Katia Arena</i> <sup>*(1)</sup> , <i>Francesco Cacciola</i> <sup>(2)</sup> , <i>Paola Dugo</i> <sup>(3)</sup> , <i>Luigi Mondello</i> <sup>(3)</sup> .....	141
<b>L73</b> <b>ANALYTICAL DEVELOPMENTS AND CHALLENGES FOR MICRO-/NANOPLASTICS ANALYSIS IN FOOD</b> <i>Haiyan Li</i> <sup>*(1)</sup> , <i>Lin Min Lee</i> <sup>(1)</sup> , <i>Joanna Khoo</i> <sup>(1)</sup> , <i>Sheot Harn Chan</i> <sup>(1)</sup> , <i>Dingyi Yu</i> <sup>(1)</sup> .....	142

# CONTENT

<b>L74</b>	
<b>THE FRACTIONATION PROCESSING AFFECTS THE AMOUNT OF ANTINUTRIENTS IN DIFFERENT PLANT-BASED FOOD INGREDIENTS</b>	
<u>Giovanni D'Auria</u> <sup>*(1)</sup> , Chiara Nitride <sup>(1)</sup> , Alfonso Piscitelli <sup>(1)</sup> , Ever Hernandez Olivas <sup>(2)</sup> , Andre Brodkorb <sup>(2)</sup> , Pasquale Ferranti <sup>(1)</sup>	143
<b>L75</b>	
<b>PROTEOMICS TO ANSWER PROTEIN-RELATED FOOD SAFETY QUESTIONS</b>	
<u>Yvonne Westphal</u> <sup>*(1)</sup> , Floor Leurs <sup>(1)</sup> , Twan America <sup>(2)</sup> , Aard De Jong <sup>(3)</sup> , Nathalie Smits <sup>(1)</sup> , Marco Blokland <sup>(1)</sup>	144
<b>L76</b>	
<b>PROTEOMIC IDENTIFICATION OF INSECT SPECIES IN FOOD PRODUCTS USING MASS SPECTROMETRY</b>	
<u>Stepanka Kuckova</u> <sup>*(1)</sup> , Tatiana Smirnova <sup>(1)</sup> , David Straka <sup>(1)</sup> , Alena Meledina <sup>(1)</sup> , Jiri Santrucek <sup>(1)</sup>	145
<b>L77</b>	
<b>QUANTITATIVE PEPTIDE PROFILING OF HYDROLYSED INFANT FORMULA BY AN UNTARGETED DIAPASEF APPROACH ON A MICROLIC-IM-QTOF-MS/MS</b>	
<u>Alina Kupfer</u> <sup>(1)</sup> , Joachim Stützer <sup>(1)</sup> , Sabrina Gensberger-Reigl <sup>(2)</sup> , Monika Pischetsrieder <sup>*(2)</sup>	146
<b>L78</b>	
<b>INNOVATIONS AND CHALLENGES IN QUANTITATIVE VOLATILOMICS: THE ROLE OF FID/MS CHROMATOGRAM FUSION IN ENHANCING PATTERN RECOGNITION</b>	
<u>Andrea Caratti</u> <sup>*(1)</sup> , Angelica Fina <sup>(2)</sup> , Fulvia Trapani <sup>(2)</sup> , Carlo Bicchi <sup>(2)</sup> , Stephen E. Reichenbach <sup>(3)</sup> , Qingping Tao <sup>(4)</sup> , Daniel Geschwender <sup>(4)</sup> , Chiara Cordero <sup>(1)</sup>	147
<b>L79</b>	
<b>MACHINE-LEARNING BASED VOLATILOMICS FOR FAST SCREENING OF CULTURED-YEAST FERMENTED COFFEE</b>	
<u>Catherine Kiefer</u> <sup>*(1)</sup> , Steffen Schwarz <sup>(2)</sup> , Sascha Rohn <sup>(3)</sup> , Philipp Weller <sup>(1)</sup>	148
<b>L80</b>	
<b>SPECTROSCOPIC APPROACHES FOR RAPID FOOD AUTHENTICITY SCREENING - RECENT APPLICATIONS AT FAO/IAEA</b>	
<u>Alina Mihailova</u> <sup>*(1)</sup> , Marivil Islam <sup>(1)</sup> , Simon Kelly <sup>(1)</sup> , Britt Maestroni <sup>(1)</sup> , Aiman Abraham <sup>(1)</sup> , Islam Hamed <sup>(1)</sup> , Christina Vlachou <sup>(1)</sup>	149
<b>L81</b>	
<b>PORTABLE NIR SPECTROSCOPY AND MACHINE LEARNING FOR A GLOBAL HONEY AUTHENTICATION AND FRAUD DETECTION</b>	
<u>Andrea Mara</u> <sup>*(1)</sup> , Marco Caredda <sup>(2)</sup> , Marco Ciulu <sup>(3)</sup> , Francesca Tilocca <sup>(1)</sup> , Ilaria Langasco <sup>(1)</sup> , Javier Saurina <sup>(4)</sup> , Maria I. Pilo <sup>(1)</sup> , Nadia Spano <sup>(1)</sup> , Gavino Sanna <sup>(1)</sup> , Oscar Nunez <sup>(5)</sup> , Sonia Sentellas <sup>(5)</sup>	150
<b>L82</b>	
<b>ELEMENTAL PROFILE OF HONEY: INSIGHTS RELATED TO THE ENVIRONMENTAL INFLUENCES AND THE DEVELOPMENT OF AUTHENTICATION MODELS</b>	
<u>Maria David</u> <sup>*(1)</sup> , Ariana Raluca Hategan <sup>(1)</sup> , Adriana Dehelean <sup>(2)</sup> , Romulus Puscas <sup>(2)</sup> , Gabriela Cristea <sup>(2)</sup> , Dana Alina Magdas <sup>(1)</sup>	151
<b>L83</b>	
<b>SOYA ELEMENTOMIC FINGERPRINTING AND PREDICTIVE MODELING: PAVING THE WAY TOWARDS ADDRESSING DEFORESTATION CHALLENGES</b>	
<u>Maria del Mar Aparicio-Muriana</u> <sup>*(1)</sup> , Yunhe Hong <sup>(1)</sup> , Brian Quinn <sup>(1)</sup> , Nicholas Birse <sup>(1)</sup> , Chris Elliott <sup>(1)</sup>	152
<b>L84</b>	
<b>SPECIES IDENTIFICATION OF SEAFOOD IN COMPLEX FOOD MATRICES USING NEXT GENERATION SEQUENCING TECHNOLOGY</b>	
<u>Julia Andronache</u> <sup>*(1)</sup> , Margit Cichna-Markl <sup>(2)</sup> , Stefanie Dobrovlny <sup>(1)</sup> , Rupert Hochegger <sup>(1)</sup>	153
<b>L85</b>	
<b>AUTHENTICATION OF RAW NUT PRODUCTS: IDENTIFICATION OF UNEXPECTED ADULTERATIONS BY TRULY UNTARGETED PEPTIDE PROFILE ANALYSIS VIA MICROLIC-IM-QTOF-MS/MS AND DE NOVO SEQUENCING</b>	
<u>Sammy Venegas</u> <sup>(1)</sup> , Andreas Mauser <sup>(1)</sup> , Sabrina Gensberger-Reigl <sup>(2)</sup> , Monika Pischetsrieder <sup>*(2)</sup>	154
<b>L86</b>	
<b>SPECIES AND CULTIVAR DIFFERENTIATION BY DNA BARCODING COUPLED TO HIGH RESOLUTION MELTING ANALYSIS TO DETECT FOOD ADULTERATION</b>	
<u>Jakob Scheibelreiter</u> <sup>(1)</sup> , Michaela Wildbacher <sup>(1)</sup> , Isabel Niederkofler <sup>(1)</sup> , Sofie Schandl <sup>(1)</sup> , Klementina Yakova <sup>(1)</sup> , Katha Pühringer <sup>(1)</sup> , Julia Andronache <sup>(2)</sup> , Stefanie Dobrovlny <sup>(2)</sup> , Rupert Hochegger <sup>(2)</sup> , Thomas Rühmer <sup>(3)</sup> , Barbara Siegmund <sup>(4)</sup> , Margit Cichna-Markl <sup>*(1)</sup>	155
<b>L87</b>	
<b>AN HOLISTIC SAFETY ASSESSMENT OF PLANT-BASED PROTEIN SOURCES: WHERE WE ARE AND WHAT THE MYCOBEANS ALLIANCE CAN DO</b>	
<u>Chiara Dall'Asta</u> <sup>*(1)</sup> , Luisa Calcinai <sup>(1)</sup> , Sara Cutroneo <sup>(1)</sup> , Octavian Mihalache <sup>(1)</sup> , Raquel Torrijos <sup>(2)</sup> , Tullia Tedeschi <sup>(1)</sup>	156

# CONTENT

<b>L88</b> <b>MYCOSMART: AN ON-SITE MYCOTOXINS DETECTION SYSTEM FROM THE SYNERGY OF EU-SE ASIA COLLABORATION</b> <u>Nitsara Karoonuthaisiri</u> * <sup>(1)</sup> .....	157
<b>L89</b> <b>RAPID NATURAL TOXIN TESTING - EU AND SE ASIA PERSPECTIVES</b> <u>Nicholas Birse</u> * <sup>(1)</sup> .....	158
<b>L90</b> <b>EVALUATING THE RISKS ASSOCIATED TO CHRONIC EXPOSURE TO CHEMICAL CONTAMINANTS IN FOOD</b> <u>Rudolf Krska</u> * <sup>(1)</sup> , <u>Chris Elliott</u> <sup>(2)</sup> .....	159
<b>L91</b> <b>SIMULTANEOUS DETECTION OF 29 ANTIMICROBIAL RESIDUES: A SUCCESSFUL STORY OF THAI-UK COLLABORATION</b> <u>Awanwee Petchkongkaew</u> * <sup>(1)</sup> .....	160
<b>L92</b> <b>PROMOTING MULTI-OMICS APPROACHES FOR BLACK TEA GEOGRAPHICAL INDICATIONS TRACING WITH AI AND MACHINE LEARNING</b> <u>Yicong Li</u> <sup>(1)</sup> , <u>Simon Haughey</u> <sup>(1)</sup> , <u>Nicholas Birse</u> <sup>(1)</sup> , <u>Natasha Logan</u> <sup>(1)</sup> , <u>Chris Elliott</u> <sup>(1)</sup> , <u>Di Wu</u> * <sup>(1)</sup> .....	161
<b>L93</b> <b>NON-TARGETED DETECTION OF FOOD ADULTERATION USING AN ENSEMBLE MACHINE-LEARNING MODEL</b> <u>Terence Lok Ting Lau</u> * <sup>(1)</sup> , <u>Teresa Chung</u> <sup>(1)</sup> , <u>Yan Yan Nelly Lam</u> <sup>(1)</sup> .....	162
<b>L94</b> <b>BAD APPLES OR BAD BARRELS - INTEGRITY IN EUROPEAN AND ASIAN FOOD BUSINESSES</b> <u>Saskia Van Ruth</u> * <sup>(1)</sup> .....	163
<b>L95</b> <b>DEVELOPMENT OF PORTABLE DETECTION TECHNOLOGY FOR FOOD AUTHENTICITY</b> <u>Guoliang Li</u> * <sup>(1)</sup> , <u>Jianghua Liu</u> <sup>(1)</sup> , <u>Zhuoqun Su</u> <sup>(1)</sup> , <u>Yiheng Shi</u> <sup>(1)</sup> .....	164
<b>L96</b> <b>NATURAL DEEP EUTECTIC SOLVENTS FOR GREEN SAMPLE PREPARATION: FROM BIOACTIVES TO CONTAMINANTS EXTRACTION</b> <u>Laura Carbonell-Rozas</u> * <sup>(1)</sup> , <u>Laura Righetti</u> <sup>(2)</sup> , <u>Roberto Romero-Gonzalez</u> <sup>(3)</sup> , <u>Ivan Aloisi</u> <sup>(4)</sup> , <u>Hans Mol</u> <sup>(4)</sup> , <u>Antonia Garrido Frenich</u> <sup>(3)</sup> .....	165
<b>L97</b> <b>MULTIDETERMINATION OF MYCOTOXINS IN SOLID AND LIQUID FOODSTUFFS USING SUPRAMOLECULAR SOLVENTS COMBINED WITH LC-MS/MS</b> <u>Luis Muniz-Bustamante</u> * <sup>(1)</sup> , <u>Noelia Caballero-Casero</u> <sup>(1)</sup> , <u>Soledad Rubio</u> <sup>(1)</sup> .....	166
<b>L98</b> <b>PROFILING OF PHENOLIC COMPOUNDS IN HERBS AND SPICES BY A SUSTAINABLE EXTRACTION COMBINED WITH A MINIATURIZED LC METHOD</b> <u>Francesca Rigano</u> * <sup>(1)</sup> , <u>Katia Arena</u> <sup>(1)</sup> , <u>Roberto Lagana Vinci</u> <sup>(1)</sup> , <u>Roberta La Tella</u> <sup>(1)</sup> , <u>Paola Dugo</u> <sup>(2)</sup> , <u>Luigi Mondello</u> <sup>(2)</sup> .....	167
<b>L99</b> <b>STRAIGHTFORWARD WHEAT QUALITY ASSESSMENT BY COMBINING NEAR-INFRARED SPECTROSCOPY AND ANALYSIS OF VARIANCE SIMULTANEOUS COMPONENT ANALYSIS</b> <u>Stephan Freitag</u> * <sup>(1)</sup> , <u>Maximilian Anlanger</u> <sup>(1)</sup> , <u>Maximilian Lipp</u> <sup>(2)</sup> , <u>Klemens Mechtler</u> <sup>(3)</sup> , <u>Elisabeth Reiter</u> <sup>(2)</sup> , <u>Heinrich Grausgruber</u> <sup>(4)</sup> , <u>Rudolf Krska</u> <sup>(5)</sup> .....	168
<b>L100</b> <b>ADVANCED MATHEMATICAL MODELLING APPLIED TO WATER-BASED EXTRACTION OF BIOACTIVE COMPOUNDS FROM APPLE POMACE USING RSM, ANNS AND RANDOM FOREST MODELS</b> <u>Francesca Carra</u> * <sup>(1)</sup> , <u>Maurizio Rinaldi</u> <sup>(2)</sup> , <u>Vincenzo Disca</u> <sup>(3)</sup> , <u>Chiara Carini</u> <sup>(1)</sup> , <u>Fabiano Travaglia</u> <sup>(1)</sup> , <u>Marco Arlorio</u> <sup>(1)</sup> .....	169
<b>L101</b> <b>GREENER FLAVOROMICS IN QUALITY CONTROL: FAST HS-GC-IMS IN COMBINATION WITH MACHINE LEARNING FOR ANALYSIS OF COMPLEX FOOD SAMPLES</b> <u>Lukas Bodenbender</u> * <sup>(1)</sup> , <u>Sascha Rohn</u> <sup>(2)</sup> , <u>Philipp Weller</u> <sup>(1)</sup> .....	170
<b>L102</b> <b>MICROWAVE-ASSISTED METHODS FOR GREENER AND MORE PRACTICAL (BLUENESS) FATTY ACID PROFILING IN FOOD</b> <u>Donatella Ferrara</u> <sup>(1)</sup> , <u>Marco Beccaria</u> <sup>(2)</sup> , <u>Chiara Emilia Irma Cordero</u> <sup>(3)</sup> , <u>Giorgia Purcaro</u> * <sup>(4)</sup> .....	171

# CONTENT

<b>L103</b>	
<b>DEVELOPMENT AND VALIDATION OF A RAPID AND GREEN ANALYTICAL TOOL FOR TOMATO PUREES AUTHENTICATION BY HIGH RESOLUTION MASS SPECTROMETRY BASED FLOW INJECTION ANALYSIS</b>	
Antonella Lamonaca <sup>(1)</sup> , Anna Luparelli <sup>(2)</sup> , Elisabetta De Angelis <sup>(2)</sup> , Linda Monaci <sup>(2)</sup> , <u>Rosa Pilolli</u> <sup>*(2)</sup> .....	172
<b>L104</b>	
<b>QUALITY ASPECTS OF PLANT PROTEINS FOR MEAT AND DAIRY ALTERNATIVES: MOLECULES AND METHODS</b>	
<u>Hans-Gerd Janssen</u> <sup>*(1)</sup> , Boudewijn Hollebrands <sup>(1)</sup> .....	173
<b>L105</b>	
<b>ARE MEAT ANALOGUES ADEQUATE TO SUBSTITUTE MEAT? A CASE STUDY ON SOY-BASED BURGER PROTOTYPES: NUTRITIONAL ADEQUACY, PROTEIN DIGESTIBILITY, AND SAFETY ASSESSMENT</b>	
Sara Cutroneo <sup>*(1)</sup> , Barbara Prandi <sup>(1)</sup> , Octavian Augustin Mihalache <sup>(1)</sup> , Marie Cristin Baune <sup>(2)</sup> , Florian Singer <sup>(2)</sup> , Nino Terjung <sup>(2)</sup> , Chiara Dall'Asta <sup>(1)</sup> , Tullia Tedeschi <sup>(1)</sup> .....	174
<b>L106</b>	
<b>SAFETY OF NEW PROTEIN SOURCES: HOW EFSA'S UPDATED NOVEL FOOD GUIDANCE SAFEGUARDS CONSUMERS' HEALTH WHILE SUPPORTING INNOVATION IN THE EU AGRIFOOD SYSTEM</b>	
<u>Ermolaos Ververis</u> <sup>*(1)</sup> , Reinhard Ackerl <sup>(2)</sup> , Oceane Albert <sup>(2)</sup> , Domenico Azzollini <sup>(2)</sup> , Elisa Beneventi <sup>(2)</sup> , Agnes De Sesmaisons Lecarre <sup>(2)</sup> , Antonio Fernandez Dumont <sup>(2)</sup> , Esther Garcia Ruiz <sup>(2)</sup> , Andrea Germini <sup>(2)</sup> , Maria Glymenaki <sup>(3)</sup> , Georges E.N.Kass <sup>(2)</sup> , Eirini Kouloura <sup>(2)</sup> , Marcello Laganaro <sup>(2)</sup> , Maura Magani <sup>(2)</sup> , Leonard Matijevic <sup>(2)</sup> , Vania Mendes <sup>(2)</sup> , Estefania Noriega Fernandez <sup>(4)</sup> , Irene Nuin Garciaarena <sup>(2)</sup> , Gabriela Precup <sup>(2)</sup> , Pablo Rodriguez Fernandez <sup>(2)</sup> , Ruth Roldan-Torres <sup>(2)</sup> , Annamaria Rossi <sup>(2)</sup> , Francesco Suriano <sup>(2)</sup> , Emanuela Turla <sup>(2)</sup> , Silvia Valtuena Martinez <sup>(2)</sup> , Wolfgang Gelbmann <sup>(2)</sup> .....	175
<b>L107</b>	
<b>METROLOGY FOR FOOD SAFETY IN THE CIRCULAR ECONOMY: TARGETED AND SCREENING METHODS FOR CONTAMINANTS IN FOOD AND RECYCLED PACKAGING</b>	
<u>Chiara Portesi</u> <sup>*(1)</sup> , Roland Becker <sup>(2)</sup> , Tea Zuliani <sup>(3)</sup> , Burcu Binici <sup>(4)</sup> , Christopher Hopley <sup>(5)</sup> , Laura Martin <sup>(6)</sup> , Consolato Schiavone <sup>(1)</sup> , Luigi Bergamaschi <sup>(1)</sup> , Charlotte Hauschildt <sup>(2)</sup> , Michal Strzelec <sup>(7)</sup> , Aida Jotanovic <sup>(8)</sup> , Ian Allan <sup>(9)</sup> , Kuria Ndungu <sup>(9)</sup> , Bert van Bavel <sup>(9)</sup> , Taner Gokcen <sup>(4)</sup> , Erika Bester <sup>(10)</sup> , Huiling Liu <sup>(11)</sup> , Christina Riemenschneider <sup>(12)</sup> , Alexander Schaechtele <sup>(12)</sup> , Tommy Licht Cederberg <sup>(13)</sup> , Agnieszka Anna Niklas <sup>(14)</sup> , Alexander Montoya <sup>(13)</sup> , Elias Kakoulides <sup>(15)</sup> , Ludger Bruehl <sup>(16)</sup> , Patrycja Wojciechowska <sup>(17)</sup> , Karolina Wiszumirska <sup>(17)</sup> , Els Van Hoek <sup>(18)</sup> , Karlien Cheyns <sup>(18)</sup> , Ivo Leito <sup>(19)</sup> , Irja Helm <sup>(19)</sup> , Matthias Groschke <sup>(20)</sup> , Sasa Hren <sup>(21)</sup> , Thomas Brandsch <sup>(22)</sup> , Evangelia Kristalli <sup>(23)</sup> , Nizar Benismail <sup>(24)</sup> , Thierry Diaz <sup>(24)</sup> , Nadine Bohni <sup>(25)</sup> , Maurus Biedermann <sup>(25)</sup> , Sarah Hill <sup>(5)</sup> , Marina Zbinden <sup>(26)</sup> , Raymond Schelker <sup>(27)</sup> , Melanie Haupt <sup>(27)</sup> , Jan Hanusch <sup>(28)</sup> , Marlena Roguszewska <sup>(29)</sup> , Andrea Mario Rossi <sup>(30)</sup> , Francesco Romaniello <sup>(1)</sup> .....	176
<b>L108</b>	
<b>DRIVERS FOR INCREASE OF CIRCULARITY IN THE FEED SECTOR VERSUS CONTROL MEASURE CHALLENGES</b>	
<u>Wilma Taverne</u> <sup>*(1)</sup> .....	178
<b>L109</b>	
<b>FOOD AND FEED SAFETY VULNERABILITIES IN THE CIRCULAR ECONOMY</b>	
<u>Angelo Maggiore</u> <sup>*(1)</sup> , Bernard Bottex <sup>(1)</sup> , Michele Ardizzone <sup>(2)</sup> .....	179
<b>L110</b>	
<b>140 YEARS OF AOAC INTERNATIONAL: ADDRESSING FOOD ANALYSIS CHALLENGES THROUGH COLLABORATION AND CONSENSUS</b>	
<u>Katerina Mastovska</u> <sup>*(1)</sup> .....	180
<b>L111</b>	
<b>ANALYSIS OF DIETARY CARBOHYDRATES</b>	
<u>Barry McCleary</u> <sup>*(1)</sup> , Yannis Vrasadis <sup>(2)</sup> , Sean Austin <sup>(3)</sup> .....	181
<b>L112</b>	
<b>AOAC INTERNATIONAL INITIATIVES IN CHEMICAL CONTAMINANT AND RESIDUE ANALYSIS</b>	
<u>Lukas Vaclavik</u> <sup>*(1)</sup> .....	182
<b>L113</b>	
<b>PFAS: AOAC ACTIVITIES AND COMPARISON OF TWO SINGLE LAB VALIDATED METHODS IN FOOD</b>	
<u>Susan Genuajdi</u> <sup>*(1)</sup> , Manjula Sunkara <sup>(1)</sup> , Lowri DeJager <sup>(1)</sup> , Ashley Griffin <sup>(2)</sup> , Ernest Capraro <sup>(2)</sup> , Ashten Gibson <sup>(2)</sup> , Andrew Savage <sup>(2)</sup> .....	183
<b>L114</b>	
<b>AOAC GUIDANCE FOR FOOD ALLERGEN METHOD VALIDATION</b>	
<u>Melanie Downs</u> <sup>*(1)</sup> .....	184
<b>L115</b>	
<b>CHALLENGES TO ASSESS SAFETY OF MIXTURES: "BEST PRACTICES FOR BIOASSAY TESTING OF FOOD AND OTHER COMPLEX MIXTURES"</b>	
<u>Maricel Marin-Kuan</u> <sup>*(1)</sup> .....	185



# CONTENT

<b>L116</b> <b>HARMONIZATION ON NON-TARGETED TESTING. BABYLONIAN LINGUISTIC DIVERSITY FOR MASS SPECTROMETRY</b> <u>Marios G. Kostakis</u> <sup>(1)</sup> , Thomas Gude <sup>(2)</sup> .....	186
<b>L117</b> <b>PFAS EXPOSURE - SENSE AND NONSENSE</b> <u>Jacob de Boer</u> <sup>(1)</sup> .....	187
<b>L118</b> <b>OCCURRENCE AND EVALUATION OF ENVIRONMENTAL RISKS OF PFAS</b> <u>Ondrej Parizek</u> <sup>(1)</sup> , Veronika Svobodova <sup>(1)</sup> , Darina Dvorakova <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Jakub Tomasko <sup>(1)</sup> , Jana Pulkrabova <sup>(1)</sup> ...	188
<b>L119</b> <b>COCKTAILS OF ENDOCRINE DISRUPTORS IN THE DIFFERENT DIETS OF FRENCH CONSUMERS</b> <u>Gaud Dervilly</u> <sup>(1)</sup> , Manon Bourdeau <sup>(1)</sup> , Bruno Le Bizec <sup>(1)</sup> , Helene Moche <sup>(2)</sup> , Anne Platel <sup>(2)</sup> , Marie-Christine Chagnon <sup>(3)</sup> , Isabelle Severin <sup>(3)</sup> , Fabrice Nesslany <sup>(2)</sup> .....	189
<b>L120</b> <b>ULTRA-TRACE ANALYSIS OF EXPOSURE CHEMICALS IN PLACENTAL TISSUE, MATERNAL- AND UMBILICAL CORD BLOOD</b> <u>Max Lennart Feuerstein</u> <sup>(1)</sup> , Sebastian Granitzer <sup>(2)</sup> , Julia Füreder <sup>(3)</sup> , Kathrin Sigl <sup>(3)</sup> , Tina Bürki-Thurnherr <sup>(4)</sup> , Claudia Gundacker <sup>(2)</sup> , Benedikt Warth <sup>(1)</sup> .....	190
<b>L121</b> <b>ADVANCEMENTS IN MULTI-ELEMENT SPECIATION: A NOVEL APPROACH FOR THE IDENTIFICATION OF CHELATING COMPOUNDS USING SEC-ICP-MS/MS AND SEC-QTOF-MS WITH A FOCUS ON CADMIUM IN PLANT-BASED FOODS</b> <u>Julian Cardini</u> <sup>(1)</sup> , Jens J. Sloth <sup>(1)</sup> , Katrin Löschner <sup>(1)</sup> .....	191
<b>L122</b> <b>SILICONE WRISTBANDS AS PASSIVE SAMPLERS TO MONITOR PESTICIDE EXPOSURE DURING FARMING PRACTICES</b> <u>Chaymae El Amraoui Aarab</u> <sup>(1)</sup> , Chunfang Xiao <sup>(2)</sup> , Merel Van der Meer <sup>(1)</sup> , Marie-Laure Aiello <sup>(3)</sup> , Wei-Hong Zhang <sup>(4)</sup> , Benjamin Couvreur <sup>(3)</sup> , Maxime Bonnave <sup>(3)</sup> , Nicolas Vidick <sup>(1)</sup> , Els Van Hoeck <sup>(5)</sup> , Maryse Ledent <sup>(1)</sup> , François Serneels <sup>(3)</sup> , Joris Van Loco <sup>(5)</sup> , Eva De Clercq <sup>(1)</sup> , Laure Joly <sup>(1)</sup> .....	192
<b>L123</b> <b>COMBINED HILIC-HRMS AND CE-HRMS APPROACHES TO CHARACTERIZE THE POLAR METABOLOME. APPLICATION TO THE BIOMARKERS OF EFFECT RELATED TO PCBs AND BPA IN HUMAN ANIMAL MODEL (PIG)</b> <u>Maykel Hernandez-Mesa</u> <sup>(1)</sup> , Luca Narduzzi <sup>(2)</sup> , Alfonso Narvaez <sup>(2)</sup> , Maria del Mar Delgado-Povedano <sup>(2)</sup> , Bruno Le Bizec <sup>(3)</sup> , Ana María García-Campaa <sup>(2)</sup> , Gaud Dervilly <sup>(3)</sup> .....	193
<b>L124</b> <b>LIFESTYLE PRODUCTS FOR OUR HEALTH: CAN WE ALWAYS TRUST IN PRODUCT LABELLING?</b> <u>Christian W. Klampf</u> <sup>(1)</sup> , Bernhard Thalhamer <sup>(1)</sup> , Markus Himmelsbach <sup>(1)</sup> .....	194
<b>L125</b> <b>ANALYSING FOOD ADDITIVES: THE GOOD, THE BAD AND THE UGLY</b> <u>Joris Van Loco</u> <sup>(1)</sup> , Séverine Gosciny <sup>(1)</sup> .....	195
<b>L126</b> <b>VALIDATION AND APPLICATION OF AN LC-ESI-MS/MS MULTI-COMPOUND METHOD SIMULTANEOUSLY DETERMINING SIX B-VITAMINS IN A BROAD RANGE OF FOODS</b> <u>Zylla Marie Oane</u> <sup>(1)</sup> , Annbjørg Bøkevoll <sup>(1)</sup> , Susanna Marie Petrova <sup>(1)</sup> , Tone Myhra <sup>(1)</sup> , <u>Eystein Oveland</u> <sup>(1)</sup> .....	196
<b>L127</b> <b>DETERMINATION OF BIOACTIVE COMPOUNDS IN 19 ITALIAN VARIETIES OF CHILI PEPPERS BY MEANS OF LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY AND IN VITRO ENZYME INHIBITORY EFFECT</b> <u>Cinzia Cafarella</u> <sup>(1)</sup> , Francesca Rigano <sup>(1)</sup> , Aristeidis S. Tsagkaris <sup>(2)</sup> , Anna Louckova <sup>(2)</sup> , Katia Arena <sup>(1)</sup> , Luigi Mondello <sup>(3)</sup> , Paola Dugo <sup>(3)</sup> , Jana Hajslova <sup>(2)</sup> .....	197
<b>L128</b> <b>ADVANCED CHARACTERIZATION OF NOVEL CANNABIS CULTIVARS</b> <u>Matej Maly</u> <sup>(1)</sup> , Frantisek Benes <sup>(1)</sup> , Zuzana Binova <sup>(1)</sup> , Federica Pollastro <sup>(2)</sup> , Stefano Salomone <sup>(2)</sup> , Gianpaolo Grassi <sup>(3)</sup> , Vincenzo Disca <sup>(2)</sup> , Jana Hajslova <sup>(1)</sup> .....	198

# CONTENT

<b>L129</b>	
<b>SUPPORTING THE OFFICIAL SENSORY EVALUATION OF VIRGIN OLIVE OILS WITH INNOVATIVE STRATEGIES BASED ON VOLATILE COMPOUNDS ANALYSIS: THE ROLE OF HS-GC-IMS AND MACHINE LEARNING</b>	
<u>Enrico Valli</u> <sup>(1)</sup> , Rosalba Tucci <sup>(2)</sup> , Ilaria Grigoletto <sup>(2)</sup> , Enrico Casadei <sup>(1)</sup> , Chiara Cevoli <sup>(1)</sup> , Silvia Mingione <sup>(3)</sup> , Nicola Balzani <sup>(3)</sup> , Sara Barbieri <sup>(2)</sup> , Cesare Rossini <sup>(4)</sup> , Alessandro Battaglia <sup>(4)</sup> , Fabio Battaglia <sup>(4)</sup> , Andrea Giomo <sup>(5)</sup> , Alessandra Bendini <sup>(1)</sup> , Tullia Gallina Toschi <sup>(1)</sup> .....	199
<b>L130</b>	
<b>ANALYSIS OF VOLATILES AND A-DICARBONYL COMPOUNDS IN MAILLARD REACTION PRODUCTS DERIVED FROM 2'-FUCOSYLACTOSE AND AMINO ACIDS</b>	
<u>Kwang-Geun Lee</u> <sup>(1)</sup> .....	200
<b>L131</b>	
<b>UNTARGETED BENCHTOP VOLATILOMICS AT TRACE LEVELS IN FOODS AND FERMENTATIONS - WHY THE GAS PHASE IS SO MUCH MORE THAN HOT AIR</b>	
<u>Philipp Weller</u> <sup>(1)</sup> .....	201
<b>L132</b>	
<b>ENTOMETABOLOMICS OF EDIBLE INSECT SPECIES REARED UNDER DIFFERENT CONDITIONS</b>	
<u>Katerina Sebelova</u> <sup>(1)</sup> , Katerina Sonntag <sup>(1)</sup> , Ondrej Pospisil <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	202
<b>L133</b>	
<b>TRANSLATION OF DART-HRMS TOOLS FROM THE BENCH TO THE INDUSTRIAL POULTRY PRODUCTION: A QUICK GUIDANCE FOR THE STANDARDIZATION OF POULTRY MEAT PIGMENTATION</b>	
<u>Alessandra Tata</u> <sup>(1)</sup> , Carmela Zacometti <sup>(1)</sup> , Andrea Massaro <sup>(1)</sup> , Sonia Falappa <sup>(2)</sup> , Simona Ceroni <sup>(2)</sup> , Salvatore Catania <sup>(1)</sup> .....	203
<b>L134</b>	
<b>QUECHUP A COMPREHENSIVE SAMPLE PREPARATION PARTICULARLY RELEVANT FOR THE FOOD EXPOSOME CHARACTERIZATION</b>	
<u>Julien Parinet</u> <sup>(1)</sup> , Thierno Diallo <sup>(1)</sup> , Yassine Makni <sup>(1)</sup> , Thierry Guerin <sup>(2)</sup> .....	204
<b>L135</b>	
<b>ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ION MOBILITY-HIGH-RESOLUTION MASS SPECTROMETRY METABOLOMICS ON PIG ORGAN EXPOSED TO ANTIBIOTICS FOR FOOD SAFETY ASSESSMENT</b>	
<u>Nicolo Riboni</u> <sup>(1)</sup> , Maurizio Piergiovanni <sup>(1)</sup> , Maria Pia Fabbri <sup>(2)</sup> , Monica Mattarozzi <sup>(1)</sup> , Antonella Cavazza <sup>(1)</sup> , Maria Olga Varra <sup>(2)</sup> , Giovanni Loris Alborali <sup>(3)</sup> , Federico Scali <sup>(3)</sup> , Adriana Ianieri <sup>(2)</sup> , Sergio Ghidini <sup>(4)</sup> , Emanuela Zanardi <sup>(2)</sup> , Federica Bianchi <sup>(5)</sup> , Maria Careri <sup>(5)</sup> .....	205
<b>L136</b>	
<b>FIRST PROOF OF CONCEPT: APPLYING UNTARGETED METABOLOMICS AND MOLECULAR NETWORKING VIA LC-DDA-HRMS/MS TO HIGHLIGHT QUALITY AND REACTION MARKERS IN PROCESSED FOODS</b>	
<u>Soha Farah</u> <sup>(1)</sup> , Mathieu Cladiere <sup>(1)</sup> , Melina Ramos <sup>(1)</sup> , Even Le Roux <sup>(1)</sup> , Barbara Rega <sup>(1)</sup> , Valerie Camel <sup>(1)</sup> .....	206
<b>L137</b>	
<b>NMR METABOLOMICS TO STUDY THE EFFECT OF DIFFERENT DRYING TECHNIQUES ON EDIBLE INSECTS: THE CASE STUDY OF ACHETA DOMESTICUS (HOUSE CRICKET)</b>	
<u>Mattia Spano</u> <sup>(1)</sup> , Giuseppina Adiletta <sup>(2)</sup> , Irene Ferri <sup>(3)</sup> , Stefania Garzoli <sup>(1)</sup> , Paola Russo <sup>(2)</sup> , Luisa Mannina <sup>(1)</sup> .....	207
<b>L138</b>	
<b>RECENT DEVELOPMENTS AND OUTLOOK OF EU POLICY ON CONTAMINANTS WITH A FOCUS ON RELATED ANALYTICAL CHALLENGES</b>	
<u>Frans Verstraete</u> <sup>(1)</sup> .....	208
<b>L139</b>	
<b>ADDRESSING EXPOSOME CHALLENGES WITH STREAMLINED FOOD SAMPLE PREPARATION BASED ON SUPRAMOLECULAR SOLVENTS</b>	
<u>Noelia Caballero-Casero</u> <sup>(1)</sup> , Ana Ballesteros-Gomez <sup>(1)</sup> , Soledad Rubio <sup>(1)</sup> .....	209
<b>L140</b>	
<b>CHALLENGES IN QUANTITATIVE VOLATILOMICS OPEN NEW OPPORTUNITIES IN FOOD QUALITY ASSESSMENT: THE ROLE OF MULTIDIMENSIONAL ANALYTICAL PLATFORMS</b>	
Andrea Caratti <sup>(1)</sup> , Angelica Fina <sup>(2)</sup> , Fulvia Trapani <sup>(2)</sup> , Simone Squara <sup>(2)</sup> , Erica Liberto <sup>(1)</sup> , <u>Chiara Cordero</u> <sup>(1)</sup> , Stephen E Reichenbach <sup>(3)</sup> , Qingping Tao <sup>(4)</sup> , Daniel Geschwender <sup>(4)</sup> .....	210

# CONTENT

<b>POSTER SESSIONS .....</b>	<b>211</b>
<b>ALLERGENS .....</b>	<b>212</b>
<b>A1</b>	
<b>UNINTENDED ALLERGEN PRESENCE (UAP): CROSS-CONTACT, CONTAMINATION OR FRAUD. REAL-LIFE CASES FROM THE FOOD INDUSTRY</b>	
<u>Marleen Voorhuijzen</u> <sup>(1)</sup> , <u>Nathalie Smits</u> <sup>(1)</sup> , <u>Andries Koops</u> <sup>(1)</sup> , <u>Yvonne Westphal</u> <sup>(1)</sup> .....	213
<b>A2</b>	
<b>MULTI-ALLERGEN QUANTIFICATION IN FOOD USING CONCATEMER-BASED ISOTOPE DILUTION MASS SPECTROMETRY: A COLLABORATIVE STUDY</b>	
<u>Maxime Gavage</u> <sup>(1)</sup> , <u>Kaatje Van Vlierberghe</u> <sup>(2)</sup> , <u>Marc De Loose</u> <sup>(2)</sup> , <u>Christof Van Poucke</u> <sup>(2)</sup> , <u>Anne-Catherine Huet</u> <sup>(3)</sup> , <u>Nathalie Gillard</u> <sup>(3)</sup> .....	214
<b>A3</b>	
<b>DETECTION OF FOOD ALLERGENS IN MICROBIAL FERMENTATION FOOD PRODUCTS USING UNTARGETED LC-HRMS PROTEOMICS</b>	
<u>Christof Van Poucke</u> <sup>(1)</sup> , <u>Marie-Alice Fraiture</u> <sup>(2)</sup> , <u>Nancy H.C. Roosens</u> <sup>(2)</sup> , <u>Rik Orval</u> <sup>(2)</sup> , <u>Frederic Debode</u> <sup>(3)</sup> , <u>Mathieu Dubois</u> <sup>(4)</sup> , <u>Nathalie Gillard</u> <sup>(4)</sup> , <u>Marc De Loose</u> <sup>(1)</sup> .....	215
<b>A4</b>	
<b>IDENTIFICATION OF GLUTEN-SPECIFIC DNA MARKERS</b>	
<u>Tamara Kutateladze</u> <sup>(1)</sup> , <u>Tamar Koberidze</u> <sup>(2)</sup> , <u>Kakha Bitskinashvili</u> <sup>(1)</sup> , <u>Nelly Datukishvili</u> <sup>(2)</sup> , <u>Boris Vishnepolsky</u> <sup>(1)</sup> .....	216
<b>A5</b>	
<b>EFFECT OF FOOD PROCESSING ON PCR-BASED DETECTION OF MAIZE AND WHEAT ALLERGENS</b>	
<u>Tata Ninidze</u> <sup>(1)</sup> , <u>Tamar Koberidze</u> <sup>(1)</sup> , <u>Kakha Bitskinashvili</u> <sup>(2)</sup> , <u>Tamara Kutateladze</u> <sup>(2)</sup> , <u>Boris Vishnepolsky</u> <sup>(2)</sup> , <u>Nelly Datukishvili</u> <sup>(1)</sup> .....	217
<b>A6</b>	
<b>COMPARISON OF FIVE ELISA KITS FOR THE DETERMINATION OF SOY IN PEA-BASED PRODUCTS</b>	
<u>Anne-Catherine Huet</u> <sup>(1)</sup> , <u>Amandine Lamote</u> <sup>(2)</sup> , <u>Rogier De Back</u> <sup>(3)</sup> , <u>Frederic Mansy</u> <sup>(3)</sup> , <u>Nathalie Gillard</u> <sup>(1)</sup> .....	218
<b>A7</b>	
<b>PROFICIENCY-TESTING SCHEME FOR HISTAMINE DETECTION IN FISHERY PRODUCTS</b>	
<u>Sabrina Bouakache</u> <sup>(1)</sup> , <u>Abdelkader Boubetra</u> <sup>(1)</sup> , <u>Antoine Gorse</u> <sup>(1)</sup> , <u>Anne Tirard</u> <sup>(1)</sup> .....	219
<b>A8</b>	
<b>VALIDATION STUDY OF LATERAL FLOW DEVICE FOR DETECTING SHELLFISH PROTEINS</b>	
<u>Takao Kurata</u> <sup>(1)</sup> , <u>Kenichi Aburatani</u> <sup>(1)</sup> , <u>Daichi Tanaka</u> <sup>(1)</sup> , <u>Masatoshi Sakai</u> <sup>(1)</sup> , <u>Masayoshi Tomiki</u> <sup>(1)</sup> , <u>Takako Nishi</u> <sup>(1)</sup> .....	220
<b>A9</b>	
<b>ALLERLIST: A PROOF-OF-PRINCIPLE DATA SPACE TO IMPROVE POST-MARKETING MONITORING OF POTENTIAL NEW FOOD ALLERGENS</b>	
<u>Christof Van Poucke</u> <sup>(1)</sup> , <u>Maxime Gavage</u> <sup>(2)</sup> , <u>Femke Van Zwynsvoorde</u> <sup>(1)</sup> , <u>Samuel Bosch</u> <sup>(1)</sup> , <u>Anne-Catherine Huet</u> <sup>(2)</sup> , <u>Romy Gaddiseur</u> <sup>(3)</sup> , <u>Isabel Taverniers</u> <sup>(1)</sup> , <u>Marc De Loose</u> <sup>(1)</sup> , <u>Nathalie Gillard</u> <sup>(2)</sup> .....	221
<b>A10</b>	
<b>2023-2024 ALLERGENS IN FOOD MONITORING IN LOMBARDIA AND EMILIA ROMAGNA REGIONS (ITALY), THE EXPERIENCE OF FOOD CONTROL DEPARTMENT, IZSLER, BRESCIA</b>	
<u>Barbara Bertasi</u> <sup>(1)</sup> , <u>Laura Ragni</u> <sup>(1)</sup> , <u>Renato Bertolassi</u> <sup>(1)</sup> , <u>Sara Arnaboldi</u> <sup>(1)</sup> , <u>Marina Nadia Losio</u> <sup>(1)</sup> , <u>Enrico Pavoni</u> <sup>(1)</sup> .....	222
<b>A11</b>	
<b>LC-HRMS/MS FOR DETECTION OF MUSTARD AND GLUTEN IN BAKERY PRODUCTS</b>	
<u>Sabina Noviello</u> <sup>(1)</sup> , <u>Manila Bianchi</u> <sup>(2)</sup> , <u>Sara Morello</u> <sup>(2)</sup> , <u>Chiara Dalvit</u> <sup>(1)</sup> , <u>Giancarlo Biancotto</u> <sup>(1)</sup> , <u>Roberto Stella</u> <sup>(1)</sup> .....	223
<b>A12</b>	
<b>VALIDATION OF A HIGHLY SENSITIVE PEANUT ELISA KIT FOR DETECTING PEANUT IN PROCESSED FOODS</b>	
<u>Masayoshi Tomiki</u> <sup>(1)</sup> , <u>Mitsuko Takahashi</u> <sup>(1)</sup> , <u>Masatoshi Sakai</u> <sup>(1)</sup> , <u>Takao Kurata</u> <sup>(1)</sup> , <u>Takako Nishi</u> <sup>(1)</sup> .....	224
<b>A13</b>	
<b>VALIDATION OF A GREEN EXTRACTION PROTOCOL FOR THE DETECTION AND QUANTIFICATION OF GLUTEN PROTEINS USING A LATERAL FLOW TEST</b>	
<u>Evanthia Angeli</u> <sup>(1)</sup> , <u>Dimitrios Foulos</u> <sup>(1)</sup> , <u>Theodora Kapsali</u> <sup>(1)</sup> , <u>Georgios Papageorgiou</u> <sup>(1)</sup> , <u>Antonios Ntantasios</u> <sup>(1)</sup> .....	225

# CONTENT

<b>ALTERNATIVE PROTEINS.....</b>	<b>226</b>
<b>B1</b>	
<b>EVALUATION OF LOCUSTA MIGRATORIA PROTEIN DIGESTIBILITY</b>	
Marta Barberis <sup>(1)</sup> , Raffaella Colombo <sup>(2)</sup> , Adele Papetti <sup>*(1)</sup> .....	227
<b>B2</b>	
<b>NOVEL PROTEINS IN THE EU: EXPLORING THE INTERPLAY BETWEEN RISK ASSESSMENT AND SOCIETAL INSIGHTS FOR COMMUNICATION</b>	
Marcello Laganaro <sup>*(1)</sup> , Giorgia Zamariola <sup>(1)</sup> , Domagoj Vrbos <sup>(1)</sup> , Esther Garcia Ruiz <sup>(1)</sup> , Maria Glymenaki <sup>(2)</sup> , Vania Mendes <sup>(1)</sup> , Alejandra Munoz Gonzalez <sup>(1)</sup> , Irene Nuin Garcarena <sup>(1)</sup> , Gabriela Precup <sup>(1)</sup> , Ruth Roldan-Torres <sup>(1)</sup> , Ermolaos Ververis <sup>(3)</sup> , Andrea Germini <sup>(1)</sup> , Anthony Smith <sup>(1)</sup> .....	228
<b>B3</b>	
<b>BLACK SOLDIER FLY (HERMETIA ILLUCENS L.) WHOLE AND FRACTIONATED LARVAE: IN VITRO PROTEIN DIGESTIBILITY AND EFFECT OF LIPID AND CHITIN REMOVAL</b>	
Maria Giulia Bonomini <sup>*(1)</sup> , Barbara Prandi <sup>(1)</sup> , Augusta Caligiani <sup>(1)</sup> .....	229
<b>B4</b>	
<b>CHARACTERIZATION OF EGGS FROM CHICKENS FED WITH INSECT-ENRICHED DIETS</b>	
Maria Giulia Bonomini <sup>*(1)</sup> , Katia D'ambra <sup>(2)</sup> , Giovanna Minelli <sup>(2)</sup> , Domenico Pietro Lo Fiego <sup>(2)</sup> , Augusta Caligiani <sup>(1)</sup> .....	230
<b>B5</b> .....	<b>231</b>
<b>RAPID AND EFFICIENT MICROWAVE DIGESTION FOR TRACE METALS ANALYSIS OF ALTERNATIVE PROTEINS</b>	
Samuel Heckle <sup>(1)</sup> , Macy Harris <sup>(1)</sup> , Layla Abu-Al-Halaweh <sup>(1)</sup> , Axel Schoener <sup>(1)</sup> , Bobbie McManus <sup>*(1)</sup> .....	231
<b>B6</b>	
<b>EMULSIFYING AND PHYSICOCHEMICAL PROPERTIES OF MAILLARD REACTION PRODUCTS FORMED BY SPIRULINA PROTEIN EXTRACT WITH SACCHARIDES</b>	
Dohyoung Lee <sup>(1)</sup> , Kwang-Geun Lee <sup>*(1)</sup> .....	232
<b>B7</b>	
<b>PLANT-BASED PROTEINS: EVALUATING THE POTENTIAL APPLICATIONS AS ALTERNATIVE PROTEINS IN TOMATO POWDER</b>	
Neriman Ezgi Cifte <sup>(1)</sup> , Mecit Halil Oztop <sup>*(1)</sup> .....	233
<b>B8</b>	
<b>CHARACTERIZATION OF BIOACTIVE SENSORY SIGNIFICANT SUBSTANCES IN PLANT-BASED PROTEINS</b>	
Anna Louckova <sup>(1)</sup> , Jan Kyselka <sup>(2)</sup> , Frantisek Benes <sup>(1)</sup> , Jana Hajslova <sup>*(1)</sup> .....	234
<b>B9</b>	
<b>ANALYSIS OF INSECT-BASED FEED: INTEGRATION OF CHITIN DETERMINATION INTO THE WEENDE METHOD</b>	
Michael Hellwig <sup>*(1)</sup> , Patrick Sudwischer <sup>(2)</sup> , Werner Sitzmann <sup>(2)</sup> , Björn Krüger <sup>(3)</sup> .....	235
<b>AUTHENTICITY, TRACEABILITY, FRAUD .....</b>	<b>236</b>
<b>C1</b>	
<b>DETECTION OF 5-HMF CONTENT AS AN INDICATOR OF HONEY ADULTERATION WITH INVERTED SUGAR SYRUP</b>	
Somaiah Alzubaydah <sup>(1)</sup> , Amani Albalawi <sup>(1)</sup> , Abdullah Akbar <sup>(1)</sup> , Tagreed Alsalem <sup>*(2)</sup> .....	237
<b>C2</b>	
<b>EVALUATION OF INNOVATIVE TOOLS FOR RAPID AND ACCURATE AUTHENTICATION OF GROUND BLACK PEPPER</b>	
Ilona Nordhorn <sup>(1)</sup> , Andrea Massaro <sup>(2)</sup> , Michele Suman <sup>(3)</sup> , Roberto Piro <sup>(2)</sup> , Alessandra Tata <sup>(2)</sup> , Carmela Zacometti <sup>*(2)</sup> , Carsten Baessmann <sup>(1)</sup> .....	238
<b>C3</b>	
<b>COMBATING HAZELNUT FRAUD: A SYSTEMATIC COMPARISON BETWEEN MULTI-ISOTOPE, SPECTROSCOPIC AND LIPIDOMIC FINGERPRINTING METHODS</b>	
Berta Torres-Cobos <sup>*(1)</sup> , Soriana Beatrice Nicotra <sup>(1)</sup> , Monica Rosell <sup>(2)</sup> , Albert Soler <sup>(2)</sup> , Merce Rovira <sup>(3)</sup> , Agusti Romero <sup>(3)</sup> , Vincent Baeten <sup>(4)</sup> , Juan Antonio Fernandez Pierna <sup>(4)</sup> , Francesc Guardiola <sup>(1)</sup> , Alba Tres <sup>(1)</sup> , Stefania Vichi <sup>(1)</sup> .....	239
<b>C4</b>	
<b>DETECTION OF GELATIN SPECIATION, HEAVY METAL CONTAMINATION AND FRAUDULENT LABELING OF UNREGISTERED DIETARY SUPPLEMENTS AVAILABLE IN ONLINE STORES</b>	
Alanoud Alsaqabi <sup>*(1)</sup> , Khalid Alshehail <sup>(1)</sup> , Faisal Alrowais <sup>(1)</sup> , Hassan Almalki <sup>(1)</sup> , Bandar Bin Helel <sup>(1)</sup> , Mohammed Alkhidhr <sup>(1)</sup> .....	240

# CONTENT

<b>C5</b>	<b>NON-TARGETED LC-HRMS FINGERPRINT AND MULTIVARIATE CHEMOMETRIC METHODS FOR CHARACTERIZATION AND CLASSIFICATION OF HONEY FROM DIFFERENT GEOGRAPHICAL ORIGINS</b> <i>Benedetta Fanesi</i> <sup>(1)</sup> , Danica Mostoles <sup>(2)</sup> , Andrea Mara <sup>(3)</sup> , Gavino Sanna <sup>(3)</sup> , Sonia Sentellas <sup>(4)</sup> , Javier Saurina <sup>(2)</sup> , Oscar Nunez <sup>(4)</sup> , Deborah Pacetti <sup>(1)</sup> , Paolo Lucci <sup>(1)</sup> .....	241
<b>C6</b>	<b>CHARACTERIZATION AND DETECTION OF FRASS FROM TENEBRIO MOLITOR LARVAE BY NEAR INFRARED SPECTROSCOPY TECHNIQUES</b> <i>Abigaël Anselmo</i> <sup>(1)</sup> , Pascal Veys <sup>(1)</sup> , Audrey Pissard <sup>(1)</sup> , Vincent Baeten <sup>(1)</sup> .....	242
<b>C7</b>	<b>ADVANCE OF PCR APPROACHES FOR EFFICIENT TRACKING OF OIL CROPS IN PROCESSED FOODS</b> <i>Nelly Datukishvili</i> <sup>(1)</sup> , Tamara Kutateladze <sup>(2)</sup> , Kakha Bitskinashvili <sup>(2)</sup> , Kakha Karchkhadze <sup>(1)</sup> , Boris Vishnepolsky <sup>(2)</sup> , Tata Ninidze <sup>(1)</sup> .....	243
<b>C8</b>	<b>EXPLORING THE CHEMICAL PROFILE OF GREEK HONEY VARIETIES VIA UHPLC-TIMS-QTOF-MS: CHEMICAL CHARACTERIZATION AND STATISTICAL ANALYSIS</b> <i>Panagiotis Loukas Gialouris</i> <sup>(1)</sup> , Eleni Nastou <sup>(1)</sup> , Dafni Preza-Mayo-Kataki <sup>(1)</sup> , Reza Aalizadeh <sup>(1)</sup> , Marilena Dasenaki <sup>(1)</sup> , Nikolaos Thomaidis <sup>(1)</sup> .....	244
<b>C9</b>	<b>DIRECT ANALYSIS IN REAL TIME COMBINED WITH HIGH RESOLUTION MASS SPECTROMETRY FOR RAPID FOOD AUTHENTICITY TESTING: EXAMPLE OF BLACK TRUFFLES</b> <i>Ilona Nordhorn</i> <sup>(1)</sup> , Klemens Losso <sup>(2)</sup> , Matthias Rainer <sup>(3)</sup> , Carsten Baessmann <sup>(1)</sup> .....	245
<b>C10</b>	<b>SAMPLE PREPARATION- AND CHROMATOGRAPHY-FREE ANALYSIS OF CINNAMON PRODUCTS ENABLED BY DIRECT ANALYSIS IN REAL TIME</b> <i>Ilona Nordhorn</i> <sup>(1)</sup> , Carsten Baessmann <sup>(1)</sup> .....	246
<b>C11</b>	<b>USE OF CHEMICAL AND ISOTOPIC FINGERPRINTS TO ESTABLISH THE GEOGRAPHICAL ORIGIN OF "NERO DEI NEBRODI" PIG MEAT</b> <i>Federica Litrenta</i> <sup>(1)</sup> , Matteo Perini <sup>(2)</sup> , Carmelo Cavallo <sup>(3)</sup> , Silvia Pianezze <sup>(2)</sup> , Vincenzo Lo Turco <sup>(1)</sup> , Angela Giorgia Potorti <sup>(4)</sup> , Giuseppa Di Bella <sup>(4)</sup> , Luigi Liotta <sup>(3)</sup> .....	247
<b>C12</b>	<b>ENSURING TRACEABILITY OF ITALIAN CURED MEAT PRODUCTS TREATED WITH HIGH-PRESSURE PROCESSING USING NEAR-INFRARED SPECTROSCOPY</b> <i>Maria Olga Varra</i> <sup>(1)</sup> , Sergio Ghidini <sup>(2)</sup> , Alessandra Negri <sup>(3)</sup> , Riccardo Ghidini <sup>(3)</sup> , Riccardo Soldesti <sup>(3)</sup> , Adriana Ianieri <sup>(1)</sup> , Emanuela Zanardi <sup>(1)</sup> .....	248
<b>C13</b>	<b>AUTHENTICITY OF PIEDMONT EXTRA VIRGIN OLIVE OIL: ADVANCED AND NON-TARGETED ANALYSIS AND CHEMOMETRICS TO PROMOTE AN UNDERSTUDIED FUNCTIONAL OIL</b> <i>Ambra Bonciolini</i> <sup>(1)</sup> , Giulia Potenziani <sup>(1)</sup> , Virginia Glicerina <sup>(1)</sup> , <i>Vladimiro Cardenia</i> <sup>(1)</sup> .....	249
<b>C14</b>	<b>FOOD FRAUD OR GMP DEFECTS? A PRAGMATIC APPROACH TO AUTHENTICITY ASSESSMENT OF CITRUS JUICES</b> <i>Markus Jungen</i> <sup>(1)</sup> .....	250
<b>C15</b>	<b>NMR-BASED METABOLOMICS TO VALIDATE FINGERPRINTS OF DIFFERENT SAUDI HONEYS</b> <i>Somaiah Almubayedh</i> <sup>(1)</sup> , Amani Albalawi <sup>(1)</sup> , Upendra Singh <sup>(2)</sup> , Mariusz Jaremko <sup>(2)</sup> , Abdul-Hamid Emwas <sup>(2)</sup> .....	251
<b>C16</b>	<b>DETECTION OF PEPPER ADULTERATION USING MALDI-TOF SPECTROMETRY</b> <i>Paul Rentz</i> <sup>(1)</sup> , Martine Bergaentzle <sup>(2)</sup> , Morane Lointier <sup>(3)</sup> , Elisa Bard <sup>(3)</sup> , Dalal Werner <sup>(3)</sup> .....	252
<b>C17</b>	<b>REGULATORY REGIME AND FOOD FRAUD VULNERABILITY: IMPACT OF BREXIT</b> <i>Amodya Theekshani Kolamunna</i> <sup>(1)</sup> , Fiona Lalor <sup>(1)</sup> , Sinead Furey <sup>(2)</sup> .....	253
<b>C18</b>	<b>METHOD FOR THE DETERMINATION OF PARAFFIN AND STEARIN/STEARIC ACID FRACTIONS IN BEESWAX BY USING GC/MS</b> <i>Chiara Manzinello</i> <sup>(1)</sup> , Franco Mutinelli <sup>(1)</sup> .....	254
<b>C19255</b>	<b>ANALYSIS OF MELAMINE AND CYANURIC ACID IN DAIRY PRODUCTS: INTERLABORATORY TESTS AS A QUALITY TOOL</b> <i>Julien Leprovost</i> <sup>(1)</sup> , <i>Antoine Gorse</i> <sup>(1)</sup> , Anne Tirard <sup>(1)</sup> , François Roussey <sup>(1)</sup> , Abdelkader Boubetra <sup>(1)</sup> .....	255

# CONTENT

<b>C20</b>	
<b>ORGANIC ACIDS AS MARKERS FOR WINE FRAUDS IDENTIFICATION</b>	
<u>Gianina Marian (Toca)</u> <sup>(1)</sup> , Gabriela Rapeanu <sup>*(1)</sup> .....	256
<b>C21</b>	
<b>HONEY AUTHENTICITY: THE POWER OF ICP-MS TRIPLE QUADRUPOLE TO DETERMINE THE GEOGRAPHICAL AND BOTANICAL ORIGINS OF HONEY</b>	
<u>Laurent Naels</u> <sup>*(1)</sup> , Jean-François Garnier <sup>(1)</sup> .....	257
<b>C22</b>	
<b>COMPOSITIONAL PROFILING FOR THE QUALITY ASSESSMENT OF CANADIAN HONEYS</b>	
<u>Mike Shao</u> <sup>(1)</sup> , Asma Mdimagh <sup>(1)</sup> , Lan Liu <sup>(1)</sup> , Stephane Bayen <sup>(1)</sup> , Salwa Karboune <sup>*(1)</sup> .....	258
<b>C23</b>	
<b>RAPESEED OR NOT RAPESEED? RAPID ANOMALY DETECTION USING RAMAN SPECTROSCOPY COMBINED WITH ONE CLASS CLASSIFICATION</b>	
Lena Weckend <sup>(1)</sup> , Michael Pfister <sup>(1)</sup> , Janet Riedl <sup>(1)</sup> , Carsten Fauh-Hassek <sup>(1)</sup> , Tanja Schwerdtle <sup>(1)</sup> , Marcus Glomb <sup>(2)</sup> , <u>Felix Wustrack</u> <sup>*(1)</sup> .....	259
<b>C24</b>	
<b>UNIVARIATE AND MULTIVARIATE QUANTIFICATION AND AUTHENTICATION OF BEER ON THE BASIS OF 1H-NMR SPECTRA</b>	
<u>Sabine Milbert</u> <sup>(1)</sup> , Olga Krenz <sup>(2)</sup> , Teresa M. Müller <sup>(2)</sup> , Johannes Fuchs <sup>(2)</sup> , Leane Lehmann <sup>(3)</sup> , Steffen T. Seifert <sup>*(2)</sup> .....	260
<b>C25</b>	
<b>FOODSAFER: IS TURMERIC A SUPER-FOOD OR A SUPER-FRAUD?</b>	
<u>Simon Haughey</u> <sup>*(1)</sup> , Wilfred Abia <sup>(1)</sup> , Holly Montgomery <sup>(1)</sup> , Heidi Russel <sup>(1)</sup> , Paul Williams <sup>(1)</sup> , Chris Elliott <sup>(2)</sup> .....	261
<b>C26</b>	
<b>MONOFLORAL HONEY AUTHENTICATION BY LC-HRMS/MS PROFILING</b>	
<u>Roberto Stella</u> <sup>*(1)</sup> , Andrea Massaro <sup>(1)</sup> , Carmela Zacometti <sup>(1)</sup> , Alessandra Tata <sup>(1)</sup> , Giancarlo Biancotto <sup>(1)</sup> .....	262
<b>C27</b>	
<b>CREATING A RELIABLE LC-HRMS-BASED METABOLOMICS APPROACH TO ENSURE AUTHENTICITY OF PDO FETA CHEESE FROM RAW MATERIAL TO THE FINAL PRODUCT</b>	
<u>Eleni Nastou</u> <sup>(1)</sup> , Sofia Karanikolopoulou <sup>(1)</sup> , Anastasia Kritikou <sup>(1)</sup> , Panagiotis Loukas Gialouris <sup>(1)</sup> , Nikolaos Thomaidis <sup>*(1)</sup> .....	263
<b>C28</b>	
<b>A METABOLOMICS-BASED MODEL TO ASSESS THE ANTIBIOTIC-FREE LABEL CLAIM IN PORK</b>	
Maria Pia Fabrice <sup>(1)</sup> , <u>Maria Olga Varra</u> <sup>(1)</sup> , Sergio Ghidini <sup>(2)</sup> , Giovanni Loris Alborali <sup>(3)</sup> , Nicolo Riboni <sup>(4)</sup> , Maurizio Piergiovanni <sup>(4)</sup> , Monica Mattarozzi <sup>(4)</sup> , Federico Scali <sup>(3)</sup> , Maria Medugno <sup>(1)</sup> , Adriana Ianieri <sup>(1)</sup> , Federica Bianchi <sup>(4)</sup> , Maria Careri <sup>(4)</sup> , Emanuela Zanardi <sup>*(1)</sup> .....	264
<b>C29</b>	
<b>HEAT-STABLE PEPTIDE MARKERS OF BOVINE HEART, KIDNEY, LIVER, LUNG, AND SPLEEN FOR AUTHENTICITY-TESTING OF MINCED BEEF PRODUCTS</b>	
Thomas Braunersreuther <sup>(1)</sup> , Dagmar Brüggemann <sup>(1)</sup> , <u>Wolfgang Jira</u> <sup>*(1)</sup> , Andreas Römpf <sup>(2)</sup> .....	265
<b>C30</b>	
<b>THE NEXT GENERATION LC-IRMS FOR HONEY AUTHENTICITY INVESTIGATION</b>	
Daniel Felsmann <sup>(1)</sup> , Nils Stoeber <sup>(1)</sup> , Qiong Li <sup>(1)</sup> , <u>Mario Tuthorn</u> <sup>*(1)</sup> .....	266
<b>C31</b>	
<b>HIGH PRECISION ISOTOPE RATIO-ORBITRAP-MS - A NEW TOOL FOR VANILLIN AND CAFFEINE AUTHENTICATION</b>	
<u>Mario Tuthorn</u> <sup>*(1)</sup> , Nils Kuhlbusch <sup>(1)</sup> , Dieter Juchelka <sup>(1)</sup> , Andreas Hilker <sup>(1)</sup> , Qiong Li <sup>(1)</sup> .....	267
<b>C32</b>	
<b>NON-TARGETED ANALYSIS OF WHISKY USING SPME ARROW AND ORBITRAP EXPLORIS GC 240 MASS SPECTROMETER</b>	
<u>Adam Ladak</u> <sup>*(1)</sup> , Dominic Roberts <sup>(1)</sup> , Giulia Giulia <sup>(1)</sup> , Nicholas Warner <sup>(1)</sup> , Daniel Kutscher <sup>(1)</sup> .....	268
<b>C33</b>	
<b>MEAT AND POULTRY ORIGIN WITH ISOTOPE RATIO MASS SPECTROMETRY: A RAPID AND ROBUST SAMPLE PREPARATION METHOD</b>	
<u>Alexander Scherl</u> <sup>*(1)</sup> , Khadijetou Ahmed Ethmane <sup>(2)</sup> , Pierre Zimmerli <sup>(1)</sup> , Didier Ortelli <sup>(1)</sup> , Patrick Edder <sup>(1)</sup> .....	269
<b>C34</b>	
<b>CONFRONTING FOOD FRAUD IN A "GREEN" WAY: DEVELOPMENT AND OPTIMIZATION OF A CHROMATOGRAPHY-FREE METHODOLOGY UTILIZING DART-HRMS FOR THE DETECTION OF EXTRA VIRGIN OLIVE OIL ADULTERATION</b>	
<u>Dimitrios Papelis</u> <sup>(1)</sup> , Sofia Drakopoulou <sup>(1)</sup> , Eleni Nastou <sup>(1)</sup> , Evangelos Gikas <sup>(1)</sup> , Ilona Nordhorn <sup>(2)</sup> , Carsten Baessmann <sup>(2)</sup> , Nikolaos Thomaidis <sup>*(1)</sup> .....	270

# CONTENT

<b>C35</b>	<b>DETECTION OF FOOD COMMODITIES ADULTERATION USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)</b>	
	<i>Paul Rogeboz</i> <sup>(1)</sup> , <i>Helia Latado</i> <sup>(1)</sup> , <i>Julie Zwahlen</i> <sup>(1)</sup> , <i>Mathieu Dubois</i> <sup>(1)</sup> , <i>Loic Dayon</i> <sup>(1)</sup> , <i>Amaury Patin</i> <sup>(1)</sup> , <i>Maricel Marin-Kuan</i> <sup>(1)</sup>	271
<b>C36</b>	<b>APPLICATION OF CARBON AND NITROGEN ISOTOPE RATIOS IN THE AUTHENTICATION OF LOCALLY GROWN VERSUS IMPORTED POTATOES IN THE CANARY ISLANDS</b>	
	<i>Raul S. Suarez-Torres</i> <sup>(1)</sup> , <i>Gabriel Santanta-Diepa</i> <sup>(1)</sup> , <i>Samara M. Dionis</i> <sup>(1)</sup> , <i>Ricardo Diaz-Diaz</i> <sup>(1)</sup>	272
<b>C37</b>	<b>ASSESSMENT AND AUTHENTICATION OF EDIBLE INSECTS AVAILABLE AT THE MARKET</b>	
	<i>Ondrej Pospisil</i> <sup>(1)</sup> , <i>Katerina Sebelova</i> <sup>(1)</sup> , <i>Jana Hajslova</i> <sup>(1)</sup>	273
<b>C38</b>	<b>A SUITE OF FOUR LC-MS/MS ASSAYS FOR ANALYSIS OF PHARMACEUTICAL ADULTERANTS IN DIETARY SUPPLEMENT PRODUCTS</b>	
	<i>John Schmitz</i> <sup>(1)</sup> , <i>Lukas Vaclavik</i> <sup>(2)</sup> , <i>Michael Buhrman</i> <sup>(1)</sup> , <i>Grace Bandong</i> <sup>(1)</sup> , <i>Jeff Stassi</i> <sup>(1)</sup>	274
<b>C39</b>	<b>UNTARGETED ANALYSIS WITH LC-HRMS AS A TOOL FOR AUTHENTICATION OF PEPPER</b>	
	<i>Vojtech Hrbek</i> <sup>(1)</sup> , <i>Karel Wunsche</i> <sup>(1)</sup> , <i>Kamila Stehnova</i> <sup>(1)</sup> , <i>Kamila Bechynska</i> <sup>(1)</sup> , <i>Vit Kosek</i> <sup>(1)</sup> , <i>Jana Hajslova</i> <sup>(1)</sup>	275
<b>C40</b>	<b>METABOLOMIC AND STABLE ISOTOPE SIGNATURES FOR WINE AUTHENTICATION AND CLASSIFICATION</b>	
	<i>Marius Gheorghe Miricioiu</i> <sup>(1)</sup> , <i>Oana Botoran</i> <sup>(1)</sup> , <i>Diana Costinel</i> <sup>(1)</sup> , <i>Roxana Elena Ionete</i> <sup>(1)</sup>	276
<b>C41</b>	<b>INVESTIGATION OF GEOGRAPHIC ORIGIN OF SUSPICIOUS "CYPRIOT" POTATOES FROM THE MARKET BY PERFORMING ISOTOPIC ANALYSES AND CHEMOMETRICS</b>	
	<i>Maria Tarapoulouzi</i> <sup>(1)</sup> , <i>Charalampos Louka</i> <sup>(2)</sup> , <i>Elena Ioannou Papayianni</i> <sup>(2)</sup> , <i>Eleni Tzioni</i> <sup>(2)</sup> , <i>Chara Savvidou</i> <sup>(2)</sup> , <i>Constantinos Damaskinos</i> <sup>(2)</sup> , <i>Rebecca Kokkinofa</i> <sup>(2)</sup>	277
	<b>BIOANALYTICAL METHODS FOR FOOD CONTROL.....</b>	<b>278</b>
<b>D1</b>	<b>MILLIFLUIDIC CELL-BASED BIOASSAY AS NEW PROMISING TOOL TO INVESTIGATE FOOD COMPOUNDS BIOACCESSIBILITY</b>	
	<i>Rafaella Colombo</i> <sup>(1)</sup> , <i>Mayra Paolillo</i> <sup>(1)</sup> , <i>Adele Papetti</i> <sup>(2)</sup>	279
<b>D2</b>	<b>ASSESSING THE AUTHENTICITY AND PURITY OF COMMERCIAL RENNET PRODUCTS THROUGH (META)GENOMICS APPROACHES</b>	
	<i>Yari Van Laere</i> <sup>(1)</sup> , <i>Marie-Alice Fraiture</i> <sup>(1)</sup> , <i>Andrea Gobbo</i> <sup>(1)</sup> , <i>Tom Van Nieuwenhuysen</i> <sup>(1)</sup> , <i>Julien Masquelier</i> <sup>(1)</sup> , <i>Sigrid De Keersmaecker</i> <sup>(1)</sup> , <i>Kathleen Marchal</i> <sup>(2)</sup> , <i>Nancy Roosens</i> <sup>(1)</sup> , <i>Kevin Vanneste</i> <sup>(1)</sup>	280
<b>D3</b>	<b>IDENTIFICATION OF PAPS DERIVING FROM AUTHORIZED INSECTS IN FEED BY HIGH-RESOLUTION LC-MS/MS</b>	
	<i>Elisabeth Reiter</i> <sup>(1)</sup> , <i>Irmengard Strnad</i> <sup>(1)</sup> , <i>Sara Schlachter</i> <sup>(1)</sup> , <i>Stefano D'Amico</i> <sup>(1)</sup> , <i>Evelyn Rampler</i> <sup>(2)</sup>	281
<b>D4</b>	<b>LABEL-FREE RELATIVE QUANTIFICATION OF MAJOR PROTEINS AND TRYPSIN INHIBITOR TYPES IN SOY BY HIGH-RESOLUTION LC-MS/MS</b>	
	<i>Elisabeth Reiter</i> <sup>(1)</sup> , <i>Stefano D'Amico</i> <sup>(1)</sup> , <i>Sara Schlachter</i> <sup>(1)</sup> , <i>Irmengard Strnad</i> <sup>(1)</sup> , <i>Evelyn Rampler</i> <sup>(2)</sup> , <i>Matthias Trimmel</i> <sup>(3)</sup>	282
<b>D5</b>	<b>DIRECT &amp; REAL-TIME HEADSPACE ANALYSIS OF ESSENTIAL OILS BY SICRIT-WATERS ACQUITY™ QDA™ MS DETECTOR</b>	
	<i>Jan Bucek</i> <sup>(1)</sup> , <i>Ciara Conway</i> <sup>(1)</sup> , <i>Jan-Christoph Wolf</i> <sup>(1)</sup>	283
<b>D6</b>	<b>YEAST-BASED ASSAY FOR THE DETECTION OF THYROIDAL ACTIVITY IN FOOD AND FOOD CONTACT MATERIAL</b>	
	<i>Martin Jähne</i> <sup>(1)</sup> , <i>Karina Hettwer</i> <sup>(2)</sup> , <i>Kapil Nichani</i> <sup>(2)</sup> , <i>Kirsten Simon</i> <sup>(1)</sup> , <i>Steffen Uhlig</i> <sup>(2)</sup>	284

# CONTENT

## CIRCULARITY & SUSTAINABILITY ..... 285

### E1

#### UTILIZING WASTE FROM THE AGRI-FOOD CHAIN FOR THE PRODUCTION OF ACTIVE PACKAGING

Francesco Romaniello<sup>\*(1)</sup>, Consolato Schiavone<sup>(1)</sup>, Andrea Mario Rossi<sup>(1)</sup>, Andrea Mario Giovannozzi<sup>(1)</sup>, Gabriele Alo<sup>(2)</sup>, Pierangela Rovellini<sup>(3)</sup>, Paola Fusari<sup>(3)</sup>, Antonio Perna<sup>(3)</sup>, Chiara Portesi<sup>(1)</sup>.....286

### E2

#### RECOVERY OF CAROTENOIDS AND EXTRACTABLE POLYPHENOLS FROM GRAPEFRUIT PEELS BY MICROWAVE-ASSISTED EXTRACTION WITH BIOBASED SOLVENTS AND SUBSEQUENT SUSTAINABLE EXTRACTION OF NON-EXTRACTABLE POLYPHENOLS

Gloria Dominguez-Rodriguez<sup>\*(1)</sup>, Maria Concepción Garcia<sup>(1)</sup>, Maria Castro-Puyana<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>.....287

### E3

#### SUSTAINABLE RECOVERY OF PROTEINS FROM LIME PEELS USING SUBCRITICAL WATER EXTRACTION COMBINED WITH ENZYME-ASSISTED EXTRACTION

Rosa María Palma-Manrique<sup>(1)</sup>, Maria Luisa Marina<sup>\*(2)</sup>, Maria Concepcion Garcia<sup>(2)</sup>, Maria Castro-Puyana<sup>(2)</sup>.....288

### E4

#### CHARACTERIZATION OF HYDROLYSATES FROM LIME PEELS PROTEIN EXTRACTS. PEPTIDE AND POLYPHENOL IDENTIFICATION BY UHPLC-HRMS

Rosa María Palma-Manrique<sup>(1)</sup>, Esther Gomez-Mejia<sup>(2)</sup>, Maria Castro-Puyana<sup>(3)</sup>, Maria Concepcion Garcia<sup>(3)</sup>, Maria Luisa Marina<sup>\*(3)</sup>.....289

### E5

#### SUSTAINABLE EMERGING TECHNIQUES FOR A SEQUENTIAL EXTRACTION OF BIOACTIVE COMPOUNDS FROM CITRUS PEELS

Gloria Dominguez-Rodriguez<sup>\*(1)</sup>, Victor M. Amador-Luna<sup>(2)</sup>, Maria Castro-Puyana<sup>(3)</sup>, Elena Ibanez<sup>(2)</sup>, Maria Luisa Marina<sup>(3)</sup>...290

### E6

#### CLOVAMIDE EXTRACTION THROUGH UNCONVENTIONAL TECHNIQUES FROM COCOA BEAN SHELLS

Vincenzo Disca<sup>\*(1)</sup>, Fabiano Travaglia<sup>(1)</sup>, Chiara Carini<sup>(1)</sup>, Jean Daniel Coisson<sup>(1)</sup>, Gian Carlo Cravotto<sup>(2)</sup>, Marco Arlorio<sup>(1)</sup>, Monica Locatelli<sup>(1)</sup>.....291

### E7

#### INVESTIGATION OF A WHEAT-BASED PHARMACEUTICAL WASTE PRODUCT TO ANALYZE ITS PROPERTIES AND EXPLORE POTENTIAL NOVEL APPLICATIONS

Lidia Ciriaco<sup>\*(1)</sup>, Antonio Paolillo<sup>(1)</sup>, Ivana Ledenko<sup>(1)</sup>, Vincenzo Armini<sup>(1)</sup>, Sonia Lombardi<sup>(1)</sup>, Barbara Maglione<sup>(2)</sup>, Paolo Grieco<sup>(3)</sup>, Luana Izzo<sup>(1)</sup>.....292

### E8

#### EVALUATION OF SELECTED QUALITY PARAMETERS OF MUFFINS ENRICHED WITH UPCYCLED INGREDIENTS, A REVIEW

Ewa Biazik<sup>\*(1)</sup>, Alicja Kulik<sup>(2)</sup>.....293

### E9

#### A BIOREFINERY PROCESS FOR THE EXTRACTION OF TERPENES AND PHENOLIC COMPOUNDS FROM EUCALYPTUS GLOBULUS LABILL. AND SALVIA OFFICINALIS L.

Enrico Romano<sup>(1)</sup>, Gloria Dominguez-Rodriguez<sup>\*(2)</sup>, Mattia Spano<sup>(1)</sup>, Luisa Mannina<sup>(1)</sup>, Elena Ibanez<sup>(3)</sup>, Alejandro Cifuentes<sup>(3)</sup>.....294

### E10

#### THE HEPARIN REVOLUTION: ENZYMATIC SWINE LIVER EXTRACTION FOR NEXT-LEVEL HEMODIALYSIS

Maria Martingo<sup>\*(1)</sup>, Sara Baptista-Silva<sup>(1)</sup>, Manuela Pintado<sup>(1)</sup>, Ines Cruz<sup>(2)</sup>.....295

### E11

#### ALTERNATIVE HIGH-QUALITY PROTEIN INGREDIENTS AS KEY NITROGEN SOURCE FOR PROBIOTICS

Maria Leonor Teixeira Pinto de Castro<sup>\*(1)</sup>, Sandra Borges<sup>(1)</sup>, Ana Rosa<sup>(2)</sup>, André Almeida<sup>(3)</sup>, Manuela Pintado<sup>(1)</sup>.....296

### E12

#### VALORIZING GRAPE POMACE AS INGREDIENT OF BEVERAGES WITH FUNCTIONAL PROPERTIES

Alessio Buono<sup>\*(1)</sup>, Francesco Romaniello<sup>(2)</sup>, Simona Lucia Bavaro<sup>(3)</sup>, Loredana Loredana Tarraran<sup>(3)</sup>, Giovanna Giovinazzo<sup>(3)</sup>, Carmela Gerardi<sup>(3)</sup>.....297

### E13

#### BIOBASED PACKAGING FOR W-3 FORTIFIED BISCUITS: AROMA QUALITY STABILITY OVER TIME

Eloisa Bagnulo<sup>(1)</sup>, Erica Liberto<sup>\*(2)</sup>, Chiara Cordero<sup>(2)</sup>, Monica Locatelli<sup>(3)</sup>.....298

### E14

#### SUSTAINABLE PACKAGING EDIBLE FILMS WITH ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES FROM DIFFERENT VEGETABLE WASTES: A COMPARATIVE STUDY

Nicoleta Corina Predescu<sup>\*(1)</sup>, Gheorghe Valentin Goran<sup>(1)</sup>, Carmen Daniela Petcu<sup>(1)</sup>, Camelia Papuc<sup>(2)</sup>.....299



# CONTENT

## E15

### PHYSICO-CHEMICAL CHARACTERIZATION OF INDUSTRIAL AND AGRICULTURAL BY-PRODUCTS FOR SUSTAINABLE BIOFERTILIZER DEVELOPMENT IN ORGANIC RASPBERRY CULTIVATION

Oana Romina Botoran<sup>(1)</sup>, Elisabeta-Irina Geana<sup>(1)</sup>, Corina-Teodora Ciucure<sup>(1)</sup>, Andreea Maria Iordache<sup>(1)</sup>, Claudia Sandru<sup>(1)</sup>, Marius Gheorghe Miricioiu<sup>(1)</sup>, Roxana Elena Ionete<sup>(1)</sup>.....300

## E16

### ENHANCING BIOMASS YIELD AND ELEMENTAL COMPOSITION OF ALFALFA (MEDICAGO SATIVA L.) PLANTS VIA BIOSTIMULANT APPLICATION COMBINED WITH FOLIAR FERTILIZATION

Ebenezer Ayew Appiah<sup>(1)</sup>, Erika Kutasy<sup>(1)</sup>.....301

## DATA HANDLING & ARTIFICIAL INTELLIGENCE IN FOOD ANALYSIS ..... 302

### F1

#### MINERALS AND AMINO ACIDS PROFILE OF BEE POLLEN FROM A SELECTED APIARY IN SOKOTO STATE, NIGERIA

Muhammad Sirajo<sup>(1)</sup> .....303

### F2

#### A TECHNOLOGICAL FRAMEWORK FOR ENHANCED FOOD QUALITY CONTROL AND TRANSPARENCY IN FOOD SUPPLY CHAINS

Paraskevas Bourgos<sup>(1)</sup>, Amalia Ntemou<sup>(1)</sup>, Ioanna Drigkopoulou<sup>(1)</sup>, Eleni Provopoulou<sup>(1)</sup>, Pantelis Lappas<sup>(1)</sup>, Spyros Evangelatos<sup>(1)</sup>.....304

### F3

#### NOVEL MACHINE LEARNING PIPELINES FOR THE DISCOVERY OF FOOD AUTHENTICITY MARKERS VIA NON-TARGETED LC-MS ANALYSIS: HONEY AS A CASE STUDY

Stephane Bayen<sup>(1)</sup>, Shawninder Chahal<sup>(1)</sup>, Lei Tian<sup>(1)</sup>, Shaghig Bilamjian<sup>(1)</sup>, Ferenc Balogh<sup>(2)</sup>, Lorna De Leoz<sup>(3)</sup>, Tarun Anumol<sup>(3)</sup>, Daniel Cuthbertson<sup>(3)</sup>.....305

## FLAVOUR & OFF-FLAVOUR SIGNIFICANT COMPOUNDS ..... 306

### G1

#### IMPROVING TASTE AND FLAVOR IN DAIRY PRODUCT THROUGH MILK ANALYSIS OF FREE FATTY BY MID-INFRARED (MIR) SPECTROMETRY

Octave Christophe<sup>(1)</sup>, Romain Reding<sup>(2)</sup>, Julie Leblois<sup>(3)</sup>, Denis Pittois<sup>(4)</sup>, Cedric Guignard<sup>(4)</sup>, Frederic Dehareng<sup>(1)</sup>.....307

### G2

#### PHYSICO-CHEMICAL PROPERTY AND SENSORY ATTRIBUTES OF NUT-BASED MILK COFFEE

Woorin Lee<sup>(1)</sup>, Kwang-Geun Lee<sup>(1)</sup>.....308

### G3

#### COMPARISON AND DIFFERENTIATION OF FLAVOR PROFILES IN VEGAN FOOD AND THEIR NON-VEGAN PRECURSORS BY A NOVEL GC-HRMS TECHNOLOGY

Marleen Vetter<sup>(1)</sup>, Eliška Ceznerova<sup>(1)</sup>, Sonja Klee<sup>(1)</sup>, Steffen Bräkling<sup>(1)</sup>, Arnd Ingendoh<sup>(2)</sup>.....309

### G4

#### THE CHEMISTRY BEHIND CHANGES IN COFFEE FLAVOR DURING STORAGE IN COMPOSTABLE AND STANDARD CAPSULES

Giulia Strocchi<sup>(1)</sup>, Eloisa Bagnulo<sup>(1)</sup>, Gloria Pellegrino<sup>(2)</sup>, Carlo Bicchi<sup>(1)</sup>, Erica Liberto<sup>(3)</sup>.....310

### G5

#### FOODOMICS APPROACH FOR THE CHARACTERIZATION DEFECTIVE COCOA LIQUORS IN ENSURING COCOA QUALITY

Eloisa Bagnulo<sup>(1)</sup>, Cristian Bortolini<sup>(2)</sup>, Alessandro Guglielmetti<sup>(2)</sup>, Irene Chetschik<sup>(3)</sup>, Carlo Bicchi<sup>(4)</sup>, Chiara Cordero<sup>(5)</sup>, Erica Liberto<sup>(5)</sup>.....311

### G6

#### COMPARATIVE VOLATILOMIC STUDY OF GRANA PADANO-TYPE CHEESE USING DIFFERENT RENNET TYPES

Manuela Giordano<sup>(1)</sup>, Vladimiro Cardenia<sup>(1)</sup>, Marta Bertolino<sup>(1)</sup>, Daniela Ghirardello<sup>(1)</sup>, Giuseppe Zeppa<sup>(1)</sup>.....312

### G7

#### AROMATIC FINGERPRINTING OF VALUABLE PRUNUS SEED OILS FOR THEIR CHARACTERIZATION THROUGH HS-SPME-GC-MS AND GC-IMS ANALYSIS OF VOCS

Francesca Carra<sup>(1)</sup>, Lorenzo Cecchi<sup>(2)</sup>, Vincenzo Disca<sup>(1)</sup>, Yassine Jaouhari<sup>(1)</sup>, Matteo Bordiga<sup>(1)</sup>, Marco Arlorio<sup>(1)</sup>.....313

### G8

#### EVALUATING BASIL VARIETIES: SENSORY, CHEMICAL, AND VISUAL ANALYSES FOR PESTO INDUSTRY APPLICATIONS

Giulia Tapparo<sup>(1)</sup>, Erica Liberto<sup>(2)</sup>, Cosetta Galamini<sup>(3)</sup>, Gianluca Cucco<sup>(3)</sup>, Marta Bertolino<sup>(1)</sup>.....314

# CONTENT

## G9

### VOLATILE OFF-FLAVOURS IN PLANT PROTEIN CONCENTRATES

Maria Filatova\*<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>.....315

## FOOD ADDITIVES..... 316

### H1

#### IDENTIFICATION AND DETERMINATION OF SYNTHETIC COLORS IN FINE BAKERY WARES (CAKES) SOLD IN RIYADH CITY, SAUDI ARABIA: A COMPARATIVE STUDY BETWEEN THE SPECIFICATIONS LAID OUT IN THE GULF STANDARDIZATION ORGANIZATION AND THE EUROPEAN UNION

Mazen AlFayez\*<sup>(1)</sup> .....317

### H2

#### DEVELOPMENT OF A HPLC-MS/MS MULTI-METHOD FOR THE ANALYSIS OF FOOD ADDITIVES WITH SPECIAL FOCUS ON SWEETENERS

Svenja Schneider\*<sup>(1)</sup>, Sabrina Darge<sup>(1)</sup>, Susanne Esslinger<sup>(1)</sup> .....318

### H3

#### PHYSICO-CHEMICAL AND SENSORY CHARACTERIZATION OF FRUIT JUICES ENRICHED WITH TANNINS

Daniela Ghirardello\*<sup>(1)</sup>, Manuela Giordano<sup>(1)</sup>, Giuseppe Zeppa<sup>(1)</sup>, Samuele Giovando<sup>(2)</sup>, Michele Battaglia<sup>(2)</sup>.....319

### H4

#### ADDED PHOSPHATES ANALYSIS IN FOOD: A NON COMPLETELY SOLVED ISSUE YET

Josep Calderon\*<sup>(1)</sup>, Sonia Abuin<sup>(1)</sup>, Soledad Soler<sup>(1)</sup>, Matilde Alvarez<sup>(1)</sup>, Víctor Pavon<sup>(1)</sup>, David Samblas<sup>(1)</sup>.....320

### H5

#### EXPANDING THE BACTERIOPHAGE ARSENAL FOR ALICYCLOBACILLUS SPOILAGE PREVENTION IN THE FOOD INDUSTRY

Helena Ferreira<sup>(1)</sup>, Ines Carvalho Leonardo<sup>(1)</sup>, Ana Patricia Quendera<sup>(1)</sup>, Maria Teresa Barreto Crespo<sup>(1)</sup>, Carlos Sao-Jose<sup>(2)</sup>, Frederic Bustos Gaspar\*<sup>(1)</sup> .....321

### H6

#### EXPLORING THE ANTIBACTERIAL POTENTIAL OF A GEOBACILLIN-26 HOMOLOG FROM ALICYCLOBACILLUS ACIDOTERRESTRIS DSM 3922T: A NOVEL APPROACH TO COMBAT SPOILAGE IN FOOD PRODUCTS

Ines Carvalho Leonardo<sup>(1)</sup>, Ana Patricia Quendera<sup>(1)</sup>, Helena Ferreira<sup>(1)</sup>, Maria Teresa Barreto Crespo<sup>(1)</sup>, Carlos Sao-Jose<sup>(2)</sup>, Frederic Bustos Gaspar\*<sup>(1)</sup> .....322

### H7

#### QUANTITATIVE LC ANALYSIS OF RARE SUGARS IN FOOD

Vadim Kraft\*<sup>(1)</sup>, Annika Malz<sup>(1)</sup>, Uwe Oppermann<sup>(1)</sup>.....323

## FOOD CONTAMINANTS (ENVIRONMENTAL) ..... 324

### I1

#### PRELIMINARY RISK EXPOSURE ASSESSMENT OF CONTAMINANTS OF EMERGING CONCERN THROUGH THE CONSUMPTION OF TAP WATER

Luis Muniz-Bustamante\*<sup>(1)</sup>, Noelia Caballero-Casero<sup>(1)</sup>, Soledad Rubio<sup>(1)</sup>.....325

### I2

#### FLUORINATED FEATURES IN FRESHWATER FISH: UNCOVERING UNKNOWN PFASS USING NON-TARGETED SCREENING AND MASS BALANCE EVALUATION

Bas Van Dijk\*<sup>(1)</sup>, Rosalie Nijssen<sup>(1)</sup>, Ruben Kause<sup>(1)</sup>, Federico Padilla Gonzalez<sup>(1)</sup>, Stefan Van Leeuwen<sup>(1)</sup> .....326

### I3

#### DIETARY EXPOSURE ASSESSMENT TO INORGANIC ARSENIC AND SMALL ORGANOARSENIC SPECIES IN THE EUROPEAN POPULATION

Jose Angel Gomez Ruiz\*<sup>(1)</sup>.....327

### I4

#### REGENERATION OF MAGNETIC NANOSTRUCTURED MATERIALS FOR REPEATED USES TO REMOVE CYANOTOXINS FROM WATER

Alejandro Cao<sup>(1)</sup>, Natalia Vilarino\*<sup>(1)</sup>, Lisandra De Castro Alves<sup>(2)</sup>, Jose Rivas<sup>(2)</sup>, Yolanda Pineiro<sup>(2)</sup>, Luis Rodriguez-Santos<sup>(1)</sup>, M. Carmen Louzao<sup>(1)</sup>, Sandra Raposo-García<sup>(1)</sup>, Luis M. Botana<sup>(1)</sup>.....328

### I5

#### OCCURRENCE OF QUATERNARY AMMONIUMS IN FOODSTUFFS OF ANIMAL ORIGIN: 3RD FRENCH TOTAL DIET STUDY

Kahina Slimani\*<sup>(1)</sup>, Annaëlle Chailleux<sup>(1)</sup>, Dominique Hurtaud-Pessel<sup>(1)</sup>.....329

# CONTENT

<b>16</b>	<b>QUANTIFICATION OF PFAS IN RICE AND MAIZE: VALIDATION OF A UHPLC-HRMS/MS ISOTOPIC DILUTION APPROACH IN SUPPORT TO FOOD SAFETY</b>	
	<i>Consolato Schiavone</i> <sup>(1)</sup> , Francesco Romaniello <sup>(1)</sup> , Andrea Mario Rossi <sup>(2)</sup> , Chiara Portesi <sup>(1)</sup> , Rosa Avolio <sup>(3)</sup> , Maria Cesarina Abete <sup>(3)</sup> .....	330
<b>17</b>	<b>SIMULTANEOUS DETERMINATION OF C10-17 POLYCHLORINATED ALKANES IN FOOD BY A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY</b>	
	<i>Laura Lazdina</i> <sup>(1)</sup> , Ingus Perkons <sup>(2)</sup> .....	331
<b>18</b>	<b>HIGH METAL CONTENT IN MUSHROOMS OF THE GENUS MORCHELLA GROWN AFTER A FIRE</b>	
	<i>Elena Ortega Caneda</i> <sup>(1)</sup> , Estrella Espada Bellido <sup>(1)</sup> , Alejandro R. Lopez <sup>(1)</sup> , Damiano Monticelli <sup>(2)</sup> , José Gerardo Lopez Castillo <sup>(3)</sup> , Miguel Palma Lovillo <sup>(1)</sup> , Gerardo F. Barbero <sup>(1)</sup> .....	332
<b>19</b>	<b>OCCURRENCE AND DIETARY EXPOSURE OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN COMMONLY CONSUMED FOOD IN SINGAPORE</b>	
	<i>Ignatius Lim</i> <sup>(1)</sup> , Ping Shen <sup>(2)</sup> , Wesley Yu <sup>(2)</sup> , Yee Soon Chin <sup>(2)</sup> , Raymond Shi <sup>(2)</sup> , Joanne Chan <sup>(2)</sup> .....	333
<b>110</b>	<b>COMPARISON OF ANALYTICAL TECHNIQUES FOR THE COMPREHENSIVE PFAS DETERMINATION: A MULTI-METHOD APPROACH</b>	
	<i>Veronika Svobodova</i> <sup>(1)</sup> , Ondrej Parizek <sup>(1)</sup> , Darina Dvorakova <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Denisa Parizkova <sup>(1)</sup> , Aristeidis Tsagkaris <sup>(1)</sup> , Jana Pulkrabova <sup>(1)</sup> .....	334
<b>111</b>	<b>SPECIATION ANALYSIS BY IC-ICP-MS – ARSENIC SPECIES AND METHYL MERCURY – AN OVERVIEW</b>	
	<i>Klaudija Cavlovic</i> <sup>(1)</sup> , Clara John <sup>(1)</sup> , Morhaf Ghadri <sup>(1)</sup> , Pascal Schreiter <sup>(1)</sup> , Lennart Schmiedeken <sup>(1)</sup> , Mareike Reichel <sup>(1)</sup> , Jan Sebastian Maenz <sup>(1)</sup> .....	335
<b>112</b>	<b>STREAMLINED ANALYSIS OF SHORT-CHAIN PFAS USING DUAL-COLUM INTEGRATION: A NOVEL APPROACH FOR HIGH-THROUGHPUT LABORATORIES</b>	
	<i>Victoria Erdelyi</i> <sup>(1)</sup> , Torben Leifels <sup>(1)</sup> , Claudia Rathmann <sup>(2)</sup> , Linda Schlittenbauer <sup>(3)</sup> .....	336
<b>113</b>	<b>MINERAL OIL ANALYSIS: A QUALITATIVE &amp; QUANTITATIVE WORKFLOW USING GC×GC-TOFMS/FID</b>	
	<i>Sebastiano Panto</i> <sup>(1)</sup> , Dmitrii Rakov <sup>(1)</sup> , Aous Khalefa <sup>(1)</sup> , Lena Dubois <sup>(1)</sup> , Nick Jones <sup>(1)</sup> .....	337
<b>114</b>	<b>AUTOMATED PFAS EXTRACTION FROM DIFFICULT FOOD AND FOOD PACKAGING MATRICES</b>	
	<i>Alicia Stell</i> <sup>(1)</sup> , Benedict Liu <sup>(1)</sup> , Brittany Fessler <sup>(1)</sup> , <i>Bobbie McManus</i> <sup>(1)</sup> .....	338
<b>115</b>	<b>IN-HOUSE VALIDATION OF A SIMPLE RP-LC-ICPMS METHOD FOR MERCURY SPECIATION ANALYSIS: APPLICATION TO DANISH SEAFOOD SAMPLES</b>	
	<i>Doaa Abouelenein</i> <sup>(1)</sup> , Yuka Omura Lund <sup>(1)</sup> , Maria Haar <sup>(2)</sup> , Katrin Loeschner <sup>(1)</sup> , Lene Duedahl-Olesen <sup>(1)</sup> , Jens J. Sloth <sup>(1)</sup> .....	339
<b>116</b>	<b>DEVELOPMENT OF A CANARY BIOSENSOR FOR RAPID AND SENSITIVE DETECTION OF SHIGA TOXIN-PRODUCING E. COLI IN FOOD</b>	
	<i>Christina Tam</i> <sup>(1)</sup> , Wen-Xian Du <sup>(1)</sup> , <i>Xiaohua He</i> <sup>(1)</sup> .....	340
<b>117</b>	<b>DEVELOPMENT AND OPTIMIZATION OF AN SS AND NTS STRATEGY TO EXTEND CONSUMER EXPOSOME CHARACTERIZATION TO OVER 900 CHEMICAL RESIDUES AND CONTAMINANTS</b>	
	<i>Cassandre Jeannot</i> <sup>(1)</sup> , Bruno Le Bizec <sup>(1)</sup> , Gaud Dervilly <sup>(1)</sup> , Julien Parinet <sup>(2)</sup> .....	341
<b>118</b>	<b>DETERMINATION OF FURANIC COMPOUNDS IN SOUTH AFRICAN TRADITIONAL FOOD PRODUCTS USING HEADSPACE SOLID-PHASE MICROEXTRACTION WITH GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR</b>	
	<i>Vusumzi Pakade</i> <sup>(1)</sup> , <i>Lawrence Madikizela</i> <sup>(1)</sup> .....	342
<b>119</b>	<b>THE EMERGENCE OF PHARMACEUTICAL RESIDUES IN HUMAN FOOD CROPS AND GARDEN-SOIL CULTIVATED UNDER ACTUAL ENVIRONMENTAL CONDITIONS</b>	
	<i>Lawrence Madikizela</i> <sup>(1)</sup> , <i>Ronewa Ronewa</i> <sup>(1)</sup> .....	343
<b>120</b>	<b>DETERMINATION OF NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> AND N-NITROSAMINES IN VEGETABLES STORED AND PREPARED UNDER DIFFERENT CONDITIONS</b>	
	<i>Daniel Clayton-Cuch</i> <sup>(1)</sup> , Agnieszka Anna Niklas <sup>(2)</sup> , Heidi Amlund <sup>(1)</sup> , Leise Leise Kjellmann <sup>(1)</sup> .....	344

# CONTENT

<b>I21</b>	<b>DEVELOPMENT OF METHODS TO IMPROVE AND VALIDATE MEASUREMENTS OF PFAS USING GAS CHROMATOGRAPHY - MASS SPECTROMETRY</b>	
	<i>Charlotte Hauschildt</i> <sup>(1)</sup> , Christian Jung <sup>(1)</sup> , Roland Becker <sup>(1)</sup> .....	345
<b>I22</b>	<b>CONTRIBUTION TO TOXICITY OF PCDD AND PCDF IN PUYE (GALAXIAS SPP) COLLECTED FROM FRESH WATER, IN SOUTH OF CHILE</b>	
	<i>Rodrigo Loyola-Sepulveda</i> <sup>(1)</sup> , Marco Salamanca O. <sup>(2)</sup> , Claudia Figueroa Sn. M. <sup>(1)</sup> , Alex Mendoza <sup>(1)</sup> .....	346
<b>I23</b>	<b>THE EFFECT OF VARIOUS WASHING METHODS ON PESTICIDE RESIDUES, TOXIC AND ESSENTIAL ELEMENTS REMOVAL IN RICE</b>	
	<i>Khulood Alnabati</i> <sup>(1)</sup> , Dalal Alqahtani <sup>(1)</sup> , Rakan Alajami <sup>(1)</sup> , Tagreed Alsalem <sup>(1)</sup> , Sara Almanna <sup>(1)</sup> .....	347
<b>I24</b>	<b>CHEMICAL HAZARD PROFILING OF BLACK SOLDIER FLY LARVAE (HERMETIA ILLUCENS)</b>	
	<i>Marie Papin</i> <sup>(1)</sup> , Erwan Engel <sup>(1)</sup> , Christelle Planche <sup>(1)</sup> , Patrick Borel <sup>(2)</sup> .....	348
<b>I25</b>	<b>A NOVEL APPROACH IN ADDRESSING THE CHALLENGES OF MONITORING MULTI-CLASSES OF DIOXINS AND OTHER POP IN A SINGLE RUN BY GC-ION MOBILITY-HRMS</b>	
	<i>Carsten Baessmann</i> <sup>(1)</sup> , Javier Lopez <sup>(2)</sup> , Miguel Angel Perez <sup>(2)</sup> , Arnd Ingendoh <sup>(1)</sup> , Hugo Mueller <sup>(3)</sup> , Gauthier Eppe <sup>(3)</sup> .....	349
<b>I26</b>	<b>DETECTING PFAS BEYOND THE CURRENT REGULATIVE REQUEST: A COMPREHENSIVE OVERVIEW OF THE CONTAMINATION IN DUTCH SURFACE WATER AND EFFLUENTS BY UPHLC-ION MOBILITY-HRMS</b>	
	<i>Carsten Baessmann</i> <sup>(1)</sup> , David Liwara <sup>(2)</sup> , Ilona Nordhorn <sup>(1)</sup> , Birgit Schneider <sup>(1)</sup> , Sicco Brandsma <sup>(2)</sup> , Pim Leonards <sup>(2)</sup> .....	350
<b>I27</b>	<b>ULTRA-SENSITIVE PFAS ANALYSIS ACCORDING TO EU REGULATIONS IN WATER AND FOOD</b>	
	<i>Diego Martin Ortiz</i> <sup>(1)</sup> , Javier Lopez Flores <sup>(1)</sup> , Rafaela Martin <sup>(1)</sup> .....	351
<b>I28</b>	<b>PEROXIDASE MIMICKING AND SERS ACTIVITY OF <math>\gamma</math>-CYCLODEXTRIN SYNTHESIZED GOLD AND SILVER NANOPARTICLES FOR THE DETECTION OF PESTICIDES</b>	
	<i>Natasha Logan</i> <sup>(1)</sup> , Cuong Cao <sup>(2)</sup> , <i>Xiaotong Liu</i> <sup>(1)</sup> .....	352
<b>I29</b>	<b>ACHIEVING EXCEPTIONAL ROBUSTNESS FOR PFAS ANALYSIS IN FOOD WITH THE NEXT-GENERATION SCIEX 7500+ SYSTEM</b>	
	<i>Holly Lee</i> <sup>(1)</sup> , Ian Moore <sup>(1)</sup> , Craig Butt <sup>(1)</sup> , Elliott Jones <sup>(1)</sup> .....	353
<b>I30</b>	<b>ADVANCED ULTRA-SHORT-CHAIN PFAS ANALYSIS USING - UNEXPECTEDLY - REVERSED PHASE CHROMATOGRAPHY</b>	
	<i>Ruben Kause</i> <sup>(1)</sup> , Bas Van Dijk <sup>(1)</sup> , Katja Van Dongen <sup>(1)</sup> , Elise Hoek <sup>(1)</sup> , Stefan Van Leeuwen <sup>(1)</sup> .....	354
<b>I31</b>	<b>IS THE STRATEGIC APPROACH OF SENTINEL ANIMAL SPECIES REALISTIC FOR RAPIDLY IDENTIFYING HALOGENATED EMERGING CONTAMINANTS IN THE HUMAN FOOD CHAIN?</b>	
	<i>Antonin Padioleau</i> <sup>(1)</sup> , Ronan Cariou <sup>(1)</sup> , Bruno Le Bizec <sup>(1)</sup> , <i>Gaud Dervilly</i> <sup>(1)</sup> .....	355
<b>I32</b>	<b>MICROBIOLOGICAL STATUS OF DONOR HUMAN MILK INTENDED FOR PREMATURE INFANTS</b>	
	<i>Elena Sinkiewicz-Darol</i> <sup>(1)</sup> , Iwona Adamczyk <sup>(1)</sup> , Katarzyna Kaliszczak <sup>(2)</sup> , Krzysztof Skowron <sup>(3)</sup> , Katarzyna Grudlewska-Buda <sup>(3)</sup> , Magdalena Twaruzek <sup>(4)</sup> .....	356
<b>I33</b>	<b>THE ANALYSIS OF PFAS IN MILK BY LC-MS/MS</b>	
	<i>Tina Brandscher</i> <sup>(1)</sup> , Sandra Ruiz Perez <sup>(1)</sup> , Jamie York <sup>(2)</sup> .....	357
<b>I34</b>	<b>CONCENTRATIONS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN EGGS: PRELIMINARY RESULTS IN THE REPUBLIC OF CROATIA</b>	
	<i>Ines Varga</i> <sup>(1)</sup> , Jelena Kaurinovic <sup>(1)</sup> , Nina Bilandzic <sup>(1)</sup> , Maja Dokic <sup>(1)</sup> , Ivana Varenina <sup>(1)</sup> , Bozica Solomun Kolanovic <sup>(1)</sup> , Marija Sedak <sup>(1)</sup> , Bruno Calopek <sup>(1)</sup> , Luka Cvetnic <sup>(2)</sup> .....	358
<b>I35</b>	<b>PRESENCE OF POLYCHLORINATED BIPHENYLS IN DIFFERENT FISH FROM THE ADRIATIC SEA</b>	
	<i>Nina Bilandzic</i> <sup>(1)</sup> , Maja Dokic <sup>(1)</sup> , Tamara Nekic <sup>(1)</sup> , Marija Sedak <sup>(1)</sup> , Bruno Calopek <sup>(1)</sup> , Ivana Varenina <sup>(1)</sup> , <i>Ines Varga</i> <sup>(1)</sup> , Bozica Solomun Kolanovic <sup>(1)</sup> .....	359

# CONTENT

<b>I36</b>	<b>PFAS AND ONE HEALTH APPROACH. IMPROVEMENTS IN THE ENVIRONMENTAL, FOOD, PACKAGING, COSMETIC AND PHARMA SECTORS. WHAT ELSE?</b>	
	<u>Emiliano De Dominicis</u> <sup>(1)</sup> , Federica Cattapan <sup>(1)</sup> , Andrea Vittadello <sup>(1)</sup> , Paola Verza <sup>(1)</sup> , Barbara Scantamburlo <sup>(1)</sup> , Alessio Mattiazzo <sup>(1)</sup> , Marco Redaelli <sup>(1)</sup> , Michela Casarotto <sup>(1)</sup> , Giulia Valente <sup>(1)</sup> , Claudia Piazza <sup>(1)</sup> , Cinzia Pellicorio <sup>(1)</sup> , Francesca Faraon <sup>(1)</sup> , Valentina Abbondandolo <sup>(1)</sup> , Sylvain Dahiot <sup>(1)</sup> , Sophie Robert <sup>(2)</sup> , Natalia Serrano <sup>(3)</sup> , Pascal Gonzalez <sup>(3)</sup> , Giampaolo Perinello <sup>(4)</sup> , Thomas Behnke <sup>(5)</sup> , Jay Alappat <sup>(6)</sup> , Walter Brandl <sup>(6)</sup> , Juan Carrasco <sup>(7)</sup> , Michael Ndlovu <sup>(8)</sup> , Robert Deng <sup>(9)</sup> , Asish Chakraborty <sup>(10)</sup> , Paolo Matteini <sup>(11)</sup> , Giuseppe Sammarco <sup>(11)</sup> , Francesco Rosi <sup>(11)</sup> , Cecilia Socolsky <sup>(11)</sup> .....	360
<b>I37</b>	<b>PRELIMINARY STUDY OF PCNS CONCENTRATION IN FARM SAMPLES</b>	
	<u>Sebastian Maszewski</u> <sup>(1)</sup> , Malgorzata Warenik-Bany <sup>(1)</sup> .....	361
<b>I38</b>	<b>QUANTIFICATION OF 60 PLASTICIZERS AND OTHER SELECTED ADDITIVES BY GC- AND LC-MS/MS IN GERMANY'S FIRST TOTAL DIET STUDY "BFR MEAL"</b>	
	<u>Torsten Bruhn</u> <sup>(1)</sup> , Andriy Kuklya <sup>(1)</sup> , Elisabeth Koch <sup>(1)</sup> , Ezgi Eyluel Bankoglu <sup>(1)</sup> , Irmela Sarvan <sup>(1)</sup> , Sebastian Zellmer <sup>(1)</sup> , Oliver Kappenstein <sup>(1)</sup> , Andreas Luch <sup>(1)</sup> , Thomas Tietz <sup>(1)</sup> , Alexander Roloff <sup>(1)</sup> .....	362
<b>I39</b>	<b>WHAT DO 3-MCPD AND 2-CHLOROETHANOL HAVE IN COMMON? STUDY ON THE SOURCE FOR FALSE POSITIVE FINDINGS FOR 2-CHLOROETHANOL IN FOOD SUPPLEMENTS</b>	
	<u>Anna Romanotto</u> <sup>(1)</sup> , Deborah Bertuola <sup>(1)</sup> , Bruno Ramm <sup>(1)</sup> , Maike Greunwald <sup>(1)</sup> .....	363
<b>I40</b>	<b>TRACE ANALYSIS POLYCHLORINATED DIBENZO-P-DIOXINS/FURANS ANALYSIS USING GC-MS/MS IN ACCORDANCE WITH AMENDMENTS TO EU REGULATIONS 644/2017 AND 771/2017 FOR FOOD AND FEED</b>	
	<u>Adam Ladak</u> <sup>(1)</sup> , Nicholas Warner <sup>(1)</sup> , Daniel Kutscher <sup>(1)</sup> , Kerstin Krätschmer <sup>(2)</sup> , Alexander Schächtele <sup>(3)</sup> .....	364
<b>I41</b>	<b>A FAST AND NOVEL WORKFLOW FOR SCREENING SMOKE FROM FOREST FIRES AFFECTING FOOD QUALITY BY SPESH-DART-MS/MS</b>	
	Terry L. Bates <sup>(1)</sup> , Francois Espourteille <sup>(1)</sup> , <u>Eva-Maria Niehaus</u> <sup>(2)</sup> .....	365
<b>I42</b>	<b>ENHANCED COMPOUND IDENTIFICATION IN NON-TARGET ANALYSIS USING A NOVEL GC-HRMS WITH SIMULTANEOUS EI AND CI</b>	
	Eliska Ceznerova <sup>(1)</sup> , Sonja Klee <sup>(1)</sup> , Steffen Bräkling <sup>(1)</sup> , Arnd Ingendoh <sup>(2)</sup> , <u>Eva-Maria Niehaus</u> <sup>(2)</sup> .....	366
<b>I43</b>	<b>DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN VEGETABLE ORIGIN FOODSTUFFS BY QUECHERS EXTRACTION AND LC-MS/MS DETECTION</b>	
	<u>Simone Pellegrini</u> <sup>(1)</sup> , Gianluca Facchinetti <sup>(1)</sup> .....	367
<b>I44</b>	<b>DETERMINING PFAS IN FRUITS, VEGETABLES AND BABY FOOD USING LC-MS/MS</b>	
	<u>Claudia Rathmann</u> <sup>(1)</sup> , Nicola Dreolin <sup>(2)</sup> , Kari Organtini <sup>(2)</sup> , Stuart Adams <sup>(2)</sup> , Oliver Burt <sup>(2)</sup> .....	368
<b>I45</b>	<b>IDENTIFICATION OF SHORT CHAIN PER AND POLYFLUORINATED ALKYL SUBSTANCES (PFAS) USING ION RATIOS WITH LOW MASS PRODUCT IONS</b>	
	<u>Claudia Rathmann</u> <sup>(1)</sup> , Stuart Adams <sup>(2)</sup> , David Gordon <sup>(2)</sup> , Kate Whyatt <sup>(2)</sup> .....	369
<b>I46</b>	<b>SIMPLE AND EFFICIENT SPE AUTOMATION AND UPLC-MS/MS ANALYSIS OF PFAS IN MILK</b>	
	<u>Cecile Pinto</u> <sup>(1)</sup> , Daniel Ng <sup>(2)</sup> , Marcus Devakishen <sup>(3)</sup> , Kai Xiang Kuah <sup>(3)</sup> , Stuart Adams <sup>(4)</sup> , Joanne Ho <sup>(4)</sup> .....	370
<b>I47</b>	<b>SIMPLE AND FAST METHOD FOR PAHS QUANTIFICATION IN OIL &amp; OIL-RICH FOOD USING MOLECULARLY IMPRINTED POLYMERS EXTRACTION</b>	
	Gisele Usanase <sup>(1)</sup> , Aurelie Cortejade <sup>(1)</sup> , Cecile Vincent <sup>(1)</sup> , Roxane Chene <sup>(1)</sup> , Sami Bayouhd <sup>(2)</sup> , <u>Michel Arotçarena</u> <sup>(2)</sup> , Kaynoush Naraghi <sup>(2)</sup> , Mickaël Nicolas <sup>(1)</sup> .....	371
<b>I48</b>	<b>TRACE ANALYSIS OF 30 PERFLUORINATED COMPOUNDS IN SALMON TROUT AND TAP WATER USING SOLID PHASE EXTRACTION (SPE)</b>	
	Sami Bayouhd <sup>(1)</sup> , Michel Arotçarena <sup>(1)</sup> , Kaynoush Naraghi <sup>(1)</sup> , Corentin Germain <sup>(1)</sup> , Juliette Gouley <sup>(1)</sup> , <u>Louna Moles</u> <sup>(1)</sup> .....	372
<b>I49</b>	<b>ULTRA-FAST MRM ACQUISITION AND QUANTITATION OF FOOD CONTAMINANTS IN MULTIPLE FOOD MATRICES</b>	
	Jianru Stahl-Zeng <sup>(1)</sup> , <u>Andre Schreiber</u> <sup>(1)</sup> , Gitte Barknowitz <sup>(1)</sup> , Ian Moore <sup>(1)</sup> , Hassan El Khallabi <sup>(2)</sup> .....	373

# CONTENT

<b>150</b>	<b>QUANTITATION OF PFAS IN FOOD WITH PARTS PER TRILLION LEVELS OF SENSITIVITY</b>	
	Daniel McMillan <sup>(1)</sup> , Jianru Stahl-Zeng <sup>(1)</sup> , Michael Scherer <sup>(1)</sup> , Xanthippe Theurillat <sup>(2)</sup> , Ashley Griffin <sup>(3)</sup> .....	374
<b>151</b>	<b>UNVEILING THE COMPLEXITY OF ADVANCED GLYCATION END PRODUCTS (AGES): A NEW HILIC MS/MS METHOD FOR SIMULTANEOUS ANALYSIS OF 19 AGES AND THEIR COELUTING ISOMERS IN FOOD MODEL SYSTEMS</b>	
	Maria-Anna Kolovou <sup>(1)</sup> , Misha Vrolijk <sup>(2)</sup> , Dick Sijm <sup>(3)</sup> , Frederik-Jan Schooten <sup>(2)</sup> , Stefan Van Leeuwen <sup>(1)</sup> .....	375
<b>152</b>	<b>BENCHMARK OF NON-TARGETED LC-HRMS DATA PROCESSING SOFTWARE TO DEVELOP A STANDARDIZED WORKFLOW FOR ASSESSING CONTAMINATION OF DIFFERENT FOOD MATRICES</b>	
	Deborah Martin <sup>(1)</sup> , Julien Parinet <sup>(2)</sup> .....	376
<b>153</b>	<b>FASTER ANALYSIS FOR FOREVER CHEMICALS: ACCELERATING PFAS ANALYSIS WITH UPLC-HRIM-MS</b>	
	Alan McKenzie-Coe <sup>(1)</sup> , Jeremy Koelmel <sup>(2)</sup> , Emma Rennie <sup>(3)</sup> , David Weil <sup>(3)</sup> , Paul Stelben <sup>(3)</sup> , Sarah Stow <sup>(3)</sup> , Krystal Pollitt <sup>(4)</sup> , David Godri <sup>(5)</sup> , Frederick Strathmann <sup>(1)</sup> , Daniel DeBord <sup>(1)</sup> .....	377
<b>154</b>	<b>SIMULTANEOUS ANALYSIS OF PESTICIDES IN WATER USING AI PEAK INTEGRATION</b>	
	Waldemar Weber <sup>(1)</sup> .....	378
<b>155</b>	<b>VACUUM AND MINIATURIZED SOLID PHASE MICROEXTRACTION ANALYSIS OF PESTICIDES IN GRAPES</b>	
	Mereke Alimzhanova <sup>(1)</sup> , Yerkanat Syrgabek <sup>(1)</sup> .....	379
<b>156</b>	<b>ANALYSIS OF GROUNDWATER CONTAMINATION BY PETROLEUM PRODUCTS USING MINI-SPME WITH GC-MS</b>	
	Mereke Alimzhanova <sup>(1)</sup> , Bauyrzhan Abdykarimov <sup>(1)</sup> .....	380
<b>157</b>	<b>CONTAMINANTS IN FOOD STUFF: SOURCES AND STRATEGIES FOR DETECTION AND RESOLUTION BY LC/MS-MS</b>	
	Alexandra Paladi <sup>(1)</sup> , Alejandro Ladreyt <sup>(1)</sup> .....	381
<b>158</b>	<b>A SURFACE ENHANCEMENT RAMAN SPECTROSCOPY (SERS) INVESTIGATION OF POTENTIAL RESIDUE CONTAMINATION OF MILK PRODUCTS FROM THE USE OF CHLORINE-FREE CLEANING PRODUCTS</b>	
	Daniela Lopez <sup>(1)</sup> , Krishnakumar Chullipallyalil <sup>(1)</sup> , Killian Barton <sup>(1)</sup> , Michael McAuliffe <sup>(1)</sup> , Ambrose Furey <sup>(2)</sup> .....	382
<b>159</b>	<b>CHEMICAL IONIZATION MASS SPECTROMETRY FOR REAL-TIME MONITORING OF PFAS</b>	
	Manuel Hutterli <sup>(1)</sup> , Luca Cappellin <sup>(2)</sup> , Spiro Jorga <sup>(1)</sup> , Abigail Koss <sup>(1)</sup> , Omar El Hajj <sup>(1)</sup> , Veronika Pospisilova <sup>(1)</sup> .....	383
<b>160</b>	<b>AUTOMATED SAMPLE PREPARATION FOR TARGETED QUANTITATION AND NON-TARGETED ANALYSIS OF PFAS WITH HPLC-HRMS ANALYSIS BASED ON ORBITRAP TECHNOLOGY</b>	
	Aristide Ganci <sup>(1)</sup> , Valerie Thibert <sup>(1)</sup> , Jean-François Garnier <sup>(1)</sup> , Bénédicte Gauriat <sup>(1)</sup> , Richard Cochran <sup>(1)</sup> , Lee Ferguson <sup>(2)</sup> .....	384
<b>161</b>	<b>PFAS ANALYSIS STRATEGY STORY - DIRECT INJECTION, DLLME, LC-MS/MS, LC-ORBITRAP / GC-ORBITRAP</b>	
	Benedicte Gauriat <sup>(1)</sup> , Valerie Thibert <sup>(1)</sup> , Aristide Ganci <sup>(1)</sup> , Jean-François Garnier <sup>(1)</sup> .....	385
<b>162</b>	<b>ROUTINE PFAS TESTING IN WASTEWATER USING TOP ASSAY AND ACQUITY™ QDA™ II MASS DETECTOR</b>	
	Henry Foddy <sup>(1)</sup> , Cristian Cojocariu <sup>(1)</sup> , Vladimir Nikiforov <sup>(2)</sup> .....	386
<b>163</b>	<b>ELEVATING SPECIFICITY AND IDENTIFICATION CONFIDENCE FOR PFAS LIQUID CHROMATOGRAPHY AND CYCLIC ION MOBILITY MASS SPECTROMETRY</b>	
	Susan Slade <sup>(1)</sup> , Michael McCullagh <sup>(1)</sup> , Georgios Theodoridis <sup>(2)</sup> , Robert Plumb <sup>(1)</sup> , Stuart Adams <sup>(1)</sup> , Sarah Dowd <sup>(1)</sup> , Artemis Lioupi <sup>(2)</sup> .....	387
<b>164</b>	<b>DETERMINATION OF VARIOUS PFAS IN EGG MATRIX USING STACKED INJECTION ON-LINE SPE COUPLED TO LC-MS/MS</b>	
	Uwe Oppermann <sup>(1)</sup> , Anja Grüning <sup>(1)</sup> , Stephane Moreau <sup>(1)</sup> .....	388

# CONTENT

## FOOD SUPPLEMENTS ..... 389

### J1

#### PIGMENTS, NITRATES AND STANDARDIZATION: PROFILING RED BEETROOT (BETA VULGARIS L.) FOOD INGREDIENTS AND SUPPLEMENTS

Marco Gozzi\*<sup>(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Martina Cirlini<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>, Renato Bruni<sup>(1)</sup> .....390

### J2

#### OPTIMIZING HEMICELLULOSE HYDROTHERMAL EXTRACTION FROM LIGNOCELLULOSIC BIOMASSES USING AN ENZYMATIC APPROACH: EFFECT OF TEMPERATURE, ENZYMES, AND MATRIX

Pio Viscusi\*<sup>(1)</sup>, Veronica Lolli<sup>(1)</sup>, Augusta Caligiani<sup>(1)</sup> .....391

### J3

#### A COMPREHENSIVE COMPARISON OF VARIOUS TECHNIQUES FOR CHARACTERISATION AND AUTHENTICATION OF FISH OIL-BASED FOOD SUPPLEMENTS

Chi Mai Nguyenova\*<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>, Marek Dolezal<sup>(1)</sup>, Jan Poustka<sup>(1)</sup> .....392

### J4

#### QUANTIFICATION OF 19 CANNABINOIDS IN COMMERCIAL CBD OILS AND SAFETY IMPLICATIONS OF THEIR CBD AND Δ9-THC CONTENTS

Niklas Lindekamp<sup>(1)</sup>, Stefan Weigel\*<sup>(1)</sup>, Benjamin Sachse<sup>(2)</sup>, Bernd Schäfer<sup>(2)</sup>, Sascha Rohn<sup>(3)</sup>, Nadja Triesch<sup>(1)</sup> .....393

### J5

#### DATE SYRUP: HEALTHY AND SAFE SWEETENER

Benedetta Sgro\*<sup>(1)</sup>, Qada Benameur<sup>(2)</sup>, Nadra Rechidi-Sidhoum<sup>(3)</sup>, Vincenzo Lo Turco<sup>(4)</sup>, Angela Giorgia Potorti<sup>(4)</sup>, Giuseppa Di Bella<sup>(4)</sup> .....394

### J6

#### EFFECT OF IN VITRO DIGESTION ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF GINGER AND PROPOLIS EXTRACTS

Katie Nina Doran\*<sup>(1)</sup>, Liliana Ordóñez<sup>(1)</sup>, Marc Griffiths<sup>(1)</sup>, Alexandros Ch Stratakos<sup>(1)</sup> .....396

### J7

#### SMOOTH THE PATH FOR HIGH THROUGHPUT LABORATORIES - A SIMPLE QUECHERS BASED- EXTRACTION MULTI-COMPOUND LC-MS/MS METHOD FOR THE DETECTION OF THE VITAMINS A, E, D AND K IN FOOD

Nancy Frank\*<sup>(1)</sup>, Lucas Hemmerle<sup>(1)</sup>, Laure Poquet<sup>(1)</sup>, Philippe Eugster<sup>(1)</sup> .....397

### J8

#### QUANTIFICATION OF SEXUAL ENHANCERS IN DIETARY SUPPLEMENTS BY LC-MS/MS

Meritxell Castello\*<sup>(1)</sup>, Eva Munoz<sup>(1)</sup>, Josep Calderon<sup>(1)</sup>, Margalida Artigues<sup>(2)</sup>, Jordi Diaz<sup>(2)</sup>, Sonia Gotsens<sup>(2)</sup>, Josep Lluís Lliberia<sup>(3)</sup> .....398

### J9

#### QUALITY ASSURANCE OF SAFFRON SUPPLEMENTS VIA SUSPECT METABOLOMIC PROFILING

Hilva Gjoni\*<sup>(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Marco Biagi<sup>(1)</sup>, Renato Bruni<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup> .....399

### J10

#### IMPROVING CHROMATOGRAPHIC RESOLUTION OF THE JECFA METHOD FOR THE ANALYSIS OF STEVIOL GLYCOSIDES

Cecile Pinto<sup>(1)</sup>, Jinchuan Yang\*<sup>(2)</sup>, Paul Rainville<sup>(2)</sup>, Stephanie Harden<sup>(3)</sup> .....400

### J11

#### CHARACTERIZATION OF CANNABIS CULTIVARS FOR MEDICINAL PURPOSES

Frantisek Benes\*<sup>(1)</sup>, Zuzana Binova<sup>(1)</sup>, Matej Maly<sup>(1)</sup>, Maria Filatova<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup> .....401

### J12

#### FORMATION OF SULFORAPHANE DURING LACTIC ACID FERMENTATION OF NASTURTIUM (TROPAEOLUM MAJUS L.)

Anna Romanotto<sup>(1)</sup>, Kerstin Hansen\*<sup>(2)</sup> .....402

## GENERAL FOOD ANALYSIS ..... 403

### K1

#### HIGH-THROUGHPUT, FULLY AUTOMATED SAMPLE PREPARATION FOR CONTAMINANT ANALYSIS

Mareike Reichel\*<sup>(1)</sup>, Clara John<sup>(1)</sup>, Jan Sebastian Mänz<sup>(1)</sup> .....404

### K2

#### EFFECT-BASED ANALYSIS USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AS A TOOL TO EVALUATE COMPLEX MIXTURES

Paul Rogeboz\*<sup>(1)</sup>, Hélia Latado<sup>(1)</sup>, Claudine Cottet Fontannaz<sup>(1)</sup>, Blandine Descamps<sup>(1)</sup>, Amaury Patin<sup>(1)</sup>, Maricel Marin-Kuan<sup>(1)</sup> .....405

# CONTENT

<b>K3</b>	
<b>THE EVALUATION OF GROWTH-INHIBITORY EFFECT OF PLANT VOLATILE COMPOUNDS AGAINST FOOD PATHOGENIC MICROORGANISMS IN LIQUID AND VAPOR PHASE USING BROTH MICRODILUTION VOLATILIZATION METHOD</b>	
<u>Adrish Dutta</u> <sup>(1)</sup> , Marketa Houdkova <sup>(1)</sup> , Ladislav Kokoska <sup>(1)</sup> .....	406
<b>K4</b>	
<b>DEVELOPMENT OF AN ANTIOXIDANT ASSAY FOR ANTHOCYANINS IN RED CABBAGE USING LIQUID CHROMATOGRAPHY COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETRY</b>	
<u>Claudia Lombroni</u> <sup>(1)</sup> , Ksenia Morozova <sup>(2)</sup> , Lucrezia Angeli <sup>(2)</sup> , Giovanna Ferrentino <sup>(2)</sup> , Matteo Scampicchio <sup>(2)</sup> .....	407
<b>K5</b>	
<b>BAD AFTERTASTE IN TRUFFLE SOUP: IDENTIFYING THE CULPRITS IN A REAL-TIME NOSESPACE STUDY</b>	
<u>Philipp Sulzer</u> <sup>(1)</sup> , Andreas Mauracher <sup>(1)</sup> , Rene Gutmann <sup>(1)</sup> .....	408
<b>K6</b>	
<b>IDENTIFICATION OF PROCESS-DEPENDENT MAILLARD ADDUCTS IN WHOLEMEAL DRY PASTA BY UNTARGETED HIGH RESOLUTION TANDEM MASS SPECTROMETRY: A COMPARATIVE ANALYSIS OF DIFFERENT DRYING CYCLES AND SEMOLINA COMPOSITION</b>	
Chiara Nitride <sup>(1)</sup> , Anna Luparelli <sup>(2)</sup> , Antonella Lamonaca <sup>(3)</sup> , Elisabetta De Angelis <sup>(2)</sup> , Linda Monaci <sup>(2)</sup> , <u>Rosa Pilolli</u> <sup>(2)</sup> .....	409
<b>K7</b>	
<b>DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE METHOD FOR VETERINARY DRUGS IN ANIMAL BASED-FOOD MATRICES USING LIQUID-CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY</b>	
<u>Eloi Marilleau</u> <sup>(1)</sup> , Yvan Gru <sup>(1)</sup> , Nicolas Cimetiere <sup>(2)</sup> , Alexandre Verdu <sup>(3)</sup> .....	410
<b>K8</b>	
<b>ADVANCING SAFETY ASESMENT - APPLICATION OF NON-TARGETED ANALYSIS FOR NEW FOODS AND FOOD PRODUCTION SYSTEMS</b>	
Hui Yi Lim <sup>(1)</sup> , Bin Li <sup>(1)</sup> , Yai Foong Chew <sup>(1)</sup> , Lin Min Lee <sup>(1)</sup> , Wei Yong Desmond Teo <sup>(1)</sup> , <u>Haiyan Li</u> <sup>(1)</sup> , Joanna Khoo <sup>(1)</sup> , Sheot Harn Chan <sup>(1)</sup> , Dingyi Yu <sup>(1)</sup> .....	411
<b>K9</b>	
<b>SCREENING OF TOCOLS CONTENT IN DIFFERENT MAIZE KERNEL TYPE</b>	
<u>Jelena Vukadinovic</u> <sup>(1)</sup> , Jelena Srdic <sup>(1)</sup> , Violeta Andelkovic <sup>(1)</sup> , Nikola Grcic <sup>(1)</sup> , Milan Brankov <sup>(1)</sup> , Milomir Filipovic <sup>(1)</sup> , Snezana Mladenovic Drinic <sup>(1)</sup> .....	412
<b>K10</b>	
<b>EXPLORATION OF NEW LOW-PRESSURE GC COLUMNS FOR FOOD AND ENVIRONMENT EMERGING CONTAMINANTS</b>	
<u>Jana Hepner</u> <sup>(1)</sup> , Whitney Dudek-Salisbury <sup>(1)</sup> , Chris English <sup>(1)</sup> .....	413
<b>K11</b>	
<b>MOROCCAN AND ITALIAN CAROBS (CERATONIA SILIQUA L.): CHEMICAL COMPOSITION, LIPID FRACTION, AND MINERAL PROFILE</b>	
<u>Vincenzo Nava</u> <sup>(1)</sup> , Ambrogina Albergamo <sup>(1)</sup> , Angela Giorgia Potorti <sup>(1)</sup> , Vincenzo Lo Turco <sup>(1)</sup> , Giuseppa Di Bella <sup>(1)</sup> .....	414
<b>K12</b>	
<b>QUANTITATION OF ALLYL ESTERS, LINALOOL, CITRAL, AND LINALYL ACETATE IN YOGURT AND SOFT DRINKS BY MEANS OF GC-MS</b>	
<u>Anna Maria Gabler</u> <sup>(1)</sup> , Michal Stupak <sup>(1)</sup> , Josephine Bönick <sup>(1)</sup> , Annemarie Richter <sup>(1)</sup> , Frederike Sommerfeld <sup>(1)</sup> , Susanne Esslinger <sup>(1)</sup> .....	415
<b>K13</b>	
<b>CHARACTERIZATION OF PLANT-BASED BEVERAGES BY 1D, 2D, AND QUANTITATIVE NMR</b>	
<u>Federico Brigante</u> <sup>(1)</sup> , Pavel Solovyev <sup>(1)</sup> , Luana Bontempo <sup>(1)</sup> .....	416
<b>K14</b>	
<b>OPTIMAL SCHEME FOR STANDARD ADDITION</b>	
<u>Panagiotis Steliopoulos</u> <sup>(1)</sup> , Franziska Scharmann <sup>(1)</sup> .....	417
<b>K15</b>	
<b>EFFECT OF CLA-PRODUCING ADJUNCT CULTURES OF NUTRITIONAL VALUE OF SHEEP CHEESE</b>	
<u>Federica Litrenta</u> <sup>(1)</sup> , Georgiana Bosco <sup>(2)</sup> , Annalisa Amato <sup>(3)</sup> , Carmelo Cavallo <sup>(3)</sup> , Marco Scalisi <sup>(3)</sup> , Cinzia Lucia Randazzo <sup>(2)</sup> , Vincenzo Nava <sup>(3)</sup> , Vincenzo Lopreiato <sup>(3)</sup> , Luigi Liotta <sup>(3)</sup> .....	418
<b>K16</b>	
<b>MINERAL ELEMENTS IN COMMERCIAL BABY FOODS</b>	
Angela Giorgia Potorti <sup>(1)</sup> , <u>Irene Maria Spanò</u> <sup>(1)</sup> , Antonio Cambria <sup>(1)</sup> , Vincenzo Lo Turco <sup>(2)</sup> , Giuseppa Di Bella <sup>(1)</sup> .....	419
<b>K17</b>	
<b>WP-CBR001 - A NEW MATRIX-CERTIFIED REFERENCE MATERIAL FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND TOXIC ELEMENTS IN WHEY PROTEIN POWDER</b>	
Simon Lobsiger <sup>(1)</sup> , Silvia Mallia <sup>(1)</sup> , Lena Marki <sup>(1)</sup> , Gisela Umbricht <sup>(1)</sup> , Hanspeter Sprecher <sup>(2)</sup> , Kathrin Breitruck <sup>(2)</sup> , Markus Obkircher <sup>(3)</sup> , <u>Alexandra Jaus</u> <sup>(1)</sup> .....	420



# CONTENT

<b>K18</b>	<b>EVALUATION OF THE ANTIOXIDANT CAPACITY OF EXTRACTS OF MUSHROOMS WITH HIGH GASTRONOMIC VALUE</b>	
	<i>Elena Ortega Caneda</i> <sup>(1)</sup> , <i>Estrella Espada Bellido</i> <sup>*(1)</sup> , <i>Rosa Maria Mateos Bernal</i> <sup>(2)</sup> , <i>Ceferino Carrera Fernandez</i> <sup>(1)</sup> , <i>Nuria Chinchilla Salcedo</i> <sup>(3)</sup> , <i>Miguel Palma Lovillo</i> <sup>(1)</sup> , <i>Gerardo Fernandez Barbero</i> <sup>(1)</sup> .....	421
<b>K19</b>	<b>PHYSICOCHEMICAL AND SENSORY PROPERTIES OF SOYMILK PREPARED FROM SOYBEANS SOAKED IN D-XYLOSE AND L-LEUCINE SOLUTIONS USING ULTRASONIC TREATMENT</b>	
	<i>Eunyeong Choi</i> <sup>(1)</sup> , <i>Kwang-Geun Lee</i> <sup>*(1)</sup> .....	422
<b>K20</b>	<b>TOTAL PHOSPHORUS AND PHOSPHATES CONTENTS IN MEAT PRODUCTS FROM THE BELGRADE MARKET, SERBIA</b>	
	<i>Danijela Vranic</i> <sup>*(1)</sup> , <i>Radivoj Petronijevic</i> <sup>(1)</sup> , <i>Jasna Djinic-Stojanovic</i> <sup>(1)</sup> , <i>Vesna Djordjevic</i> <sup>(1)</sup> , <i>Srdjan Stefanovic</i> <sup>(1)</sup> , <i>Vladimir Koricanac</i> <sup>(1)</sup> , <i>Dejana Trbovic</i> <sup>(1)</sup> .....	423
<b>K21</b>	<b>ASSESSMENT OF DIETARY INTAKE OF IRON IN SERBIAN ADULT BY CONSUMPTION OF DIFFERENT MEAT PRODUCTS</b>	
	<i>Jasna Djinic-Stojanovic</i> <sup>*(1)</sup> , <i>Ivana Brankovic Lazic</i> <sup>(1)</sup> , <i>Sasa Jankovic</i> <sup>(1)</sup> , <i>Danijela Vranic</i> <sup>(1)</sup> , <i>Munevera Begic</i> <sup>(2)</sup> , <i>Ognjen Krnjaja</i> <sup>(1)</sup> , <i>Vesna Djordjevic</i> <sup>(1)</sup> .....	424
<b>K22</b>	<b>CALCIUM AND MAGNESIUM CONTENT IN BOILED SAUSAGES FROM THE SERBIAN MARKET</b>	
	<i>Jasna Djinic-Stojanovic</i> <sup>*(1)</sup> , <i>Ivana Brankovic Lazic</i> <sup>(1)</sup> , <i>Sasa Jankovic</i> <sup>(1)</sup> , <i>Danijela Vranic</i> <sup>(1)</sup> , <i>Munevera Begic</i> <sup>(2)</sup> , <i>Srdjan Stefanovic</i> <sup>(1)</sup> , <i>Vesna Djordjevic</i> <sup>(1)</sup> .....	425
<b>K23</b>	<b>OCCURRENCE OF TOTAL AFLATOXINS (B1, B2, G1, G2) IN COMMERCIAL PEANUT AND PEANUT BUTTER FROM THE SAUDI MARKET IN THE PERIOD FROM 2015 TO 2020</b>	
	<i>Yaser Almasoud</i> <sup>*(1)</sup> .....	426
<b>K24</b>	<b>ACCURATE QUANTITATIVE PROFILING OF FATTY ACIDS IN PLANT-BASED FOOD USING BY ONE-STEP MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION FOLLOWED BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH PARALLEL DETECTION BY FLAME IONIZATION DETECTOR AND MASS SPECTROMETRY</b>	
	<i>Angelica Fina</i> <sup>(1)</sup> , <i>Andrea Caratti</i> <sup>(2)</sup> , <i>Donatella Ferrara</i> <sup>(3)</sup> , <i>Giorgia Botta</i> <sup>(1)</sup> , <i>Erica Liberto</i> <sup>(2)</sup> , <i>Simone Apolloni</i> <sup>(1)</sup> , <i>Marco Beccaria</i> <sup>(4)</sup> , <i>Diego Carnaroglio</i> <sup>(5)</sup> , <i>Giorgia Purcaro</i> <sup>(3)</sup> , <i>Chiara Cordero</i> <sup>*(2)</sup> .....	427
<b>K25</b>	<b>SEMI-AUTOMATED SAMPLING DURING TEA INFUSION: DETERMINATION OF THE TRANSFER OF SESQUITERPENE LACTONES FROM BOTANICAL PREPARATIONS (ARNICA MONTANA) INTO HOT TEA INFUSIONS</b>	
	<i>Franziska Kolrep</i> <sup>*(1)</sup> , <i>Xenia Anabel Körner</i> <sup>(1)</sup> , <i>Oliver Kappenstein</i> <sup>(1)</sup> .....	428
<b>K26</b>	<b>FOLIAR APPLICATION OF SODIUM SELENATE: EFFECTS ON BIOACTIVE COMPOUNDS AND QUALITY OF RASPBERRY (RUBUS IDAEUS L.)</b>	
	<i>Manuel Martoccia</i> <sup>*(1)</sup> , <i>Helder Jose Couto Oliveira</i> <sup>(2)</sup> , <i>Maurizio Aceto</i> <sup>(3)</sup> , <i>Vincenzo Disca</i> <sup>(1)</sup> , <i>Matteo Bordiga</i> <sup>(1)</sup> , <i>Jean Daniel Coisson</i> <sup>(1)</sup> .....	429
<b>K27</b>	<b>TRANSFER OF SELECTED PERSISTENT ORGANIC POLLUTANTS IN YELLOW MEALWORM LARVAE (TENEBRIO MOLITOR)</b>	
	<i>Corinna Kurbis</i> <sup>*(1)</sup> , <i>Johanna Ziegler</i> <sup>(1)</sup> , <i>Lisa Klusmann</i> <sup>(1)</sup> , <i>Mariko Yamamoto</i> <sup>(1)</sup> , <i>Pia Sy</i> <sup>(1)</sup> , <i>Anne Jahnke</i> <sup>(1)</sup> , <i>Linda Uhr</i> <sup>(1)</sup> , <i>Anja Lüth</i> <sup>(1)</sup> , <i>David M. Schumacher</i> <sup>(1)</sup> .....	430
<b>K28</b>	<b>QUANTIFICATION OF 30 PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN FRUITS AND VEGETABLES</b>	
	<i>Anne Jahnke</i> <sup>*(1)</sup> , <i>Corinna Kurbis</i> <sup>(1)</sup> , <i>Nicolas Martin</i> <sup>(1)</sup> , <i>Kerstin Weidner</i> <sup>(1)</sup> , <i>Marvin Spieker</i> <sup>(1)</sup> , <i>Anja Lüth</i> <sup>(1)</sup> .....	431
<b>K29</b>	<b>ANALYTICAL STRATEGY FOR THE DETERMINATION OF CAROTENOIDS AND THEIR FORMS IN FEED AND ANIMAL PRODUCTS</b>	
	<i>Aliaksandra Kharoshka</i> <sup>*(1)</sup> , <i>Kamila Mimrova</i> <sup>(1)</sup> , <i>Jana Hajslova</i> <sup>(1)</sup> , <i>Vera Schulzova</i> <sup>(1)</sup> .....	432
<b>K30</b>	<b>ANALYTICAL ASSESSMENT AND HEALTH CLAIMS STRENGTHENING IN GREEK OLIVE OIL: STUDYING THE INFLUENCE OF OLIVE PITTING ON QUALITY PARAMETERS OF THE PRODUCED OLIVE OIL</b>	
	<i>Panagiotis Loukas Gialouris</i> <sup>(1)</sup> , <i>Sofia Drakopoulou</i> <sup>(1)</sup> , <i>Eleni Nastou</i> <sup>(1)</sup> , <i>Anastasia Koupa</i> <sup>(1)</sup> , <i>Georgia Sultani</i> <sup>(1)</sup> , <i>Nikolaos Thomaidis</i> <sup>*(1)</sup> .....	433

# CONTENT

<b>K31</b> <b>COMPARISON OF VARIOUS ANALYTICAL METHODS FOR MONITORING OF CHANGES IN THE CHEMICAL COMPOSITION OF SELECTED PLANT OILS DURING LONG TERM STORAGE</b> <i>Chi Mai Nguyenova</i> <sup>(1)</sup> , Marek Dolezal <sup>(1)</sup> , Jan Poustka <sup>(1)</sup> .....	434
<b>K32</b> <b>VOLATILE COMPOUNDS, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF LACTIC ACID BACTERIA-BIOCONVERTED GRAPE BY-PRODUCT (VITIS LABRUSCA B.)</b> <i>Geonhak Lee</i> <sup>(1)</sup> , Kwang-Geun Lee <sup>(1)</sup> .....	435
<b>K33</b> <b>INNOVATIVE AMINO ACID ANALYSIS: ACCURATE AND ROBUST UNDERIVATIZED AMINO ACIDS ANALYSIS IN FOOD AND FEED USING HYPERCARB COLUMN COMBINED WITH UNIQUE TANDEM LC CONFIGURATION FOR INCREASED THROUGHPUT</b> <i>Valerie Thibert</i> <sup>(1)</sup> , Maryline Carvalho <sup>(1)</sup> , Serdar Bilgesoy <sup>(2)</sup> , Andreas Becker <sup>(3)</sup> .....	436
<b>K34</b> <b>PFAS ANALYSIS IN FOOD USING AN AUTOMATED SOLVENT EXTRACTION SYSTEM AND THE LATEST LC-TQ</b> <i>Day Powell</i> <sup>(1)</sup> .....	437
<b>K35</b> <b>5-HMF QUANTIFICATION AND VOLATILE PROFILING IN HONEY BY VAC-HS-SPME-GC×GC-MS</b> Damien Eggermont <sup>(1)</sup> , Francesca Pardi <sup>(2)</sup> , <i>Giorgia Purcaro</i> <sup>(1)</sup> .....	438
<b>K36</b> <b>BIOACTIVE COMPOUNDS OF GREEK PROPOLIS: A QUALITATIVE AND QUANTITATIVE INVESTIGATION EXPLOITING UHPLC-VIP-HESI-TIMS-QTOF-MS</b> <i>Eleni Nastou</i> <sup>(1)</sup> , Dafni Preza-Mayo-Kataki <sup>(1)</sup> , Panagiotis Loukas Gialouris <sup>(1)</sup> , Nikolaos Thomaidis <sup>(1)</sup> .....	439
<b>K37</b> <b>PHYSICAL CHARACTERIZATION OF NOVEL OLIVE POWDER</b> <i>Melis Cetin Karasu</i> <sup>(1)</sup> , Mecit Halil Oztop <sup>(1)</sup> , Behic Mert <sup>(1)</sup> .....	440
<b>K38</b> <b>UNDERSTANDING TEMPERATURE-DEPENDENT CAKING MECHANISMS IN POWDERED DAIRY PRODUCTS USING TD-NMR RELAXOMETRY</b> Ozan Tas <sup>(1)</sup> , Esmanur Ilhan <sup>(1)</sup> , <i>Melis Cetin Karasu</i> <sup>(1)</sup> , Selen Guner San <sup>(1)</sup> , Mecit Halil Oztop <sup>(1)</sup> .....	441
<b>K39</b> <b>CHARACTERIZATION OF ANTHOCYANIN GLYCOSIDES FROM BERRIES CULTIVATED IN KOREA BASED ON HIGH RESOLUTION MASS SPECTROMETRY (UPLC-DAD-QTOF/MS)</b> <i>Heon-Woong Kim</i> <sup>(1)</sup> , Ryeong Ha Kwon <sup>(1)</sup> , Hyemin Na <sup>(1)</sup> , Ju Hyung Kim <sup>(1)</sup> , So Ah Kim <sup>(1)</sup> , Eun-Suk Jung <sup>(1)</sup> , Hyo Jeong Hwang <sup>(1)</sup> , Chi-Do Wee <sup>(1)</sup> , Kwang-Sik Lee <sup>(1)</sup> , Young-Hee Park <sup>(1)</sup> .....	442
<b>K40</b> <b>MASS SPECTROMETRY IN THE FACTORY: AN ASSESSMENT OF RECENT DIELECTRIC BARRIER DISCHARGE IONISATION (DBDI) MASS SPECTROMETRY APPLICATIONS WITH IN-FACTORY ANALYSIS POTENTIAL</b> Nurmeen Adil <sup>(1)</sup> , Natasha Logan <sup>(1)</sup> , Simon Haughey <sup>(1)</sup> , Chris Elliott <sup>(1)</sup> , <i>Nicholas Birse</i> <sup>(1)</sup> .....	443
<b>K41</b> <b>THE EFFECT OF SELECTED ADDITIONS ON THE COMPOSITION OF FATTY ACIDS IN COTTAGE CHEESE MADE FROM GOAT'S MILK</b> <i>Beata Paszczyk</i> <sup>(1)</sup> , Joanna Klepacka <sup>(1)</sup> , Marta Czarnowska-Kujawska <sup>(1)</sup> .....	444
<b>K42</b> <b>MONITORING ASPARAGINE CONTENT IN CEREALS: AN APPROACH TO REDUCE ACRYLAMIDE FORMATION IN BAKED GOODS</b> <i>Hana Pirova</i> <sup>(1)</sup> , Lucie Stara <sup>(1)</sup> , Beverly Hradecka <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	445
<b>K43</b> <b>FROM BEANS TO BREWING: ENHANCING COFFEE QUALITY THROUGH DYNAMIC IMAGE ANALYSIS AND ZETA POTENTIAL MEASUREMENTS</b> <i>Henrique Brasil</i> <sup>(1)</sup> , Franz Miller Branco Ferraz <sup>(1)</sup> .....	446
<b>K44</b> <b>UNLOCKING THE POTENTIAL OF MOROCCAN VIRGIN OLIVE OILS FOR A POSSIBLE RECOGNITION BY THE EUROPEAN UNION GEOGRAPHICAL INDICATION THROUGH COMPREHENSIVE ANALYTICAL CHARACTERIZATION OF THEIR QUALITY AND COMPOSITION</b> Sofia Zantedeschi <sup>(1)</sup> , Matilde Tura <sup>(2)</sup> , <i>Enrico Valli</i> <sup>(2)</sup> , Sara Barbieri <sup>(1)</sup> , Alessandra Bendini <sup>(2)</sup> , Nouredine Mokhtari <sup>(3)</sup> , Fatima Zahrae El-Mossaid <sup>(4)</sup> , Aadil Bajoub <sup>(4)</sup> , Abdelaziz Ait Elkassia <sup>(3)</sup> , Erika Ramanzini <sup>(5)</sup> , Ali Abidar <sup>(3)</sup> , Abdeslam Oughad <sup>(6)</sup> , Naima Basidi <sup>(5)</sup> , Marco Setti <sup>(1)</sup> , Tullia Gallina Toschi <sup>(2)</sup> .....	447

# CONTENT

<b>K45</b>	
<b>INFLUENCE OF TIME AND PRESSURE IN THE CHARACTERIZATION AND DEVELOPING OF PUFFED RICE</b>	
<u>Madalina-Oana Marincaș</u> <sup>(1)</sup> , Romina Marc <sup>(1)</sup> , Crina Muresan <sup>(1)</sup> .....	448
<b>K46</b>	
<b>NATURAL PREFERMENTS: ENHANCING QUALITY AND SUSTAINABILITY IN BAKING</b>	
<u>Maria-Luissa Maino</u> <sup>(1)</sup> , Romina Marc <sup>(1)</sup> , Crina Muresan <sup>(1)</sup> .....	449
<b>K47</b>	
<b>TRANS FATTY ACIDS CONTENT IN FOOD FOR INFANTS AND YOUNG CHILDREN</b>	
Hanna Mojska <sup>(1)</sup> , <u>Bogumiła Krygier</u> <sup>(1)</sup> , Edyta Jasinska-Melon <sup>(1)</sup> .....	450
<b>K48</b>	
<b>ASSESSING THE IMPLICATION OF SPAD AND NDVI VALUES IN NITROGEN APPLICATION AND GRAIN QUALITY OF SELECTED MAIZE HYBRIDS</b>	
<u>Muhoja Sylivester Nyandi</u> <sup>(1)</sup> , Peter Pepo <sup>(1)</sup> .....	451
<b>K49</b>	
<b>CHARACTERIZATION OF SECONDARY METABOLITES OF DYER'S ALKANET (ALKANNA TINCTORIA) POSSESSING ANTIMICROBIAL ACTIVITY</b>	
<u>Tereza Jaegerova</u> <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> , Hana Sykorova <sup>(2)</sup> .....	452
<b>K50</b>	
<b>ASSESSMENT OF THE PERFORMANCE OF PARTICIPANTS IN CAFFEINE ANALYSIS PROFICIENCY TESTS IN SAUDI FOOD LABORATORIES</b>	
<u>Turki Abuhamrh</u> <sup>(1)</sup> .....	453
<b>K51</b>	
<b>DYNAMIC CHARACTERISATION OF PERMEATION PROCESSES IN POLYMER PACKAGING FILMS</b>	
<u>Andreas Stenzel</u> <sup>(1)</sup> , Jonathan Beauchamp <sup>(2)</sup> , Y Lan Pham <sup>(2)</sup> .....	454
<b>K52</b>	
<b>COMPARATIVE PHARMACOKINETICS OF NANOFORMULATED CBD AND CBDA IN APOE KO MICE: A THREE-MONTH STUDY</b>	
<u>Zuzana Binova</u> <sup>(1)</sup> , Frantisek Benes <sup>(1)</sup> , Matej Maly <sup>(1)</sup> , Petr Kastanek <sup>(2)</sup> , Monika Cahova <sup>(3)</sup> , Milena Stranska <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	455
<b>K53</b>	
<b>OPTIMIZING PROFICIENCY TESTING SAMPLES IN FOOD MICROBIOLOGY</b>	
Loreto Gallego <sup>(1)</sup> , Vicente Catalan <sup>(1)</sup> , Elena Penaranda <sup>(1)</sup> , Ana De Pablo <sup>(1)</sup> , <u>Luis Maria Gallego</u> <sup>(2)</sup> .....	456
<b>K54</b>	
<b>FROM SPELT SEED TO FUNCTIONAL FOOD: IMPROVING BIOACCESSIBILITY OF PHENOLICS THROUGH BIOPROCESSING TECHNIQUES</b>	
<u>Marjeta Mencin</u> <sup>(1)</sup> , Maja Mikulic Petkovsek <sup>(2)</sup> , Petra Terpinč <sup>(2)</sup> .....	457
<b>K55</b>	
<b>EFFECT OF BUCKWHEAT HUSK ADDITION ON SENSORY PROPERTIES OF BREAD</b>	
Wajeעה Mumtaz <sup>(1)</sup> , <u>Marta Czarnowska-Kujawska</u> <sup>(1)</sup> , Joanna Klepacka <sup>(1)</sup> .....	458
<b>K56</b>	
<b>CHANGES IN THE SENSORY PROPERTIES, PHYSICO-CHEMICAL CHARACTERISTICS AND AROMAS OF SMOKED CHEESE IN DIFFERENT RIPENING STAGES</b>	
<u>Anamaria Bolbos</u> <sup>(1)</sup> , Romina Marc <sup>(2)</sup> , Crina Muresan <sup>(2)</sup> .....	459
<b>K57</b>	
<b>PROFILING OF MILK TRIGLYCERIDS BY UHPLC-MS</b>	
<u>Jean-Michel Romnee</u> <sup>(1)</sup> , Mohamed El Morabit <sup>(2)</sup> , Virginie Decruyenaere <sup>(3)</sup> , François Stevens <sup>(2)</sup> .....	460
<b>GREEN FOOD ANALYSIS.....</b>	<b>461</b>
<b>M1</b>	
<b>DES-TINATION: A SUSTAINABLE JOURNEY TO ACCURATE AFLATOXIN ANALYSIS IN PISTACHIOS</b>	
<u>Andrea Schincaglia</u> <sup>(1)</sup> , Alberto Cavazzini <sup>(1)</sup> , Luisa Pasti <sup>(1)</sup> , Giorgia Purcaro <sup>(2)</sup> , Marco Beccaria <sup>(1)</sup> .....	462
<b>M2</b>	
<b>USE OF UNIQUE GC-TOFMS TECHNOLOGY FOR ENHANCED &amp; INCREASED THROUGHPUT SCREENING OF GINGER OILS - METHOD TRANSFER FROM HELIUM TO HYDROGEN CARRIER GASES</b>	
Nick Jones <sup>(1)</sup> , Sebastiano Panto <sup>(1)</sup> , <u>Pavel Jiros</u> <sup>(1)</sup> , Lena Dubois <sup>(1)</sup> .....	463
<b>M3</b>	
<b>RECOVERY OF PROTEINS AND BIOACTIVE COMPOUNDS FROM A TURMERIC CURCUMA LONGA WASTE USING SUBCRITICAL WATER EXTRACTION AND HIGH INTENSITY FOCUSED ULTRASOUNDS. IDENTIFICATION OF COMPOUNDS BY RP-UPLC-ESI-QTOF</b>	
<u>Carlos Rubio Marin</u> <sup>(1)</sup> , Maria Luisa Marina <sup>(1)</sup> , Maria Concepcion Garcia <sup>(1)</sup> .....	464

# CONTENT

## M4

### COMPARISON OF GREEN STRATEGIES FOR MAXIMIZING THE EXTRACTION OF PROTEINS AND COMPOUNDS WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY FROM A GINGER WASTE

Carlos Rubio Marin<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>, Maria Concepcion Garcia<sup>\*(1)</sup> .....465

## M5

### MICROWAVE-ASSISTED EXTRACTION AND SAPONIFICATION TO INCREASE THE THROUGHPUT IN FOOD QUALITY ANALYSIS

Donatella Ferrara<sup>(1)</sup>, Nicola Ruin<sup>(2)</sup>, Marco Beccaria<sup>(2)</sup>, Chiara Emilia Irma Cordero<sup>(3)</sup>, Giorgia Purcaro<sup>\*(4)</sup>.....466

## M6

### PLANT-BASED BEVERAGES IN NUTRITION

Bogumiła Krygier<sup>\*(1)</sup>, Edyta Jasinska-Melon<sup>(1)</sup>, Hanna Mojska<sup>(1)</sup> .....467

## HUMAN BIOMONITORING & EXPOSOMICS ..... 468

### N1

#### MYCOTOXINS IN HUMAN URINE OF ADULTS FOLLOWING HEALTHY AND SUSTAINABLE DIETS

Octavian Augustin Mihalache<sup>\*(1)</sup>, Raquel Torrijos<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>.....469

### N2

#### MINERAL CONTENT IN SICILIAN WOMEN'S BLOOD BY ICP-MS

Irene Maria Spanò<sup>\*(1)</sup>, Caterina Faggio<sup>(2)</sup>, Ambrogina Albergamo<sup>(1)</sup>, Miriam Porretti<sup>(2)</sup>, Giuseppa Di Bella<sup>(1)</sup>.....470

### N3

#### DETERMINATION OF 30 PFAS IN BLOOD SERUM IN A CROSS-SECTIONAL STUDY IN SWITZERLAND AND LINKING THE RESULTS TO EXPOSURE SOURCES

Alexandra Jaus<sup>\*(1)</sup>, Celine Fragniere Rime<sup>(2)</sup>, Julien Riou<sup>(3)</sup>, Murielle Bochud<sup>(3)</sup>, Beat Bruschweiler<sup>(4)</sup>, Natalie von Gotz<sup>(5)</sup>.....471

### N4

#### PFAS IN HUMANS: INSIGHTS FROM HUMAN BIOMONITORING IN THE CZECH REPUBLIC

Darina Dvorakova<sup>\*(1)</sup>, Tomas Gramblicka<sup>(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Veronika Svobodova<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup> .....472

### N5

#### SILICONE WRISTBANDS AS A TOOL FOR ASSESSING HUMAN EXPOSURE TO VARIOUS ENVIRONMENTAL POLLUTANTS

Tomas Gramblicka<sup>\*(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup> .....473

### N6

#### USE OF SILICONE WRISTBANDS TO STUDY DERMAL AND INHALATION EXPOSURE TO CHLORINATED PARAFFINS

Denisa Parizkova<sup>(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Jakub Tomasko<sup>(1)</sup>, Jana Pulkrabova<sup>\*(1)</sup> .....474

### N7

#### STUDY OF THE EFFECT OF ORGANIC DIET THROUGH HUMAN BIOMONITORING OF MYCOTOXINS AND PESTICIDES

Jose A. Gallardo-Ramos<sup>(1)</sup>, Jesus Marín-Sáez<sup>(2)</sup>, Vicente Sanchis<sup>(1)</sup>, Laura Gamiz-Gracia<sup>(3)</sup>, Maykel Hernandez-Mesa<sup>(3)</sup>, German Cano-Sancho<sup>(4)</sup>, Ana M. Garcia-Campana<sup>\*(3)</sup> .....475

### N8

#### TRANS FATTY ACIDS IN BREAST MILK AS AN INDICATOR OF DIETARY EXPOSURE TO THESE COMPOUNDS

Edyta Jasinska-Melon<sup>\*(1)</sup>, Hanna Mojska<sup>(1)</sup> .....476

## MAJOR NUTRIENTS & VITAMINS ..... 477

### O1

#### APPLICATION OF FT-NIR SPECTROSCOPY COUPLED WITH CHEMOMETRICS FOR RAPID QUANTIFICATION OF MACRONUTRIENTS IN CEREAL-BASED BABY FOOD

Alexandra Nanou<sup>\*(1)</sup>, Sofia Koutroumpí<sup>(1)</sup>, Evangelia Krystalli<sup>(1)</sup>.....478

### O2

#### DEVELOPMENT OF A NEW ECO-FRIENDLY ULTRASOUND-ASSISTED EXTRACTION METHOD TO QUANTIFY TRYPTOPHAN IN WILD MUSHROOMS AND DETERMINATION OF ITS BENEFICIAL PROPERTIES

Alejandro R. Lopez<sup>\*(1)</sup>, Elena Ortega-Caneda<sup>(1)</sup>, Estrella Espada-Bellido<sup>(1)</sup>, Nuria Chinchilla<sup>(1)</sup>, Miguel Palma<sup>(1)</sup>, Maria Jose Aliano-Gonzalez<sup>(1)</sup>, Gerardo F. Barbero<sup>(1)</sup>, Ceferino Carrera<sup>(1)</sup>.....479

### O3

#### EVALUATION OF THE MINERAL COMPOSITION OF YERBA MATE (ILEX PARAGUARIENSIS)

Justyna Osko<sup>\*(1)</sup>, Aleksandra Bojarowska<sup>(1)</sup>, Kornelia Kadac-Czapska<sup>(1)</sup>, Małgorzata Grembecka<sup>(1)</sup>.....480

# CONTENT

## O4

### FATTY ACID PROFILE OF CLAMS FROM SICILIAN TRANSITIONAL WATER ZONES

Salvatore Giacobbe<sup>(1)</sup>, Benedetta Sgro<sup>\*(2)</sup>, Federica Litrenta<sup>(3)</sup>, Giuseppa Di Bella<sup>(3)</sup>, Vincenzo Lo Turco<sup>(3)</sup> .....481

## O5

### SYNBIOTIC PROPERTIES OF BREAST MILK

Katarzyna Kubicz<sup>\*(1)</sup>, Magdalena Twaruzek<sup>(1)</sup>, Elena Sinkiewicz-Darol<sup>(2)</sup>, Dorota Martysiak-Zurowska<sup>(3)</sup>, Barbara Kusznierevicz<sup>(3)</sup> .....482

## O6

### CHANGES IN THE FOLATE CONTENT OF KOMBUCHAS DERIVED FROM MILK AND PLANT DRINKS

Marta Czarnowska-Kujawska<sup>\*(1)</sup>, Joanna Klepacka<sup>(1)</sup>, Beata Paszczyk<sup>(1)</sup> .....483

## O7

### THE INFLUENCE OF BUCKWHEAT HUSK ADDITION ON THE ANTIOXIDANT PROPERTIES OF BREAD

Joanna Klepacka<sup>\*(1)</sup>, Wajeeha Mumtaz<sup>(1)</sup>, Marta Czarnowska-Kujawska<sup>(1)</sup> .....484

## O8

### THE IMPACT OF INDUSTRIAL ROASTING OF COFFEE BEANS ON THE LEVEL OF PHENOLIC COMPOUNDS

Joanna Klepacka<sup>\*(1)</sup>, Marta Czarnowska-Kujawska<sup>(1)</sup>, Beata Paszczyk<sup>(1)</sup> .....485

## O9

### OPTIMISATION OF SAMPLE PREPARATION PROCEDURE FOR THE DETERMINATION OF FOLATE IN VEGETABLES

Lenka Libenska<sup>\*(1)</sup>, Jana Pulkrabova<sup>(1)</sup>, Lucie Drabova<sup>(1)</sup> .....486

## O10

### EVALUATION OF THE VITAMIN (A, D AND E) STATUS OF CZECH MOTHERS AND THEIR NEWBORNS: DIFFERENCES BETWEEN PRE-TERM AND FULL-TERM LABORS (PILOT STUDY)

Veronika Gomersall<sup>(1)</sup>, Lenka Libenska<sup>(1)</sup>, Radim J. Sram<sup>(2)</sup>, Jiri Dusek<sup>(3)</sup>, Jana Pulkrabova<sup>(1)</sup>, Lucie Drabova<sup>\*(1)</sup> .....487

## O11

### UHPLC-Q-ORBITRAP-MS-BASED LIPIDOMIC STUDY OF SWINE SAUSAGE PRODUCED BY ADDING NATURAL INGREDIENTS TO REPLACE NITRITE AND NITRATE ADDITION

Annalisa Mentana<sup>(1)</sup>, Andrea Chiappinelli<sup>(1)</sup>, Maria Campaniello<sup>(1)</sup>, Rosalia Zianni<sup>(1)</sup>, Giovanna Berardi<sup>(1)</sup>, Marco Langianese<sup>(1)</sup>, Valeria Nardelli<sup>(1)</sup>, Marco Iammarino<sup>\*(1)</sup> .....488

## O12

### ENHANCED VITAMIN ANALYSIS: THE POWER OF IMMUNOAFFINITY CLEAN-UP AND AUTOMATION

Lochlann Walford<sup>(1)</sup>, Naomi McLachlan<sup>(1)</sup>, Carol Donnelly<sup>(1)</sup>, Claire Milligan<sup>(1)</sup>, Elizabeth Mannaing<sup>\*(1)</sup> .....489

## O13

### HEPARIN AFFINITY COLUMN: ANALYSIS OF LACTOFERRIN IN INFANT FORMULA

Stobhan Kelly<sup>(1)</sup>, Helen Cameron<sup>(1)</sup>, Carol Donnelly<sup>\*(1)</sup>, Claire Milligan<sup>(1)</sup> .....490

## METALS AND METALLOIDS & SPECIATION ..... 491

### P1

#### FRONTAL CHROMATOGRAPHY-ICP-MS FOR ANTIMONY SPECIATION IN PET ADDITIVES

Alejandro R. Lopez<sup>\*(1)</sup>, Davide Spanu<sup>(2)</sup>, Damiano Monticelli<sup>(2)</sup>, Sandro Recchia<sup>(2)</sup>, Gilberto Binda<sup>(2)</sup>, Gianluca Roncoroni<sup>(2)</sup>..492

### P2

#### RAPID AND SENSITIVE DETERMINATION OF METHYLMERCURY IN FISH AND HUMAN HAIR FOR COMPREHENSIVE BIOMONITORING

Davide Spanu<sup>\*(1)</sup>, Sandro Recchia<sup>(1)</sup>, Damiano Monticelli<sup>(1)</sup> .....493

### P3

#### ELEMENTAL PROFILING IN BRAZILIAN ARTISAN CHOCOLATES CONTAINING VARYING LEVELS OF COCOA

Giovanna Santana<sup>(1)</sup>, Romulo Silveira Rodrigues<sup>(2)</sup>, Geisamanda Pedrini Brandão<sup>(1)</sup>, Vinicius Câmara Costa<sup>(2)</sup>, Maria Tereza Weitzel Dias Carneiro<sup>\*(2)</sup> .....494

### P4

#### EXTRACTION OF IMPURITIES FROM AMORPHOUS SILICON DIOXIDE BY DILUTED HYDROCHLORIC ACID

Daniela Montalvo<sup>\*(1)</sup>, Loic Bandinelli<sup>(1)</sup>, Noa Olluin<sup>(1)</sup>, Jan Mast<sup>(1)</sup>, Karlien Cheyys<sup>(1)</sup> .....495

### P5

#### DETERMINATION OF ESSENTIAL METALS (FE, MG, K, CA, AND NA) IN CULTIVATED AND WILD EDIBLE MUSHROOMS FROM THE SOUTH OF SPAIN AND NORTH OF MOROCCO

Oscar R. Taracena-Zepeda<sup>(1)</sup>, Alejandro Ruiz-Lopez<sup>(1)</sup>, Elena Ortega-Caneda<sup>(1)</sup>, Estrella Espada-Bellido<sup>\*(1)</sup>, Miguel Palma<sup>(1)</sup>, Gerardo Fernandez-Barbero<sup>(1)</sup> .....496

### P6

#### SPECIATION OF INORGANIC ARSENIC AND METHYL MERCURY IN FOODSTUFFS USING LC-ICP-MS

Ben Watkin<sup>\*(1)</sup> .....497

# CONTENT

<b>P7</b>	
<b>ELEMENTAL COMPOSITION OF CZECH AND SLOVAK HONEY SAMPLES</b>	
<u>Anna Simankova</u> <sup>*(1)</sup> , <u>Zuzana Prochazkova</u> <sup>(1)</sup> , <u>Lucie Drabova</u> <sup>(1)</sup> , <u>Richard Koplik</u> <sup>(1)</sup> .....	498
<b>P8</b>	
<b>SURVEY OF LEAD AND OTHER TRACE ELEMENTS IN DRIED SPICES SOURCED FROM THE DANISH MARKET</b>	
<u>Lou Estines</u> <sup>(1)</sup> , <u>Dooa Diaaeldin Ahmed M Abouelenein</u> <sup>(2)</sup> , <u>Heidi Amlund</u> <sup>*(2)</sup> .....	499
<b>P9</b>	
<b>TRACE ELEMENT ANALYSIS OF GOURMET AND REFINED TABLE SALTS BY ICP-OES AND ICP-MS</b>	
<u>Paulin Moully</u> <sup>(1)</sup> , <u>Daniel Clayton-Cuch</u> <sup>(2)</sup> , <u>Heidi Amlund</u> <sup>*(2)</sup> .....	500
<b>P10</b>	
<b>PROFILING THE ELEMENTAL COMPOSITION OF WHISKY USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)</b>	
<u>Matthew Cassap</u> <sup>*(1)</sup> , <u>Bhagyesh Surekar</u> <sup>(1)</sup> , <u>Daniel Kutscher</u> <sup>(1)</sup> .....	501
<b>P11</b>	
<b>TRACE METALS ANALYSIS IN BABY FOOD USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)</b>	
<u>Matthew Cassap</u> <sup>*(1)</sup> , <u>Laurent Naels</u> <sup>(1)</sup> .....	502
<b>MIGRANTS FROM FOOD CONTACT MATERIALS ..... 503</b>	
<b>Q1</b>	
<b>INVISIBLE THREAT – MICROPLASTICS RELEASE FROM FEEDING BOTTLES</b>	
<u>Kornelia Kadac-Czapska</u> <sup>*(1)</sup> , <u>Beata Bochentyn</u> <sup>(2)</sup> , <u>Karolina Jucewicz</u> <sup>(1)</sup> , <u>Sebastian Mahlik</u> <sup>(3)</sup> , <u>Małgorzata Grembecka</u> <sup>(1)</sup> .....	504
<b>Q2</b>	
<b>MIGRATION OF PLASTICISERS FROM LID GASKETS INTO FATTY FOODS: A CONTINUING CONTAMINATION ISSUE</b>	
<u>Christoph Czerwenka</u> <sup>*(1)</sup> , <u>Daniela Reil</u> <sup>(1)</sup> , <u>Bettina Schmolka</u> <sup>(1)</sup> .....	505
<b>Q3</b>	
<b>ALTERNATIVE PLASTIC ADDITIVES IN A SPANISH GROCERY BASKET: ARE UNPACKED FOODSTUFFS LESS HARMFUL?</b>	
<u>Julio Fernandez-Arribas</u> <sup>*(1)</sup> , <u>Teresa Moreno</u> <sup>(1)</sup> , <u>Ethel Eljarrat</u> <sup>(1)</sup> .....	506
<b>Q4</b>	
<b>ANALYSIS OF POLYETHYLENE TEREPHTHALATE OLIGOMERS IN PLANT-BASED MEAT ALTERNATIVES</b>	
<u>Elнора Abdullajeva</u> <sup>(1)</sup> , <u>Bina Bhattarai</u> <sup>(1)</sup> , <u>Elena Hakme</u> <sup>(1)</sup> , <u>Lene Duedahl-Olesen</u> <sup>*(1)</sup> .....	507
<b>Q5</b>	
<b>SUSPECT SCREENING ANALYSIS OF CONTAMINANTS IN FOOD CONTACT MATERIALS USING SUPRAMOLECULAR SOLVENTS</b>	
<u>Laura Garcia-Cansino</u> <sup>(1)</sup> , <u>Noelia Caballero-Casero</u> <sup>*(2)</sup> , <u>Maria Angeles Garcia</u> <sup>(1)</sup> , <u>Maria Luisa Marina</u> <sup>(1)</sup> , <u>Soledad Rubio</u> <sup>(2)</sup> .....	508
<b>Q6</b>	
<b>BISPHENOLS IN FOOD AND FOOD CONTACT MATERIALS: TWO-YEAR-OBSERVATION AFTER THE RISK ASSESSMENT REVISION BY EFSA</b>	
<u>Thomas Behnke</u> <sup>*(1)</sup> , <u>Jörg Konetzki</u> <sup>(1)</sup> .....	509
<b>Q7</b>	
<b>SENSITIVE QUECHERS-BASED METHOD FOR THE ANALYSIS OF STYRENE IN FOOD</b>	
<u>Florian Peltier</u> <sup>*(1)</sup> , <u>Iris Wynn</u> <sup>(1)</sup> , <u>Els Van Hoeck</u> <sup>(1)</sup> , <u>Mélanie Di Mario</u> <sup>(1)</sup> .....	510
<b>Q8</b>	
<b>TOWARDS SUSTAINABLE FOOD PACKAGING: ENSURING FOOD SAFETY IN POST-CONSUMER RECYCLED PLASTIC MATERIALS</b>	
<u>Andrea Hochegger</u> <sup>*(1)</sup> , <u>Erich Leitner</u> <sup>(1)</sup> .....	511
<b>Q9</b>	
<b>ELEMENT RELEASE FROM LEAD CRYSTAL WARE AND METALLIC HIP FLASKS</b>	
<u>Oliver Kappenstein</u> <sup>(1)</sup> , <u>Sarah von Leliwa</u> <sup>*(1)</sup> , <u>Thomas Tietz</u> <sup>(1)</sup> , <u>Stefan Merkel</u> <sup>(1)</sup> , <u>Andreas Luch</u> <sup>(1)</sup> , <u>Roman Schmidt</u> <sup>(1)</sup> .....	512
<b>Q10</b>	
<b>FRENCH REGULATION FOR PRINTING INKS. IS THE 1 PPM LIMIT FOR POLYAROMATIC MOAH ANALYTICALLY FEASIBLE? A CASE STUDY</b>	
<u>Michael Koch</u> <sup>(1)</sup> , <u>Eileen Schulz</u> <sup>*(1)</sup> , <u>Agnes Buczkowski</u> <sup>(1)</sup> , <u>Thomas Behnke</u> <sup>(1)</sup> , <u>Erik Becker</u> <sup>(1)</sup> .....	513

# CONTENT

## Q11

### A COMPREHENSIVE ANALYTICAL WORKFLOW FOR SAFETY ASSESSMENT: IDENTIFICATION OF MIGRATING SUBSTANCES FROM PBS/PLA BIODEGRADABLE PACKAGING FOR FRESH-CUT PRODUCE

Jahan Zaib Ashraf<sup>(1)</sup>, Emmanouil Tsochatzis<sup>(1)</sup>, Sandra Pati<sup>(2)</sup>, Maria Luisa Amodio<sup>(2)</sup>, Annalisa Apicella<sup>(3)</sup>, Loredana Incarnato<sup>(3)</sup>, Mario M. Martinez<sup>\* (1)</sup> .....514

## Q12

### MICROWAVE-ASSISTED EXTRACTION AND UHPLC-QTRAP®-MS/MS ANALYSIS OF PLASTICISERS IN VARIOUS CHEESES

Aurelie Bugey<sup>\* (1)</sup>, Doriane Santimaria<sup>(1)</sup>, Fabrice Gillerat<sup>(1)</sup>, Ngoc-Huy Ho<sup>(1)</sup>, Didier Ortelli<sup>(1)</sup>, Patrick Edde<sup>(1)</sup> .....515

## Q13

### TRACE ANALYSIS OF BISPHENOL A IN CANNED FOOD BY DI-SPME-ARROW-GC-MS/MS

Deborah Bertuola<sup>\* (1)</sup>, Anna Romanotto<sup>(1)</sup> .....516

## Q14

### COMBINED QUALITATIVE AND QUANTITATIVE ANALYSIS OF FOOD PACKAGING MATERIALS USING QTOF MASS SPECTROMETRY

Daqiang Pan<sup>\* (1)</sup>, Clemens Bidmon<sup>(2)</sup>, Jianru Stahl-Zeng<sup>(1)</sup>, Andre Schreiber<sup>(1)</sup>, Gitte Barknowitz<sup>(1)</sup> .....517

## Q15

### BIO-BASED DISHES: SUSTAINABLE SOLUTION OR HIDDEN HEALTH RISK?

Kamila Bechynska<sup>(1)</sup>, Vit Kosek<sup>(1)</sup>, Leos Uttl<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>, Emma Bradley<sup>(2)</sup>, Claire McKillen<sup>(2)</sup>, Monika Tomaniova<sup>(1)</sup>, Jana Hajslova<sup>\* (1)</sup> .....518

## Q16

### MIGRATION TESTING OF CROTONIC ACID FROM POLYHYDROXYBUTYRATE BASED FOOD CONTACT MATERIALS BY GC-MS/MS

Uwe Oppermann<sup>(1)</sup>, Franky Puype<sup>\* (2)</sup> .....519

## Q17

### ADVANCED SEPARATION OF MINERAL OIL AROMATIC HYDROCARBONS BY NUMBER OF AROMATIC RINGS USING DONOR-ACCEPTOR-COMPLEX CHROMATOGRAPHY TO EXTEND ON-LINE COUPLED LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY

Martin Eckardt<sup>\* (1)</sup>, Martin Lommatzsch<sup>(1)</sup>, Johanna Holzapfel<sup>(2)</sup>, Sebastian Säger<sup>(1)</sup>, Thomas Simat<sup>(2)</sup> .....520

## Q18

### MIGRATION OF CYCLIC SILOXANE OLIGOMERS INTO FOOD - DETERMINATION OF D4 TO D25 VIA ONLINE COUPLED HPLC-GC-FID

Martin Eckardt<sup>\* (1)</sup>, Sebastian Säger<sup>(1)</sup>, Ann-Kathrin Seidel<sup>(2)</sup>, Thomas Simat<sup>(2)</sup> .....521

## (MULTI)OMICS IN FOOD ANALYSIS ..... 522

### R1

#### VOLATOLOMICS TO INVESTIGATE THE RESPONSE OF LISTERIA MONOCYTOGENES TO PH CHANGES IN ITS CULTURE MEDIUM

Aya Fakh<sup>\* (1)</sup>, Jeremy Ratel<sup>(1)</sup>, Dimitra Tsourekis<sup>(2)</sup>, Frederic Mercier<sup>(3)</sup>, Nathalie Kondjoyan<sup>(1)</sup>, Nelly Caccia<sup>(4)</sup>, Cristian Botta<sup>(2)</sup>, Kalliopi Rantsiou<sup>(2)</sup>, Erwan Engel<sup>(1)</sup> .....523

### R2

#### APPLICATION OF NMR SPECTROSCOPY TO STUDY THE EFFECT OF MALTING PROCESS ON LEGUME SEEDS

Mattia Spano<sup>\* (1)</sup>, Alessio Cimini<sup>(2)</sup>, Anna Maria Giusti<sup>(3)</sup>, Lorenzo Maria Donini<sup>(3)</sup>, Mauro Moresi<sup>(2)</sup>, Luisa Mannina<sup>(1)</sup> .....524

### R3

#### NONTARGETED DIRECT HEADSPACE GC-IMS OF MAMMALIAN CELL CULTURES

Richard Frey<sup>\* (1)</sup>, Sarah Winkler<sup>(1)</sup>, Rüdiger Rufolf<sup>(2)</sup>, Philipp Weller<sup>(3)</sup> .....525

### R4

#### CHEMICAL CHARACTERIZATION OF HEMP SEED (CANNABIS SATIVA L.) POLAR METABOLITES THROUGH LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY

Lorenzo Del Vecchio<sup>\* (1)</sup>, Guillem Campmajo<sup>(2)</sup>, Vita Maria Cristiana Moliterni<sup>(3)</sup>, Chiara Dall'Asta<sup>(2)</sup>, Gianni Galaverna<sup>(1)</sup>, Martina Cirlini<sup>(1)</sup> .....526

### R5

#### CHEMICAL CHARACTERIZATION BY LC-HR-MS/MS OF AN INNOVATIVE AND ENRICHED TARALLO PUGLIESE FORTIFIED WITH LEGUME END-PRODUCTS

Antonella Lamonaca<sup>(1)</sup>, Federica De Bellis<sup>(1)</sup>, Anna Luparelli<sup>(1)</sup>, Leonardo Caputo<sup>(1)</sup>, Laura Quintieri<sup>(1)</sup>, Rosa Pilolli<sup>(1)</sup>, Elisabetta De Angelis<sup>(1)</sup>, Linda Monaci<sup>\* (1)</sup> .....527

# CONTENT

## R6

### COMPUTER VISION HIGHLIGHTS QUALITY TRAITS IN ARTISANAL CHEESE: INVESTIGATING VALCASOTTO CHEESE BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND QUANTITATIVE VOLATILOMICS

Fulvia Trapani<sup>(1)</sup>, Andrea Caratti<sup>(1)</sup>, Angelica Fina<sup>(1)</sup>, Erica Liberto<sup>(1)</sup>, Francesco Ferrero<sup>(2)</sup>, Giorgio Borreani<sup>(2)</sup>, Fabio Bruno<sup>(3)</sup>, Daniele Giacccone<sup>(4)</sup>, Andrea Revello Chion<sup>(4)</sup>, Chiara Cordero<sup>\*(1)</sup>.....528

## R7

### ADVANCING FECAL VOLATILOME PROFILING BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (GC×GC-TOF MS) AND IMAGE PATTERN RECOGNITION

Fulvia Trapani<sup>(1)</sup>, Andrea Caratti<sup>(1)</sup>, Erica Liberto<sup>(1)</sup>, Ilario Ferrocino<sup>(2)</sup>, Luca Coccolin<sup>(2)</sup>, Ilaria Goitre<sup>(2)</sup>, Valentina Ponzio<sup>(2)</sup>, Simona Bo<sup>(3)</sup>, Chiara Cordero<sup>\*(1)</sup>.....529

## R8

### FOOD METABOLOMICS SUPPORTS INDUSTRIAL QUALITY RESEARCH: UNREVEALING COMPOSITIONAL CHANGES IN GERMINATED PEANUTS BY MULTIDIMENSIONAL GAS CHROMATOGRAPHY PLATFORMS

Angelica Fina<sup>(1)</sup>, Andrea Caratti<sup>(2)</sup>, Fulvia Trapani<sup>(1)</sup>, Erica Liberto<sup>(2)</sup>, Irene Cincera<sup>(3)</sup>, Giuseppe Genova<sup>(3)</sup>, Cristina Casetta<sup>(3)</sup>, Carlo Bicchi<sup>(1)</sup>, Chiara Cordero<sup>\*(2)</sup>.....530

## R9

### NON-TARGETED FINGERPRINTING OF THE VOLATILE AND NON-VOLATILE FRACTION OF SICILIAN COFFEA ARABICA L. LEAVES

Davide Rovelli<sup>\*(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Raquel Torrijos<sup>(2)</sup>, Martina Cirilini<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>.....531

## R10

### EXPLORING THE PROTEIN PROFILE OF COFFEE LEAF INFUSIONS: A STUDY ON COFFEA ARABICA L. LEAVES

Davide Rovelli<sup>\*(1)</sup>, Sara Cutroneo<sup>(1)</sup>, Tullia Tedeschi<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>.....532

## MYCOTOXINS, MARINE & PLANT TOXINS ..... 533

### S1

#### DETECTION AND MOLECULAR CHARACTERIZATION OF AFLATOXIN AND OCHRATOXIN PRODUCE ASPERGILLUS SPECIES IN CAPSICUM SPICES IN SAUDI ARABIA

Abdulmohsen Aljardani<sup>\*(1)</sup>.....534

### S2

#### ADVANCES IN MONITORING-CENTERED QUANTIFICATION OF MICROCYSTINS IN FISH MATRIX USING UHPLC-MS/MS

Wannes Hugo R. Van Hassel<sup>\*(1)</sup>, Camille Van Camp<sup>(1)</sup>, Jeroen Van Wichelen<sup>(2)</sup>, Julien Masquelier<sup>(1)</sup>.....535

### S3

#### DETERMINATION OF ERGOT ALKALOIDS: COMPARISON OF EXTRACTION EFFICIENCIES IN SEVERAL CEREAL PRODUCTION CHAINS BY UPLC-MS/MS

Eleonora Rollo<sup>(1)</sup>, Dante Cattellani<sup>(2)</sup>, Chiara Dall'Asta<sup>(3)</sup>, Nicola Dreolin<sup>(4)</sup>, Michele Suman<sup>\*(5)</sup>.....536

### S4

#### READY, SET, DETECT: SIMPLIFYING MYCOTOXIN ANALYSIS FOR THE FOOD INDUSTRY

Peter Carl<sup>\*(1)</sup>, Timm Schwaar<sup>(1)</sup>.....537

### S5

#### QUALITY ASSESSMENT AND REGULATORY IMPLICATIONS OF LIQUID MYCOTOXIN REFERENCE STANDARDS UNDER ISO 17034: ENSURING RELIABILITY AND CONSISTENCY

David Steiner<sup>\*(1)</sup>, Tibor Barók<sup>(2)</sup>, Michael Sulyok<sup>(3)</sup>, Andras Szekeres<sup>(4)</sup>, Monika Varga<sup>(4)</sup>, Levente Horvath<sup>(2)</sup>, Helmut Rost<sup>(1)</sup>...538

### S6

#### TARGET AND SUSPECT SCREENING ANALYSIS, OCCURRENCE AND RISK ASSESSMENT OF PYRROLIZINE ALKALOIDS IN HONEY SAMPLES FROM DIFFERENT FLORAL ORIGINS

Laura Carbonell-Rozas<sup>\*(1)</sup>, Paula Soto-Rosina<sup>(1)</sup>, Jose Raul Belmonte-Sanchez<sup>(1)</sup>, Roberto Romero-González<sup>(1)</sup>, Antonia Garrido Frenich<sup>(1)</sup>.....539

### S7

#### EFFECT OF AFLASAFE® TZ01 IN AFLATOXINS REDUCTION AND ITS EMERGING CHALLENGES WITH FUSARIUM MYCOTOXINS IN MAIZE

Sambwe Fundikira<sup>\*(1)</sup>, Sarah De Saeger<sup>(1)</sup>, Rashid Suleiman<sup>(2)</sup>, Marthe De Boevre<sup>(1)</sup>, Kokeb Tesfamariam<sup>(1)</sup>, Martin Kimanya<sup>(3)</sup>.....540

### S8

#### IN VITRO SYSTEM TO EXPLORE THE ABILITY OF NEW PERENNIAL GRAINS TO BIOTRANSFORM DEOXYNIVALENOL

Marco Gozzi<sup>\*(1)</sup>, Maria Cavallero<sup>(2)</sup>, Enrico Rolli<sup>(2)</sup>, Chiara Dall'Asta<sup>(1)</sup>, Laura Gazza<sup>(3)</sup>, Gianni Galaverna<sup>(1)</sup>.....541



# CONTENT

<b>S9</b>	<b>OPTIMIZATION AND VALIDATION OF A MULTI-MYCOTOXIN ANALYTICAL METHOD IN PLANT-BASED BEVERAGES APPLYING SALLE EXTRACTION COMBINED WITH UHPLC-MS/MS</b>	
	<i>Raquel Torrijos</i> <sup>(1)</sup> , <i>Octavian Augustin Mihalache</i> <sup>(2)</sup> , <i>Chiara Dall'Asta</i> <sup>(2)</sup> .....	542
<b>S10</b>	<b>SYNTHESIS AND APPLICATION OF STABLE ISOTOPICALLY LABELED ERGOT ALKALOIDS</b>	
	<i>Sven-Oliver Herter</i> <sup>(1)</sup> , <i>Hajo Haase</i> <sup>(2)</sup> , <i>Matthias Koch</i> <sup>(1)</sup> .....	543
<b>S11</b>	<b>STUDY OF THE OPTIMIZED CHEMICAL HYDROLYSIS CONDITIONS TO CONVERT GC4 AND GC5 INTO THEIR DECARBAMOYL ANALOGUES DCGTX1 AND DCGTX4</b>	
	<i>Veronica Rey</i> <sup>(1)</sup> , <i>Ana Botana</i> <sup>(1)</sup> , <i>Luis Botana</i> <sup>(1)</sup> .....	544
<b>S12</b>	<b>VALIDATION OF MULTI-ANALYTE METHODS FOR THE DETERMINATION OF QUINOLIZIDINE ALKALOIDS IN FOOD AND FEED WITH LC-MS/MS - RESULTS OF A COLLABORATIVE METHOD VALIDATION STUDY</b>	
	<i>Carolin Hoyer</i> <sup>(1)</sup> , <i>Yannick Seele</i> <sup>(1)</sup> , <i>Annika I. Ostermann</i> <sup>(1)</sup> , <i>Oliver Kappenstein</i> <sup>(1)</sup> .....	545
<b>S13</b>	<b>COMPREHENSIVE ANALYSIS AND TARGET QUANTIFICATION OF MYCOTOXINS IN FRUIT JUICE SAMPLES USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETRY</b>	
	<i>Antonio Paolillo</i> <sup>(1)</sup> , <i>Ivana Ledenko</i> <sup>(1)</sup> , <i>Lidia Ciriaco</i> <sup>(1)</sup> , <i>Vincenzo Armini</i> <sup>(1)</sup> , <i>Sonia Lombardi</i> <sup>(1)</sup> , <i>Luana Izzo</i> <sup>(1)</sup> .....	546
<b>S14</b>	<b>SEA TEMPERATURE INFLUENCES ACCUMULATION OF TETRODOTOXIN IN BRITISH BIVALVE SHELLFISH</b>	
	<i>Monika Dhanji-Rapkova</i> <sup>(1)</sup> , <i>Mickael Teixeira Alves</i> <sup>(1)</sup> , <i>Joaquin Trinanes</i> <sup>(2)</sup> , <i>Jaime Martinez-Urtaza</i> <sup>(3)</sup> , <i>David Haverson</i> <sup>(1)</sup> , <i>Kirsty Bradley</i> <sup>(1)</sup> , <i>Craig Baker-Austin</i> <sup>(1)</sup> , <i>Jennifer Ritchie</i> <sup>(4)</sup> , <i>Andrew Turner</i> <sup>(1)</sup> .....	547
<b>S15</b>	<b>ESTROGENIC IN VITRO EVALUATION OF SEVERAL PHASE II METABOLITES OF ISOFLAVONES</b>	
	<i>Elisabeth Varga</i> <sup>(1)</sup> , <i>Dino Grgic</i> <sup>(2)</sup> , <i>Dimitra Bella-Velidou</i> <sup>(2)</sup> , <i>Luca Dellafiora</i> <sup>(3)</sup> , <i>Doris Marko</i> <sup>(2)</sup> .....	548
<b>S16</b>	<b>A LC-TWIMS-HRMS WORKFLOW TO INVESTIGATE FUNGAL METABOLITES INVOLVED IN FUSARIUM PROLIFERATUM MYCOTOXIN BIOSYNTHETIC PATHWAYS</b>	
	<i>Irene Piccici</i> <sup>(1)</sup> , <i>Guillem Campmajo</i> <sup>(1)</sup> , <i>Antonio Moretti</i> <sup>(2)</sup> , <i>Chiara Dall'Asta</i> <sup>(1)</sup> .....	549
<b>S17</b>	<b>CAPILLARY ELECTROPHORESIS TANDEM MASS SPECTROMETRY AS EFFICIENT TECHNIQUE FOR MULTICLASS CYANOTOXIN ANALYSIS IN WATER AND VEGETABLE SAMPLES</b>	
	<i>Ana M. Garcia-Campana</i> <sup>(1)</sup> , <i>Rocio Carmona-Molero</i> <sup>(1)</sup> , <i>Maria del Mar Aparicio-Muriana</i> <sup>(1)</sup> , <i>Francisco J. Lara</i> <sup>(1)</sup> , <i>Monsalud Del Olmo-Iruela</i> <sup>(1)</sup> .....	550
<b>S18</b>	<b>DETERMINATION OF PHOMOPSINS IN FOOD - METHOD VALIDATION AND SURVEY</b>	
	<i>Sylvia Kalli</i> <sup>(1)</sup> , <i>Ilaria di Marco Pisciotano</i> <sup>(1)</sup> , <i>Patrick Mulder</i> <sup>(1)</sup> , <i>Josipa Grzetic Martens</i> <sup>(1)</sup> , <i>Martin Meijer</i> <sup>(2)</sup> , <i>Jos Houbraken</i> <sup>(2)</sup> .....	551
<b>S19</b>	<b>TARGETED AND UNTARGETED LC-(HR)MS STRATEGIES FOR THE DETERMINATION OF CUCURBITACINS IN FOOD</b>	
	<i>Ilaria Di Marco Pisciotano</i> <sup>(1)</sup> , <i>Sylvia Kalli</i> <sup>(1)</sup> , <i>Patrick Mulder</i> <sup>(1)</sup> , <i>Elena De Vries</i> <sup>(1)</sup> , <i>Timo Jellema</i> <sup>(1)</sup> , <i>Josipa Grzetic Martens</i> <sup>(1)</sup> .....	552
<b>S20</b>	<b>MONITORING OF TOXIN-PRODUCING PHYTOPLANKTON IN THE ADRIATIC SEA (ITALY) IN 2023-2024 USING MICROSCOPIC AND MOLECULAR METHODS</b>	
	<i>Barbara Bertasi</i> <sup>(1)</sup> , <i>Laura Ragni</i> <sup>(1)</sup> , <i>Sara Arnaboldi</i> <sup>(1)</sup> , <i>Enrico Pavoni</i> <sup>(1)</sup> , <i>Laura Pezzolesi</i> <sup>(2)</sup> , <i>Franca Guerrini</i> <sup>(2)</sup> , <i>Rossella Pistocchi</i> <sup>(2)</sup> , <i>Monica Cangini</i> <sup>(3)</sup> , <i>Irene Servadei</i> <sup>(3)</sup> , <i>Silva Rubini</i> <sup>(4)</sup> .....	553
<b>S21</b>	<b>ROBUST, HIGHLY SENSITIVE AND FAST POLARITY SWITCHING QUANTITATION OF MYCOTOXINS IN DIFFERENT MATRICES</b>	
	<i>Carlos Bueno</i> <sup>(1)</sup> , <i>Jianru Stahl-Zeng</i> <sup>(1)</sup> , <i>Michael Scherer</i> <sup>(1)</sup> .....	554
<b>S22</b>	<b>DEVELOPMENT, VALIDATION AND APPLICATION OF DILUTE AND SHOOT TECHNIQUE FOR LC-MS/MS TO DETERMINE TROPANE ALKALOIDS IN SOYBEANS</b>	
	<i>Rufielyn Gravador</i> <sup>(1)</sup> , <i>Simon Haughey</i> <sup>(1)</sup> , <i>Brett Greer</i> <sup>(1)</sup> , <i>Chelsea Ugay</i> <sup>(1)</sup> , <i>Chris Elliott</i> <sup>(2)</sup> .....	555
<b>S23</b>	<b>LEVERAGING SENSITIVITY AND SELECTIVITY OF THE SCIEX 7500 SYSTEM FOR TARGETED ANALYSIS OF MYCOTOXINS IN PLANT-BASED MEAT</b>	
	<i>Holly Lee</i> <sup>(1)</sup> , <i>Hitha PP</i> <sup>(1)</sup> , <i>Sujata Rajan</i> <sup>(1)</sup> , <i>Sashank Pillai</i> <sup>(1)</sup> , <i>Craig Butt</i> <sup>(1)</sup> .....	556

# CONTENT

<b>S24</b>	<b>HIGHLY EFFICIENT LC-MS/MS ANALYSIS OF MULTIPLE MYCOTOXINS UTILIZING BIPHENYL COLUMN SELECTIVITY WITH INERT COLUMN TECHNOLOGY</b>	
	<u>Tina Brandscher</u> <sup>(1)</sup> , Jan Pschierer <sup>(1)</sup> , Paul Connolly <sup>(2)</sup> , Diego Lopez <sup>(2)</sup> .....	557
<b>S25</b>	<b>BIOTRANSFORMATION OF HT-2 AND T-2 TOXINS USING PLANT APPARATUS AND ISOLATION OF THE RESULTING GLUCOSIDES</b>	
	<u>Adam Behner</u> <sup>(1)</sup> , Martina Travnickova <sup>(2)</sup> , Zbynek Dzuman <sup>(1)</sup> , Jana Chrpova <sup>(2)</sup> , Milena Stranska <sup>(1)</sup> .....	558
<b>S26</b>	<b>VALIDATION OF AN LC-MS/MS BASED METHOD FOR MULTIPLE MYCOTOXINS AND PLANT TOXINS IN MEAT REPLACEMENT PRODUCTS</b>	
	<u>Michael Sulyok</u> <sup>(1)</sup> , Claudia Seeber <sup>(1)</sup> , Rudolf Krska <sup>(1)</sup> .....	559
<b>S27</b>	<b>2D-LIQUID CHROMATOGRAPHY - AN EFFICIENT ALTERNATIVE TO SPE AND IAC FOR THE DETERMINATION OF MYCOTOXINS IN FOOD</b>	
	<u>Sarah Schleicher</u> <sup>(1)</sup> , Susanne Sölter <sup>(1)</sup> , Mareike Rathke <sup>(2)</sup> , Clara John <sup>(2)</sup> , Sarah Kulas <sup>(2)</sup> , Mareike Reichel <sup>(2)</sup> .....	560
<b>S28</b>	<b>A VALIDATED LC-MS/MS MULTI-METHOD FOR THE DETERMINATION OF 110 MYCOTOXINS AND PLANT TOXINS IN RAW MILK</b>	
	<u>Ahmed El-Khatib</u> <sup>(1)</sup> , Arnold Bahlmann <sup>(1)</sup> , Carolin Hoyer <sup>(1)</sup> , Christoph Hutzler <sup>(1)</sup> , Stefan Weigel <sup>(1)</sup> .....	561
<b>S29</b>	<b>A PROTEOMICS APPROACH FOR THE DETECTION AND IDENTIFICATION OF POISONING PLANTS - DATURA STRAMONIUM, DATURA METEL AND MANDRAGORA AUTUMNALIS - IN LEAFY PLANTS</b>	
	<u>Giancarlo Biancotto</u> <sup>(1)</sup> , Sabina Noviello <sup>(1)</sup> , Roberta Masin <sup>(2)</sup> , Albino Gallina <sup>(1)</sup> , Lisa Ferrari <sup>(1)</sup> , Roberto Stella <sup>(1)</sup> .....	562
<b>S30</b>	<b>NATURAL CONTAMINANTS IN PLANT-BASED MEAT ALTERNATIVES</b>	
	<u>Nela Prusova</u> <sup>(1)</sup> , Ema Vitova <sup>(1)</sup> , Tereza Dolezalova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> , Milena Stranska <sup>(1)</sup> .....	563
<b>S31</b>	<b>QUINOLIZIDINE ALKALOIDS IN FOOD: DEVELOPMENT OF AN LC-MS/MS METHOD AND OBSERVATIONS FROM ROUTINE</b>	
	<u>Jörg Konetzki</u> <sup>(1)</sup> , Juliane Böttcher <sup>(1)</sup> , Thomas Behnke <sup>(1)</sup> , Erik Becker <sup>(1)</sup> .....	564
<b>S32</b>	<b>ALPHA-TOMATINE IN CHERRY TOMATOES: AN ITALIAN CASE OF GLYCOALKALOIDS INTOXICATION</b>	
	<u>Mariantonietta Peloso</u> <sup>(1)</sup> , Ilaria Prizio <sup>(1)</sup> , Gaetan Minkoumba Sonfack <sup>(1)</sup> , Simona Pellicciotti <sup>(1)</sup> , Giorgio Fedrizzi <sup>(1)</sup> , Elisabetta Caprai <sup>(1)</sup> .....	565
<b>S33</b>	<b>EXAMINATION ON THE EFFECT OF CHLORINE-CONTAINING LIQUID SUBSTANCE ON MYCOTOXIN PRODUCING FUSARIUM SPECIES</b>	
	<u>Eniko Makkai</u> <sup>(1)</sup> , Tibor Bartok <sup>(2)</sup> , Attila K. Horvath <sup>(3)</sup> , György Cseko <sup>(3)</sup> , Zoltan Gazdag <sup>(4)</sup> , Arpad Czeh <sup>(5)</sup> .....	566
<b>S34</b>	<b>ANALYSIS OF MULTI-MYCOTOXIN CONTAMINATION OF MAIZE SAMPLES IN HUNGARY, USING MYCOTOXIN DETECTION MYCOFOSSTM PLATFORM</b>	
	<u>Anna Orsolya Laczko</u> <sup>(1)</sup> , Istvan Lakatos <sup>(2)</sup> , Petra Dorottya Tigyi <sup>(3)</sup> , Tamas Szörenyi <sup>(3)</sup> , Miklos Harasztia <sup>(3)</sup> , Andras Peter Vida <sup>(3)</sup> , Laszlo Robert Szemethy <sup>(4)</sup> , <u>Arpad Czeh</u> <sup>(5)</sup> .....	567
<b>S35</b>	<b>DIVERSITY AND DETERMINATION OF PRYMNESIUM PARVUM TOXINS</b>	
	<u>Magdalena Pöchlhammer</u> <sup>(1)</sup> , Alexander Conrad <sup>(1)</sup> , Doris Marko <sup>(2)</sup> , <u>Elisabeth Varga</u> <sup>(1)</sup> .....	568
<b>S36</b>	<b>QUANTUM GREEN: FAST AND INNOVATIVE LATERAL FLOW CASSETTES FOR QUANTIFICATION OF DEOXYNIVALENOL AND FUMONISIN IN CEREAL AND ANIMAL FEED, DETECTING TOXIN-FREE SAMPLES IN 45 SECONDS USING THE SAME EXTRACT</b>	
	<u>Sofia Iliopoulou</u> <sup>(1)</sup> , Antonios Ntanasios <sup>(1)</sup> , Vasilios Meras <sup>(1)</sup> , Theodorakis Ntoupis <sup>(1)</sup> , Sevasti Laporda <sup>(1)</sup> , Alexandra Alevra <sup>(1)</sup> , Maura Begolli <sup>(1)</sup> , Georgios Papageorgiou <sup>(1)</sup> .....	569
<b>S37</b>	<b>MULTI-MYCOTOXIN ANALYSES BY UPLC-MS/MS IN WHEAT: EMERGING MYCOTOXINS IN WALLONIA IN 2023 AND 2024</b>	
	<u>Camille Jonard</u> <sup>(1)</sup> , Anne Chandelier <sup>(2)</sup> , Damien Eylenbosch <sup>(3)</sup> , Anne-Michelle Faux <sup>(3)</sup> , Philippe Vermeulen <sup>(1)</sup> , Bruno Godin <sup>(1)</sup> , Sebastien Gofflot <sup>(1)</sup> .....	570

# CONTENT

<b>S38</b>	<b>MULTI-MYCOTOXIN ANALYSES BY UPLC-MS/MS: METHOD DEVELOPMENT AND APPLICATION TO BENINESE PEANUT PRODUCTS</b>	
	<i>Camille Jonard</i> <sup>(1)</sup> , Sébastien Gofflot <sup>(1)</sup> , O. Herbert Iko Afe <sup>(2)</sup> , Christin Sogbossi Gbetokpanou <sup>(2)</sup> , Paulin Azokpota <sup>(2)</sup> , E. Yann Madode <sup>(2)</sup> , Caroline Douny <sup>(3)</sup> , Marie-Louise Scippo <sup>(3)</sup> .....	572
<b>S39</b>	<b>OCCURRENCE OF MYCOTOXINS IN IRISH OATS OVER THE YEARS 2022-2023</b>	
	<i>Melissa Di Rocco</i> <sup>(1)</sup> , Diana Bucur <sup>(2)</sup> , Steven Kildea <sup>(2)</sup> , Martin Danaher <sup>(1)</sup> .....	572
<b>S40</b>	<b>A NEW PHYSICAL AND BIOLOGICAL STRATEGY TO REDUCE THE CONTENT OF ZEARALENONE IN INFECTED WHEAT KERNELS: THE EFFECT OF COLD NEEDLE PERFORATION, MICROORGANISMS, AND PURIFIED ENZYME</b>	
	<i>Amandine Andre</i> <sup>(1)</sup> , Katrin Hecht <sup>(2)</sup> , Sandra Mischler <sup>(1)</sup> , Rebecca Buller <sup>(2)</sup> , Susette Freimüller Leischfeld <sup>(1)</sup> , Irene Chetschik <sup>(1)</sup> , Susanne Miescher Schwenninger <sup>(1)</sup> , Nadina Müller <sup>(1)</sup> .....	573
<b>S41</b>	<b>EFFECTIVENESS OF COMMERCIAL ADSORBENTS FOR THE ELIMINATION OF MYCOTOXINS</b>	
	<i>Zbynek Dzuman</i> <sup>(1)</sup> , Milena Stranska <sup>(1)</sup> , Nela Prusova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	574
<b>S42</b>	<b>DEGLYCOSYLATION OF "MASKED" HT2/T2 TOXINS AND QUANTIFICATION IN CEREALS</b>	
	<i>Tereza Dolezalova</i> <sup>(1)</sup> , Nela Prusova <sup>(1)</sup> , Adam Behner <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Milena Stranska <sup>(1)</sup> .....	575
<b>S43</b>	<b>MATRIX EFFECTS WHEN QUANTIFYING MYCOTOXINS IN VARIOUSLY COMPLEX CRAFT BEER MATRICES - WHY DOES IT MATTER?</b>	
	<i>Tereza Dolezalova</i> <sup>(1)</sup> , Nela Prusova <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Milena Stranska <sup>(1)</sup> .....	576
<b>S44</b>	<b>A NOVEL STRATEGY FOR THE ANNOTATION OF UNKNOWN PYRROLIZIDINE ALKALOIDS</b>	
	<i>Leonie Straub</i> <sup>(1)</sup> , Federico Padilla Gonzalez <sup>(1)</sup> , Han Zuilhof <sup>(1)</sup> , Laura Righetti <sup>(1)</sup> , Patrick Mulder <sup>(2)</sup> .....	577
<b>S45</b>	<b>CO-OCCURRENCE OF T-2 AND HT-2 TOXINS AND THEIR GLUCOSIDES IN WHEAT AND OAT GRAINS</b>	
	Adam Pierzgalski <sup>(1)</sup> , <i>Marcin Bryła</i> <sup>(1)</sup> , Benedikt Cramer <sup>(2)</sup> , Hans-Ulrich Humpf <sup>(2)</sup> , Magdalena Twaruzek <sup>(3)</sup> .....	578
<b>S46</b>	<b>OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN ROOIBOS TEA FROM THE EUROPEAN MARKET</b>	
	<i>Alessandro Volkov</i> <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> , <i>Ondrej Brabec</i> <sup>(1)</sup> .....	579
<b>S47</b>	<b>MONITORING OF PYRROLIZIDINE AND TROPANE ALKALOIDS IN CZECH HONEY</b>	
	<i>Ondrej Rubas</i> <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , <i>Ondrej Brabec</i> <sup>(1)</sup> , Dalibor Titera <sup>(2)</sup> , Jana Hajslova <sup>(1)</sup> .....	580
<b>S48</b>	<b>STRUCTURAL MODIFICATIONS OF CERTAIN PYRROLIZIDINE ALKALOIDS DURING SAMPLE EXTRACTION AND ITS IMPACT ON ANALYTICAL RESULTS</b>	
	<i>Florian Kaltner</i> <sup>(1)</sup> , Lisa Monika Klein <sup>(1)</sup> .....	581
<b>S49</b>	<b>TOXIC ALKALOID OR INTERFERENCE? PITFALLS IN LC-MS ANALYSIS OF TROPANE AND PYRROLIZIDINE ALKALOIDS</b>	
	<i>Ondrej Brabec</i> <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	582
<b>S50</b>	<b>VALIDATION OF SINGLE EXTRACTION METHOD FOR THE SIMULTANEOUS ANALYSIS OF MYCOTOXINS USING IMMUNOAFFINITY COLUMNS</b>	
	Dave Leeman <sup>(1)</sup> , Andrew Allen <sup>(1)</sup> , Helen Cameron <sup>(1)</sup> , Carol Donnelly <sup>(1)</sup> , Claire Milligan <sup>(1)</sup> , Adam Tramseur <sup>(2)</sup> , Joanna Stratton <sup>(2)</sup> , Susan MacDonald <sup>(2)</sup> , <i>Elizabeth Mannaing</i> <sup>(1)</sup> .....	583
<b>S51</b>	<b>IMPROVING INTERNAL LABORATORY PROCESSES AND SUSTAINABILITY USING RAPID ONLINE AUTOMATION FOR AFLATOXIN ANALYSIS</b>	
	Sebastian Wissmueller <sup>(1)</sup> , Alexander Burkon <sup>(1)</sup> , Markus Kopp <sup>(1)</sup> , Elizabeth Manning <sup>(2)</sup> , Naomi McLachlan <sup>(2)</sup> , <i>Carol Donnelly</i> <sup>(2)</sup> .....	584
<b>S52</b>	<b>ASSESSMENT OF DIFFERENT EXTRACTION METHODS FOR THE VALIDATION OF AN ANALYTICAL MULTI-METHOD FOR THE DETERMINATION OF ALTERNARIA MYCOTOXINS IN WHEAT BY LC-MS/MS</b>	
	Vojislava Bursic <sup>(1)</sup> , <i>Aleksandar Vatazevic</i> <sup>(2)</sup> , Gorica Vukovic <sup>(2)</sup> , Bojan Konstantinovic <sup>(1)</sup> , Tijana Stojanovic <sup>(1)</sup> , Natasa Samardzic <sup>(1)</sup> , Milena Popov <sup>(1)</sup> .....	585

# CONTENT

S53

## OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN BLACK PEPPER

Aleksandar Vatazevic<sup>(1)</sup>, Gorica Vukovic<sup>\*1)</sup>, Sanja Novkovic<sup>(1)</sup>, Vojislava Bursic<sup>(2)</sup>, Nikola Puvača<sup>(3)</sup>, Bojana Spirovic-Trifunovic<sup>(4)</sup>.....586

## NANOPARTICLES ..... 587

T1

### PHYSICOCHEMICAL CHARACTERISATION OF IRON OXIDES AND HYDROXIDES APPLIED AS FOOD ADDITIVE E 172

Lisa Siciliani<sup>\*1)</sup>, Francesco Fumagalli<sup>(2)</sup>, Eveline Verleysen<sup>(3)</sup>, Jessica Ponti<sup>(2)</sup>, Francesco Cubadda<sup>(4)</sup>, Andrea Haase<sup>(5)</sup>, Sara Bals<sup>(6)</sup>, Jan Mast<sup>(3)</sup>.....588

T2

### ANALYSIS OF (NANO)PARTICLES IN FOOD CONTACT MATERIALS USING ELECTRON MICROSCOPY

Lisa Siciliani<sup>\*1)</sup>, Eveline Verleysen<sup>(2)</sup>, Sara Bals<sup>(3)</sup>, Heidi Demaegdt<sup>(2)</sup>, Salvatore Ciano<sup>(2)</sup>, Els Van Hoek<sup>(2)</sup>, Jan Mast<sup>(2)</sup>.....589

T3

### STUDY OF DETOXIFICATION IN AQUATIC ENVIRONMENTS FOR THE ELIMINATION OF DECARBAMOYL SAXITOXIN (DCSTX), USING MAGNETIC NANOPARTICLES

Carmen Lopez<sup>(1)</sup>, Alejandro Cao<sup>(1)</sup>, Ana Botana<sup>\*1)</sup>, Luis Botana<sup>(1)</sup>.....590

T4

### PREPARATION AND CHARACTERIZATION OF VITAMIN E/CALCIUM/SOY PROTEIN ISOLATE NANOPARTICLES FOR SOYBEAN MILK BEVERAGE FORTIFICATION

Neveen Agamy<sup>(1)</sup>, Amira Darwish<sup>\*2)</sup>.....591

## PROCESSING CONTAMINANTS ..... 592

U1

### APPROACHES TO MINIMIZE ACRYLAMIDE IN OXIDIZED CALIFORNIA STYLE OLIVES

Carmen Breitling-Utzmann<sup>\*1)</sup>, Nadja Bauer<sup>(1)</sup>, Alexandra Treyer<sup>(1)</sup>.....593

U2

### POLYCHLORINATED ALKANES (PCAS): INVESTIGATING LEVELS IN FOOD FROM THE DUTCH MARKET

Wout Bergkamp<sup>(1)</sup>, Kerstin Krätschmer<sup>\*1)</sup>.....594

U3

### EXPERIMENTAL INVESTIGATION AND MODELLING OF PROCESS PARAMETERS EFFECT ON GINGERBREAD QUALITY AND SAFETY

Lene Duedahl-Olesen<sup>\*1)</sup>, Elena Hakme<sup>(1)</sup>, Eda Topaloglu<sup>(1)</sup>, Denis Sehovic<sup>(1)</sup>, Aberham Hailu Feyissa<sup>(1)</sup>.....595

U4

### INFLUENCE OF THE BAKING PROCESS ON CANNABINOID CONTENTS AND PROFILES IN HEMP-CONTAINING BREAD AND SURVEY OF MARKET SAMPLES

Niklas Lindekamp<sup>(1)</sup>, Nadja Triesch<sup>(1)</sup>, Tim Rautenberg<sup>(1)</sup>, Alexander Voß<sup>(2)</sup>, Sascha Rohn<sup>(3)</sup>, Stefan Weigel<sup>\*1)</sup>.....596

U5

### TRACE ELEMENTS AND BISPHENOLS IN PROCESSED FOOD (MEAT AND FISH PRODUCTS)

Vincenzo Lo Turco<sup>(1)</sup>, Vincenzo Nava<sup>\*2)</sup>, Federica Litrenta<sup>(1)</sup>, Rossana Rando<sup>(2)</sup>, Angela Giorgia Potorti<sup>(2)</sup>, Giuseppa Di Bella<sup>(2)</sup>.....597

U6

### MONOCHLOROPROPANEDIOL AND GLYCIDYL FATTY ESTERS IN A DIVERSE RANGE OF BABY FOODS

Dimitra Diamantidou<sup>\*1)</sup>, Arvid Fromberg<sup>(1)</sup>.....598

U7

### EVALUATION OF MINERAL OIL HYDROCARBONS (MOSH AND MOAH) CONTAMINATION IN SYNTHETIC CASINGS USING GCXGC-MS

Ana M. Rodrigues<sup>(1)</sup>, Fabio L. Gonçalves<sup>(2)</sup>, Carolina Ventura<sup>(2)</sup>, António Ferreira<sup>(2)</sup>, Naiara Fernandez<sup>(2)</sup>, Catia Carmo<sup>(2)</sup>, Maria Rosario Bronze<sup>\*2)</sup>.....599

U8

### SURVEY ON MINERAL OIL HYDROCARBONS (MOH) CONTAMINANTS IN VIRGIN AND EXTRA VIRGIN OLIVE OILS

Ana M. Rodrigues<sup>(1)</sup>, Daniel Reyes Diaz<sup>(2)</sup>, Amulya Baniya<sup>(1)</sup>, Sofia Lourenço<sup>(3)</sup>, Paula Allen Lopes<sup>(3)</sup>, Luis Aparicio<sup>(3)</sup>, Naiara Fernandez<sup>(1)</sup>, Maria Rosario Bronze<sup>\*1)</sup>.....600

U9

### CHALLENGES IN THE PRODUCTION OF PROFICIENCY TESTING AND REFERENCE MATERIALS FOR FURAN, ALKYL FURANS AND BENZENE IN FOOD

Isabella Zübner<sup>\*1)</sup>, Martin Kaminski<sup>(1)</sup>.....601

# CONTENT

## U10

### COMMITMENT TO EXCELLENCE: ENSURING FOOD SAFETY AND INNOVATION IN MOSH/MOAH ANALYSIS THROUGH A DUAL APPROACH

Carlos Martin-Alberca<sup>(1)</sup>, Zuzana Zelinkova<sup>\*(2)</sup>, Raquel De Llanos<sup>(1)</sup>, Benjamin Horemans<sup>(3)</sup>, Inna Stambula<sup>(2)</sup>, Marian Steverink<sup>(1)</sup> .....602

## U11

### DETERMINATION OF MINERAL OIL AROMATIC HYDROCARBONS BY GCXGC-FID. USED ROUTINELY FOR 1.5 YEARS: AN OVERVIEW ABOUT EXPERIENCES, LIMITATIONS AND RESULTS

Michael Koch<sup>(1)</sup>, Kristin Wenzel<sup>(1)</sup>, Eileen Schulz<sup>\*(1)</sup>, Agnes Buczkowski<sup>(1)</sup>, Thomas Behnke<sup>(1)</sup>, Erik Becker<sup>(1)</sup> .....603

## U12

### NITROSAMINES 2030: FROM PHARMA & PACKAGING TO FOOD & PACKAGING. RISKS TO THINK ABOUT

Emiliano De Dominicis<sup>\*(1)</sup>, Marialuisa Piva<sup>(1)</sup>, Mario Dante<sup>(1)</sup>, Elisa Gritti<sup>(1)</sup>, Gianluigi Boschello<sup>(1)</sup>, Valentina Menegon<sup>(1)</sup>, Manuel Rubino<sup>(1)</sup>, Laura Zampieri<sup>(1)</sup>, Silvia Toffoletto<sup>(1)</sup>, Emanuele Conte<sup>(1)</sup>, Stefano Cazzaro<sup>(1)</sup>, Marco Redaelli<sup>(1)</sup>, Enrico Nieddu<sup>(1)</sup>, Cinzia Pellicorio<sup>(1)</sup>, Francesca Faraon<sup>(1)</sup>, Valentina Abbondandolo<sup>(1)</sup>, Sergio Fasan<sup>(1)</sup>, Sylvain Dahiot<sup>(1)</sup> .....604

## U13

### FIRST CERTIFIED REFERENCE MATERIAL FOR LOW LEVELS OF MCPD FATTY ACID ESTERS IN INFANT FORMULA

Berit Sejerøe-Olsen<sup>\*(1)</sup>, Penka Shegunova<sup>(1)</sup>, Andrea Held<sup>(1)</sup>, Arvid Fromberg<sup>(2)</sup> .....605

## U14

### POLISH APPROACH OF THE TRANS FATS (I-TFA) ELIMINATION FROM FOOD

Hanna Mojska<sup>(1)</sup>, Edyta Jasinska-Melon<sup>\*(1)</sup> .....606

## RESIDUES – PESTICIDES ..... 607

### V1

#### MONITORING BIOCIDES CONTAMINATION IN FOOD AND FEED USING A COMPREHENSIVE LC-HRMS METHOD

Paul Zomer<sup>\*(1)</sup>, Ingrid Duarte Marques<sup>(1)</sup>, Joëlle Wieman<sup>(1)</sup>, Hans Mol<sup>(1)</sup> .....608

### V2

#### PESTICIDE REMOVAL FROM WATER USING DIFFERENT TYPES OF ADSORBENT MATERIALS

Alejandro Cao<sup>(1)</sup>, Natalia Vilarino<sup>\*(2)</sup>, Lisandra De Castro Alves<sup>(3)</sup>, Jose Rivas<sup>(3)</sup>, Yolanda Pineiro<sup>(3)</sup>, Luis Rodriguez-Santos<sup>(2)</sup>, M. Carmen Louzao<sup>(2)</sup>, Sandra Raposo-García<sup>(2)</sup>, Luis M. Botana<sup>(2)</sup> .....609

### V3

#### EXPLORING PESTICIDE RESIDUE ANALYSIS IN FOOD USING A NITROGEN-ONLY SYSTEM FOR GC-MS/MS

Peter Hancock<sup>\*(1)</sup>, David Gould<sup>(1)</sup>, Douglas Stevens<sup>(1)</sup>, Stuart Adams<sup>(1)</sup> .....610

### V4

#### NON-TARGETED ANALYSIS OF FOOD SAMPLES BY A DUAL IONIZATION GC-HRMS USING SIMULTANEOUS EI AND CI FOR UNAMBIGUOUS IDENTIFICATION OF UNKNOWNNS

Eliska Ceznerova<sup>\*(1)</sup>, Sonja Klee<sup>(1)</sup>, Steffen Bräkling<sup>(1)</sup>, Marlene Moskowicz<sup>(2)</sup>, Javier Lopez<sup>(2)</sup>, Arnd Ingendoh<sup>(2)</sup>, Carsten Baessmann<sup>(2)</sup> .....611

### V5

#### HIGHLY EFFICIENT LC-MS/MS ANALYSIS OF ORGANOPHOSPHOROUS PESTICIDES UTILIZING ARC-18 COLUMN SELECTIVITY WITH INERT COLUMN TECHNOLOGY

Tina Brandscher<sup>(1)</sup>, Hansjörg Majer<sup>(1)</sup>, Paul Connolly<sup>(2)</sup>, Diego Lopez<sup>\*(2)</sup>, Jan Pschierer<sup>(1)</sup> .....612

### V6

#### ASSESSING THE PERFORMANCE OF NEW SORBENT COMBINATION IN $\mu$ SPE CARTRIDGES FOR PESTICIDES ANALYSES IN CEREAL BASED MATRIXES

Ederina Ninga<sup>\*(1)</sup>, Mette E. Poulsen<sup>(1)</sup> .....613

### V7

#### ENHANCED WORKFLOW FOR COMPREHENSIVE PESTICIDE RESIDUE ANALYSIS IN FOOD WITH CROSS-CONFIRMATION USING GC-MS/MS AND EITHER LC-MS/MS OR LC-HRMS

Delphine Thomas<sup>\*(1)</sup>, Bénédicte Gauriat<sup>(1)</sup>, Julie Moriceau<sup>(1)</sup>, Maryline Carvalho<sup>(1)</sup>, Valerie Thibert<sup>(1)</sup>, Aristide Ganci<sup>(1)</sup>, Jean-François Garnier<sup>(1)</sup> .....614

### V8

#### JOINT MONITORING PROGRAM OF PESTICIDE RESIDUES IN HONEY (MRM - COMPOUNDS)

Anja Muzyka<sup>(1)</sup>, Anna Buettner<sup>\*(1)</sup> .....615

### V9

#### INVESTIGATION OF THE POTENTIAL EXPOSURE OF DANISH BROWN HARES TO PESTICIDES

Elena Hakme<sup>\*(1)</sup>, Victor Cutillas Juarez<sup>(2)</sup>, Mette Erecius Poulsen<sup>(1)</sup> .....616

# CONTENT

<b>V10</b>	
<b>IMPACT OF CLIMATE CHANGE AND SUSTAINABLE APPROACHES ON PESTICIDE RESIDUES</b>	
Thomas Behnke <sup>(1)</sup> , Andreas Hentschel <sup>(1)</sup> , Paolo Matteini <sup>(2)</sup> , Giuseppe Sammarco <sup>(3)</sup> , Jay Alappat <sup>(4)</sup> , Juan Carrasco <sup>(5)</sup> , Sophie Robert <sup>(6)</sup> , Emiliano De Dominicis <sup>(7)</sup> , Cinzia Pellicorio <sup>(8)</sup> , Wessel Kriek <sup>(9)</sup> , Francesca Bellini <sup>(10)</sup> , Massimo Frera <sup>(10)</sup> .....	618
<b>V11</b>	
<b>NOVEL TRIPLE QUAD APPROACHES FOR RELIABLE AND SENSITIVE QUANTIFICATION OF A LARGE NUMBER OF PESTICIDES IN A SINGLE RUN</b>	
Diego Martin Ortiz <sup>(1)</sup> , Javier Loepez Flores <sup>(1)</sup> .....	618
<b>V12</b>	
<b>NON-TARGET SCREENING OF FOOD FOR HALOGENATED SUBSTANCES BY LC-HRMS APPLIED IN THE EFSA SCREENER PROJECT</b>	
Paul Zomer <sup>(1)</sup> , Hans Mol <sup>(1)</sup> , Wout Bergkamp <sup>(1)</sup> , Anna Undas <sup>(1)</sup> , Arjen Lommen <sup>(1)</sup> .....	619
<b>V13</b>	
<b>HIGH-THROUGHPUT AUTOMATED OFFLINE <math>\mu</math>-SPE FOR CLEANUP OF COMPLEX QUECHERS EXTRACTS IN GC-BASED MULTI-RESIDUE ANALYSIS</b>	
Ivan Aloisi <sup>(1)</sup> , Marc Tienstra <sup>(1)</sup> , Michel Willemsen <sup>(1)</sup> , Hans Mol <sup>(1)</sup> .....	620
<b>V14</b>	
<b>DEVELOPMENT AND VALIDATION OF A RESIDUE ANALYSIS METHOD FOR GLYPHOSATE, ITS METABOLITE AMPA, AND GLUFOSINATE IN HONEY BY DERIVATIZATION AND LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY</b>	
Marianna Martinello <sup>(1)</sup> , Sara Zanella <sup>(2)</sup> , Franco Mutinelli <sup>(1)</sup> .....	621
<b>V15</b>	
<b>CHLORATE, PERCHLORATE AND BROMATE IN FOOD USING A MIXED MODE REVERSED-PHASE ANIONIC EXCHANGE COLUMN</b>	
Peter Hancock <sup>(1)</sup> , Ofure Idialu <sup>(1)</sup> , Simon Hird <sup>(1)</sup> , Eimear Trivedi <sup>(1)</sup> , Euan Ross <sup>(1)</sup> .....	622
<b>V16</b>	
<b>A COMPREHENSIVE HRMS METHODOLOGY FOR THE WIDE-SCOPE TARGET SCREENING OF &gt;750 PESTICIDES IN OLIVE OIL USING LC-(ESI)-/GC-(APCI)-QTOF MS COMPLEMENTARY PLATFORMS</b>	
Sofia K. Drakopoulou <sup>(1)</sup> , Stefanos E. Kokolakis <sup>(1)</sup> , Apostolos L. Karagiannidis <sup>(1)</sup> , Marilena E. Dasenaki <sup>(2)</sup> , Niki C. Maragou <sup>(1)</sup> , Carsten Baessmann <sup>(3)</sup> , Nikolaos S. Thomaidis <sup>(1)</sup> .....	623
<b>V17</b>	
<b>QUECHERS MICRO-SPE CLEAN-UP METHOD FOR THE DETERMINATION OF PESTICIDES IN FEED SAMPLES IN GC-ORBITRAP-BASED ANALYSIS-COLLABORATIVE STUDY</b>	
Ederina Ninga <sup>(1)</sup> , Ivan Aloisi <sup>(2)</sup> , Michel Willemsen <sup>(2)</sup> , Marc Tienstra <sup>(2)</sup> , Julio Cesar Espana Amortegui <sup>(1)</sup> , Hans Mol <sup>(2)</sup> , Mette E Poulsen <sup>(1)</sup> .....	624
<b>V18</b>	
<b>EVOLUTION OF A ROBUST AND SENSITIVE METHOD FOR THE DIRECT ANALYSIS OF POLAR PESTICIDES IN FOOD</b>	
Daniel McMillan <sup>(1)</sup> , Jianru Stahl-Zeng <sup>(1)</sup> , Yoann Fillatre <sup>(1)</sup> , Wim Broer <sup>(2)</sup> .....	625
<b>V19</b>	
<b>SIMULTANEOUS DETERMINATION OF MYCOTOXINS AND PESTICIDES IN FISH FEED AND BEEKEEPING PRODUCTS BY QUECHERS-LC-MS</b>	
Maria Alvarez-Romero <sup>(1)</sup> , Jesus Marin-Saez <sup>(2)</sup> , Antonia Garrido-Frenich <sup>(3)</sup> , Ana M. Garcia-Campana <sup>(1)</sup> , Laura Gamiz-Gracia <sup>(1)</sup> , Maykel Hernandez-Mesa <sup>(4)</sup> .....	626
<b>V20</b>	
<b>SCREENING OF PESTICIDES AND MYCOTOXINS TRANSFER FROM FEED INTO EDIBLE INSECTS</b>	
Jingwen Han <sup>(1)</sup> , Dana Schusterova <sup>(1)</sup> , Tereza Dolezalova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	627
<b>V21</b>	
<b>COMPARISON OF SPE- AND QUECHERS-BASED EXTRACTION FOR THE ANALYSIS OF PESTICIDE METABOLITES IN HUMAN URINE</b>	
Kamila Stehnova <sup>(1)</sup> , Darina Dvorakova <sup>(1)</sup> , Denisa Parizkova <sup>(1)</sup> , Jana Pulkrabova <sup>(1)</sup> .....	628
<b>V22</b>	
<b>POST-HARVEST PROCESSING TECHNOLOGY OF POPPY TO ENSURE QUALITY AND SAFETY OF FINAL PRODUCT - PILOT STUDY</b>	
Dana Schusterova <sup>(1)</sup> , Katerina Sebelova <sup>(1)</sup> , Petr Mraz <sup>(1)</sup> , Leos Uttl <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	629
<b>V23</b>	
<b>MONITORING OF PESTICIDES IN OILSEEDS AND THEIR TRANSFER INTO OIL</b>	
Petr Mraz <sup>(1)</sup> , Leos Uttl <sup>(1)</sup> , Dana Schusterova <sup>(1)</sup> , Vladimir Kocourek <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	630

# CONTENT

<b>V24</b>	
<b>ARE FREEZE-DRIED STRAWBERRIES AND RASPBERRIES REALLY A HEALTHY SNACK?</b>	
<i>Eva Czmorik Ludvickova</i> <sup>(1)</sup> , Dana Schusterova <sup>(1)</sup> , Petr Mraz <sup>(1)</sup> , Zbynek Dzuman <sup>(1)</sup> , Jana Ryparova Kvirencova <sup>(1)</sup> , Vladimir Kocourek <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> , Vojtech Hrbek <sup>(1)</sup> .....	631
<b>V25</b>	
<b>FORMAT COMPARISON FOR DISPERSIVE SOLID PHASE EXTRACTION (DSPE) WORKFLOW IMPROVEMENTS FOR FOOD TESTING PRIOR TO GC/MS ANALYSIS</b>	
<i>Thomas Smith</i> <sup>(1)</sup> , Lucy Richards <sup>(1)</sup> , Lee Williams <sup>(1)</sup> , Geoff Davies <sup>(1)</sup> , Russell Parry <sup>(1)</sup> , Alan Edgington <sup>(1)</sup> , Adam Senior <sup>(1)</sup> , Helen Lodder <sup>(1)</sup> , Charlotte Hayes <sup>(1)</sup> , Zainab Khan <sup>(1)</sup> , Claire Desbrow <sup>(1)</sup> , Dan Menasco <sup>(1)</sup> .....	632
<b>V26</b>	
<b>ENANTIOSELECTIVE SEPARATION OF LAMBDA- AND GAMMA-CYHALOTHRIN BY LC-MS/MS FOR CONTROLS OF AGRICULTURAL PRACTICE</b>	
<i>Pavlna Landova</i> <sup>(1)</sup> , Kristyna Ondrova <sup>(1)</sup> , Petra Kosubova <sup>(1)</sup> .....	633
<b>V27</b>	
<b>INNOVATIVE PESTICIDE SCREENING IN COMPLEX MATRICES: THE CASE FOR HIGH RESOLUTION ION MOBILITY MASS SPECTROMETRY</b>	
Oscar Cabrices <sup>(1)</sup> , Julie Wushensky <sup>(2)</sup> , Miriam Fico <sup>(2)</sup> , <i>Frederick Strathmann</i> <sup>(2)</sup> , Mario Stefanelli <sup>(3)</sup> .....	634
<b>V28</b>	
<b>"BEYOND TARGETED MONITORING: DUAL APPROACH FOR THE EARLY DETECTION OF TOXICOLOGICAL RISKS IN AGRICULTURAL PLOTS USING HIGH RESOLUTION MASS SPECTROMETRY"</b>	
<i>Flavia Barragan</i> <sup>(1)</sup> , Jesse L. Calvillo <sup>(1)</sup> , Jose M. Perez <sup>(1)</sup> , Oliver Stolper <sup>(1)</sup> .....	635
<b>V29</b>	
<b>DIFFERENT PESTICIDE TREATMENT STRATEGIES IN LOCALLY GROWN AND IMPORTED APPLES IN THE CZECH MARKET</b>	
<i>Petra Vackova</i> <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> , Petr Mraz <sup>(1)</sup> , Leos Uttl <sup>(1)</sup> , Vladimir Kocourek <sup>(1)</sup> .....	636
<b>V30</b>	
<b>DETERMINATION OF PESTICIDES USING GC-MS/MS WITH HYDROGEN AS AN ALTERNATIVE CARRIER GAS</b>	
<i>Uwe Oppermann</i> <sup>(1)</sup> , Waldemar Weber <sup>(1)</sup> , Dominika Gruszecka <sup>(2)</sup> , Victor Cutillas <sup>(3)</sup> , Chigusa Ichikawa <sup>(4)</sup> , Alan Owens <sup>(2)</sup> , Yoshiro Hiramatsu <sup>(2)</sup> .....	637
<b>V31</b>	
<b>HIGHLY SENSITIVE ANALYSIS OF GLYPHOSATE, GLUFOSINATE, AND THEIR METABOLITES IN SOYBEANS USING ONLINE SOLID-PHASE DERIVATIZATION LC-MS/MS SYSTEM</b>	
Nozomi Maeshima <sup>(1)</sup> , Ryoichi Sasano <sup>(2)</sup> , Jun Watanabe <sup>(1)</sup> , Isao Saito <sup>(2)</sup> , Manami Kobayashi <sup>(1)</sup> , Hiroshi Akiyama <sup>(3)</sup> , <i>Stephane Moreau</i> <sup>(4)</sup> .....	638
<b>RESIDUES - VETERINARY DRUGS.....</b>	<b>639</b>
<b>W1</b>	
<b>RESIDUE DEPLETION OF NITROFURAZONE METABOLITES IN BROILER CHICKENS AFTER ORAL ADMINISTRATION</b>	
<i>Khaled El Hawari</i> <sup>(1)</sup> , Jean-François Taillandier <sup>(1)</sup> , Pierre Guichard <sup>(1)</sup> , Michel Laurentie <sup>(1)</sup> , Dominique Hurtaud-Pessel <sup>(1)</sup> , Eric Verdon <sup>(2)</sup> .....	640
<b>W2</b>	
<b>ITERATIVE DEVELOPMENT AND FINAL VALIDATION OF AN AUTOMATED METHOD FOR THE JOINT DETERMINATION OF 96 VETERINARY DRUGS FROM VARIOUS MATRICES BY LC-HRMS</b>	
<i>Nico Landsteiner</i> <sup>(1)</sup> , Clara John <sup>(1)</sup> , Jana Lücken <sup>(1)</sup> , Sonja Schittko <sup>(1)</sup> , Mareike Reichel <sup>(1)</sup> , Jan Sebastian Maenz <sup>(1)</sup> , Klaudija Cavlovic <sup>(1)</sup> .....	641
<b>W3</b>	
<b>QUANTITATIVE ANALYSIS OF ANDROGENS AND ESTROGENS IN BOVINE AND PIG URINARY SAMPLES BY LC MS/MS: NEW ASPECTS IN VETERINARY DRUG RESIDUE ANALYSIS</b>	
<i>Thomas Meikopoulos</i> <sup>(1)</sup> , Mikael Pedersen <sup>(1)</sup> .....	642
<b>W4</b>	
<b>DETERMINATION OF CHLORPROMAZINE IN ANIMAL-DERIVED FOODS USING QUECHERS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)</b>	
<i>Pak-wing Ricky Kong</i> <sup>(1)</sup> , Chi-shing Ng <sup>(1)</sup> .....	643
<b>W5</b>	
<b>DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF 21 ANTIVIRAL DRUGS IN CHICKEN MUSCLE AND LIVER</b>	
Ana Castaneira-Landeira <sup>(1)</sup> , <i>Samantha Sasse</i> <sup>(1)</sup> , Melissa Broeren <sup>(1)</sup> , Saskia Sterk <sup>(1)</sup> , Ane Arrizabalaga-Larranaga <sup>(1)</sup> .....	644

# CONTENT

<b>W6</b>	<b>ANABOLIC STEROIDS INDUCED CHANGES AT THE LEVEL OF PROTEIN EXPRESSION: EFFECTS OF PROLONGED ADMINISTRATION OF TESTOSTERONE AND NANDROLONE TO PIGS</b>	
	<i>Kristina Tosnerova</i> <sup>*<sup>(1)</sup></sup> , Jan Gebauer <sup>(1)</sup> , Nikola Hodkovicova <sup>(1)</sup> , Kristyna Skoupa <sup>(2)</sup> , Andrej Batik <sup>(2)</sup> , Iva Bartejsova <sup>(1)</sup> , Michaela Charvatova <sup>(1)</sup> , Lenka Leva <sup>(1)</sup> , Lea Jarosova <sup>(1)</sup> , Zbysek Sladek <sup>(2)</sup> , Martin Faldyna <sup>(1)</sup> , Kamil Stastny <sup>(1)</sup> .....	645
<b>W7</b>	<b>DEVELOPMENT AND VALIDATION OF AN INDIRECT COMPETITIVE LATERAL FLOW IMMUNOASSAY FOR THE DETECTION OF ACETAMINOPHEN (PARACETAMOL) IN BOVINE URINE</b>	
	<i>Samantha Sasse</i> <sup>*<sup>(1)</sup></sup> , Ariadni Geballa-Koukoulou <sup>(1)</sup> , Toine Bovee <sup>(1)</sup> .....	646
<b>W8</b>	<b>FAST SIMULTANEOUS DETERMINATION OF 23 VETERINARY DRUG RESIDUES IN FISH, POULTRY, AND RED MEAT BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY</b>	
	Ahmed Aljaber <sup>*<sup>(1)</sup></sup> , Saeed Alzahrani <sup>(1)</sup> .....	647
<b>W9</b>	<b>DETERMINATION OF 14 AMINOGLYCOSIDES IN FOODSTUFFS BY LC-MS/MS USING MOLECULARLY IMPRINTED POLYMER SOLID PHASE EXTRACTION</b>	
	<i>Michel Arotçarena</i> <sup>*<sup>(1)</sup></sup> , Sami Bayoudh <sup>(1)</sup> , Kaynoush Naraghi <sup>(1)</sup> .....	648
<b>W10</b>	<b>CONFIRMATION OF FIVE NITROFURAN METABOLITES BY LC-MS/MS IN VARIOUS FOOD MATRICES OF ANIMAL ORIGIN ACCORDING TO REGULATION (EU) 2021/808</b>	
	<i>Pierre Guichard</i> <sup>*<sup>(1)</sup></sup> , Sophie Gautier <sup>(1)</sup> , Dominique Hurtaud-Pessel <sup>(2)</sup> , Eric Verdon <sup>(3)</sup> , Laura Suaudeau <sup>(4)</sup> .....	649
<b>W11</b>	<b>A UHPLC-MS/MS METHOD FOR THE DETERMINATION OF ANTIPARASITIC DRUGS, INCLUDING AVERMECTINS, IN AVIAN EGGS, FEATURING A NOVEL STANDARD ADDITION APPROACH TO VALIDATION</b>	
	<i>Philip Mullen</i> <sup>*<sup>(1)</sup></sup> , Cheryl Rainey <sup>(1)</sup> , Matthew Walker <sup>(1)</sup> , Anna Gadaj <sup>(1)</sup> .....	650
<b>W12</b>	<b>A QUICK AND EASY LC-MS/MS METHOD FOR SEDATIVES AND TRANQUILIZERS IN KIDNEY VALIDATED UNDER REGULATION (EU) 2021/808</b>	
	Eva Munoz <sup>*<sup>(1)</sup></sup> , Meritxell Castello <sup>(1)</sup> , Josep Calderon <sup>(1)</sup> .....	651
<b>W13</b>	<b>RAPID SCREENING DETECTION OF DNSH (NIFURSOL) RESIDUES: A COST-EFFECTIVE SOLUTION FOR ENSURING EU COMPLIANCE</b>	
	Nicole Haveron <sup>(1)</sup> , Mark Squance <sup>(1)</sup> , Nicola Kane <sup>*<sup>(1)</sup></sup> , Morteza Afrasiabi <sup>(2)</sup> .....	652
<b>W14</b>	<b>ULTRA-HIGH SENSITIVITY QUANTIFICATION OF VETERINARY DRUG RESIDUES USING LC/MS WORKFLOWS</b>	
	<i>Jianru Stahl-Zeng</i> <sup>*<sup>(1)</sup></sup> , Yoann Fillatre <sup>(1)</sup> , Gregoire Bonvin <sup>(2)</sup> , Michael Scherer <sup>(1)</sup> .....	653
<b>W15</b>	<b>AN ALTERNATIVE STATISTICAL APPROACH FOR METHOD VALIDATION UNDER EU REGULATION 2021/808</b>	
	<i>Wendy Robinson</i> <sup>*<sup>(1)</sup></sup> , Roy MacArthur <sup>(1)</sup> , Gary Dowsett <sup>(1)</sup> , Warwick Anderson <sup>(1)</sup> , Katherine Weddell <sup>(1)</sup> .....	654
<b>W16</b>	<b>DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE, MULTI-CLASS METHOD FOR EVALUATING DAIRY CATTLE EXPOSURE TO ANTIBIOTICS THROUGH FAECES ANALYSIS</b>	
	Giulia Stephany <sup>(1)</sup> , Francesca Bovo <sup>(1)</sup> , Giovanni Sette <sup>(1)</sup> , Cristiana Benetti <sup>*<sup>(1)</sup></sup> .....	655
<b>W17</b>	<b>APPLICATION OF LC-HRMS FOR OFFICIAL CONTROLS IN A NEW RANDOMISED SURVEILLANCE PLAN FOR VETERINARY MEDICINAL PRODUCT RESIDUES IN FOOD PRODUCTS IN FRANCE</b>	
	<i>Dominique Hurtaud-Pessel</i> <sup>*<sup>(1)</sup></sup> , Pierrick Couedor <sup>(1)</sup> , Eric Verdon <sup>(1)</sup> .....	656
<b>W18</b>	<b>VETERINARY MEDICINES: WHEN ONE ANTIBIOTIC MOLECULE HIDES ANOTHER!</b>	
	<i>Celine Hedou</i> <sup>*<sup>(1)</sup></sup> , Valerie Gaudin <sup>(1)</sup> , Melaine Bessiral <sup>(1)</sup> , Eric Verdon <sup>(2)</sup> .....	657
<b>W19</b>	<b>EVALUATION OF SUITABLE PROCEDURAL CALIBRATION STANDARDS FOR THE MULTI-MATRICES QUANTIFICATION OF VETERINARY DRUGS BY LC-MS/MS</b>	
	<i>Gregoire Bonvin</i> <sup>*<sup>(1)</sup></sup> , Jenette Zwygart <sup>(1)</sup> , Philippe Jan <sup>(1)</sup> , Yoric Gagnebin <sup>(1)</sup> , Patrick Edder <sup>(1)</sup> , Didier Ortelli <sup>(1)</sup> .....	658
<b>W20</b>	<b>TRANSITION AND PERSISTENCE OF DOXYCYCLINE IN ORAL FLUID AND PLASMA AFTER PER OS ADMINISTRATION IN SWINE</b>	
	<i>Ewelina Nowacka-Kozak</i> <sup>*<sup>(1)</sup></sup> , Anna Gajda <sup>(1)</sup> , Artur Jablonski <sup>(2)</sup> , Małgorzata Gbylik-Sikorska <sup>(1)</sup> .....	659



# CONTENT

## W21

### TO LOOK AT CHEMICAL EXPOSURE IN A BROADER CONTEXT: MULTIDIRECTIONAL ANALYSIS OF CONTAMINANTS AND FEED ADDITIVES IN ANIMAL FEED AND WATER

Ewelina Patyra<sup>(1)</sup>, Małgorzata Warenik-Baryk<sup>(2)</sup>, Anna Gajda<sup>\*(3)</sup>, Ewelina Nowacka-Kozak<sup>(3)</sup>, Tomasz Kiljanek<sup>(3)</sup>, Katarzyna Pietruszka<sup>(3)</sup>, Konrad Pietruk<sup>(3)</sup>, Agnieszka Nawrocka<sup>(3)</sup>, Ewelina Kowalczyk<sup>(1)</sup>, Aleksandra Grelík<sup>(1)</sup>, Monika Przeniosło-Siwczyńska<sup>(1)</sup>, Krzysztof Kwiatek<sup>(1)</sup>, Jolanta G. Rola<sup>(4)</sup>, Sebastian Maszewski<sup>(2)</sup>, Szczepan Mikołajczyk<sup>(2)</sup>, Magdalena Gembał<sup>(2)</sup>, Wojciech Pietron<sup>(2)</sup>, Piotr Jedziniak<sup>(3)</sup>.....660

## W22

### THE INFLUENCE OF HEAT TREATMENTS ON THE RESIDUES OF SEVEN ANTIBACTERIALS IN BEEF MEAT

Anna Gajda<sup>\*(1)</sup>, Magdalena Bilecka<sup>(1)</sup>, Natalia Mizak<sup>(1)</sup> .....661

## W23

### SIMULTANEOUS SCREENING OF 118+ VETERINARY DRUGS IN MILK AND MUSCLE USING UNTARGETED LC-HRMS ANALYSIS

Tina Angerer<sup>\*(1)</sup>, Stina Söderlund<sup>(1)</sup>, Martina Degerman<sup>(1)</sup>, Samantha Madawala<sup>(1)</sup>, Petter Englund<sup>(1)</sup> .....662

## W24

### THE COST ACTION BESAFEBEEHONEY: A PROMISING NETWORK TO PROMOTE THE SAFETY OF BEES AND HONEY AND THE SGL'S ENGAGEMENT

Maria Strouthou<sup>(1)</sup>, Christopher Papachrysostomou<sup>(1)</sup>, M Leite<sup>(2)</sup>, Andreia Freitas<sup>\*(3)</sup> .....663

## SENSORS & PORTABLE FOOD ANALYSIS..... 664

### X1

#### ADVANCED INFRARED SPECTROSCOPIC TECHNOLOGIES: TOWARDS ON-SITE ANALYSIS OF MYCOTOXINS IN CEREALS

Polina Fomina<sup>\*(1)</sup>, Antoni Femenias<sup>(1)</sup>, Miriam Aledda<sup>(2)</sup>, Stephan Freitag<sup>(3)</sup>, Michael Sulyok<sup>(3)</sup>, Achim Kohler<sup>(2)</sup>, Rudolf Krska<sup>(3)</sup>, Boris Mizaikoff<sup>(1)</sup>.....665

### X2

#### AUTOMATED AND SENSITIVE ALLERGEN DETECTION WITH A PORTABLE MICROFLUIDIC PLATFORM INTEGRATING SAMPLE PREPARATION AND A SMART IMMUNOASSAY

Maxime Gavage<sup>\*(1)</sup>, Bastian Breiner<sup>(2)</sup>, Daniel Kainz<sup>(2)</sup>, Stefan Wagner<sup>(2)</sup>, Serhat Sahakalkan<sup>(2)</sup>, Riccardo Marega<sup>(1)</sup> .....666

### X3

#### ENZYME-FREE AMPLIFICATION DETECTION OF NOROVIRUS (NOV) RNA USING HYBRIDIZATION CHAIN REACTION AND GOLD NANOPARTICLES

Prabir Kumar Kulabhusan<sup>\*(1)</sup>, Andrey Ipatov<sup>(1)</sup>, Marta Prado<sup>(2)</sup>.....667

### X4..... 668

#### A SENSITIVE AND FAST TETRODOTOXIN LATERAL FLOW BIOSENSOR BY ANTIBODY-APTAMER SANDWICH ASSAY

Ulises Guillermo Díaz Avello<sup>(1)</sup>, Vasso Skouridou<sup>(1)</sup>, Monica Campas<sup>(2)</sup>, Ciara Kathleen O'Sullivan<sup>\*(1)</sup> .....668

### X5

#### MINIATURIZED DEVICE FOR POINT-OF-CARE TESTING OF FOODBORNE PATHOGENS

Ana Costa-Ribeiro<sup>\*(1)</sup>, Sofia Araujo Pereira<sup>(1)</sup>, Alexandre Lamas<sup>(2)</sup>, Alejandro Garrido-Maestu<sup>(3)</sup>, Marta Prado<sup>(2)</sup>, Adrian Sanchez-Visedo<sup>(1)</sup>.....669

### X6

#### NOVEL BIO-SENSING TECHNOLOGY FOR RAPID AND COST-EFFECTIVE ANALYSES OF BOAR TAINT COMPOUNDS AT THE POINT OF TEST

Olena Doran<sup>\*(1)</sup>, Adrian Crew<sup>(1)</sup>, Kelly Westmacott<sup>(1)</sup>, John Hart<sup>(1)</sup>.....670

### X7

#### APPLICATION OF FLUORESCENCE SPECTROSCOPY TO DETECT COLD CHAIN DISRUPTIONS DURING STORAGE OF MODIFIED-AIR PACKAGED MINCED PORK

Johannes Schlosser<sup>(1)</sup>, Martin Mühling<sup>(2)</sup>, Heinar Schmidt<sup>\*(3)</sup> .....671

### X8

#### FLOWSENSE: SINGLE AND MULTIPLEX ON-SITE TESTING FOR DETECTION OF ANTIBIOTICS AND MYCOTOXINS

Mark Squance<sup>(1)</sup>, Kielan Toal<sup>(1)</sup>, Nicola Kane<sup>\*(1)</sup>, Morteza Afrasiabi<sup>(2)</sup> .....672

### X9

#### RECEPTOMIX: A CUSTOMIZABLE MULTIPLEX RECEPTOR BIOCHIP FOR THE ANALYSIS OF FLAVOUR AND HEALTH PROPERTIES OF FOOD INGREDIENTS

Lorenzo Corsico<sup>\*(1)</sup>, Maurice Henquet<sup>(2)</sup>, Aria Samimi<sup>(1)</sup>, Margriet Roelse<sup>(2)</sup>, Maarten Jongmsma<sup>(2)</sup>.....673

### X10

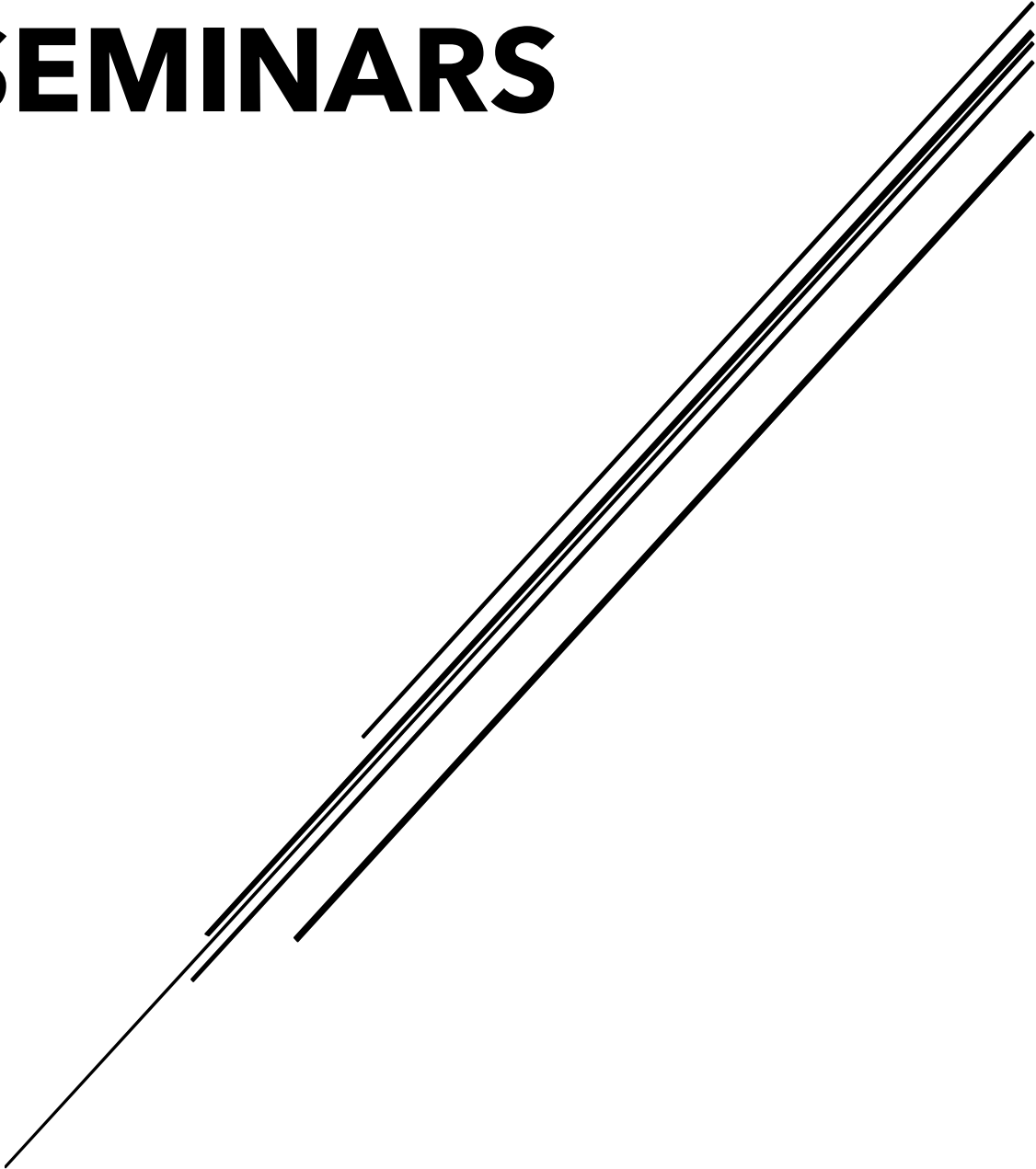
#### ENHANCING ESCHERICHIA COLI DETECTION: INTEGRATING HYBRID PMMA/PAPER MICROFLUIDIC DEVICE FOR DNA PURIFICATION, AMPLIFICATION AND COLORIMETRIC DETECTION

Sofia Araujo Pereira<sup>(1)</sup>, Ana Costa-Ribeiro<sup>(1)</sup>, Rui Campos<sup>(1)</sup>, Andrey Ipatov<sup>(1)</sup>, Adrián Sanchez-Visedo<sup>\*(1)</sup> .....674

# CONTENT

<b>X11</b>	
<b>RAPID AND PORTABLE PYRROLIZIDINE ALKALOID SCREENING IN OREGANO USING SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)</b>	
<i>Aristeidis S. Tsagkaris</i> <sup>*(1)</sup> , Ondrej Brabenec <sup>(1)</sup> , Cinzia Cafarella <sup>(1)</sup> , Jana Pulkrabova <sup>(1)</sup> , Jana Hajslova <sup>(1)</sup> .....	675
<b>X12</b>	
<b>NEXTGENMICROFLUIDICS: HIGHLY-INTEGRATED, MODULAR LAB-ON-A-CARTRIDGE DEVICES TOWARDS MULTIPLEXED CONTAMINANT/ANALYTE DETECTION FOR FOOD SAFETY AND QUALITY MONITORING</b>	
<i>George Tsekenis</i> <sup>*(1)</sup> , Nikitas Melios <sup>(1)</sup> , Joerg Nestler <sup>(2)</sup> , Jan Kafka <sup>(3)</sup> , Mirko Lohse <sup>(4)</sup> , Willi Weigel <sup>(5)</sup> , Claude Leiner <sup>(6)</sup> , Martin Smolka <sup>(6)</sup> , Pakapreud Khumwan <sup>(7)</sup> , Jenny Graunitz <sup>(8)</sup> , Anja Haase <sup>(6)</sup> , Jan Hesse <sup>(6)</sup> .....	676
<b>X13</b>	
<b>VASTLY IMPROVED LEAD TIME FOR APTASENSOR DEVELOPMENT THROUGH THE UNCONVENTIONAL USE OF CLASSICAL BIOPHYSICAL TECHNIQUES IN APTAMER SEQUENCE SELECTION, OPTIMIZATION AND ASSAY INTEGRATION</b>	
<i>George Tsekenis</i> <sup>*(1)</sup> .....	677
<b>INDEX-AUTHORS .....</b>	<b>678</b>

# **VENDOR SEMINARS**



## VENDOR SEMINARS

**November 6, 2024 (7:45-8:30)**



### VENDOR SEMINAR:

## **CCS - Catching Contaminants Speedily: The role of ion mobility in rapid contaminant detection for food and feed safety**

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### **CCS - Catching Contaminants Speedily: The role of ion mobility in rapid contaminant detection for food and feed safety**

#### **Nicholas J. Birse**

*Queen's University Belfast, Institute for Global Food Security, School of Biological Sciences, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland, UK*

Per- and polyfluoroalkyl substances (PFAS) are classes of chemicals, numbering almost 5,000 unique compounds, that can be found in a wide variety of products and processes essential to modern life. These classes of chemicals are widely used as they have desirable properties, such as imparting water or friction resistance, meaning the products and processes for which they're used are extremely wide reaching, from waterproof jackets to frying pans, fire extinguishers to carpets. The desirable properties extend to the long-lasting nature of PFAS compounds, which are extremely resistance to typical types of degradation, such as UV or ozone exposure, or mechanical stress.

The long-lasting nature of PFAS and their inclusion in a wide variety of industrial processes mean the escape of PFAS compounds into the environment was widespread until more recent times and today they can appear with alarming frequency in all types of food and animal feeds.

Testing complex food and feed matrices for PFAS retains the typical food and feed analysis problems of needing relatively slow chromatography to achieve the desired separations required to enable good compound identification, whilst needing rapid speeds to enable the high throughputs essential for good representative sampling.

Ion mobility has been demonstrated in recent years as a way to resolve compounds that cannot otherwise be separated sufficiently through chromatography and/or high-resolution mass spectrometry. Ion mobility works by determining a third dimension, the collisional cross section (CCS) value of an analyte, rather than just retention time and/or accurate mass.

In this workshop, MOBILion's high resolution ion mobility MOBIE platform for the Agilent 6545, 6545XT and 6546 mass spectrometers will be presented and its use in a rapid untargeted workflow focusing on the detection of PFAS in food and feed matrices will be discussed, with key considerations on the experimental design and method optimization requirements needed to both improve speed of analysis and/or achieve separation of analytes that are difficult or impossible to separate without the use of an ion mobility enabled mass spectrometer.

## VENDOR SEMINARS

November 6, 2024 (7:45-8:30)



### VENDOR SEMINAR:

## Expect the unexpected - Climate change and (emerging) mycotoxins

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### Expect the unexpected - Climate change and (emerging) mycotoxins

Julie Brunkhorst, Ronald Niemeijer, Emilee Easter, Jordan Steinberg

*Trilogy Analytical Laboratory*

**Holly Lee**

*Sciex*

Climate change is increasingly recognized as a critical factor influencing the prevalence and distribution of mycotoxins, toxic compounds produced by certain fungi that pose significant health risks to humans and animals. This workshop explores the complex relationship between changing climatic conditions and mycotoxin contamination in agricultural commodities. We will discuss how rising temperatures, and altered precipitation patterns, affect fungal growth and mycotoxin occurrence. Additionally, the presentation will address the emergence of new mycotoxigenic fungi in regions previously unaffected, driven by climate-induced shifts in environmental conditions.

Many studies have been performed in more recent years that are showing that mycotoxins are occurring in areas of the world that historically have not had contamination such as Aflatoxin in wheat in Europe. Mycotoxins are also occurring due to storage conditions from shipping containers that are held up for months. Advances in technology have also proven that it is common to have multiple mycotoxins occur in samples.

We will present a multi-mycotoxin analytical method, including emerging mycotoxins in cereals, finished feeds and food matrices by liquid chromatography-Tandem Quadrupole Mass Spectrometry with lower limits of detection, as well as a toolbox of quality assurance tools called QualiT™.

This method development and study objective was to quantitatively detect multiple mycotoxins in cereals, finished feed and food products at lower limits of detection. As mycotoxin regulations have been lowered in some countries around the world and emerging mycotoxins detected, a method that could extract and detect these at lower levels was developed.

## VENDOR SEMINARS

**November 6, 2024 (13:30-14:15)**



### **VENDOR SEMINAR:**

## **Optimization strategies to deliver performant PFAS and pesticide quantitation in complex food matrices**

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**End-to-end LC/MS workflows for analysis of PFAS in meat, dairy products, and fish, through to infant foods and formulations**

**Day Powell**

*Application Scientist, Agilent Technologies, UK*

This presentation describes workflows for multi-target PFAS determination in infant and adult foods covering sample preparation steps to LC-triple quad MS detection. Sample preparation exploiting traditional QuEChERS extraction followed by novel EMR mixed-mode passthrough cleanup using Captiva EMR PFAS Food cartridges is described. An Agilent 6495D LC/MS system with modifications on the LC system using PFC-free kit with large volume sandwiched injection onto a reversed phase column is deployed to enable efficient analyte separation with sensitive detection.

**Exploiting intelligent sample reinjection within a LC-HRMS workflow to improve throughput and confidence in pesticide quantitation results**

**Nicola Cimino**

*LC/MS Product Specialist, Agilent Technologies, Italy*

Here we present the exploitation of full spectra data acquisition with Revident LC/Q-TOF in combination with fast LC gradients with data dependent, intelligent sample reinjection under different chromatographic and MS acquisition modes to maximize both throughput and confidence in the final result.

## VENDOR SEMINARS

**November 6, 2024 (13:30-14:15)**



### **VENDOR SEMINAR:**

#### **Enhancing laboratory efficiency with Biotage® workflow solutions for streamlined sample preparation from a range of food matrices**

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**Enhancing laboratory efficiency with Biotage® workflow solutions for streamlined sample preparation from a range of food matrices**

**Monika Vezse**

*EMEA Analytical Market & Specialists Manager, Thomas Smith, Application Laboratory Team Leader and Fausto Chiapparini, EMEA Distributor Sales Manager of Biotage*

This workshop will be focusing on various automated techniques that can significantly improve laboratory efficiency, by using real-life applications for analytes like acrylamide and pesticides, that have been developed in-house by our R&D teams, with reference to method EN 16618: 2015 where Biotage products were featured.

Attendees will explore advancements in sample pre-treatment with the Biotage® Lysera for matrix pulverization, followed by clean-up processes utilizing ISOLUTE® and EVOLUTE® solid-phase extraction consumables, including pre-packed ISOLUTE® cSPE for QuEChERS columns.

The session will also cover automated processing protocols with the Biotage® Extrahera™ HV-5000 workstation and solvent evaporation using the TurboVap® LV. Participants will gain practical knowledge on implementing these automated solutions to enhance precision, reduce processing times, and increase throughput in their laboratories.

## VENDOR SEMINARS

**November 6, 2024 (13:30-14:15)**



### VENDOR SEMINAR:

## **Paradigm shifts in food analysis: breakthrough solutions for allergies, authenticity and traceability**

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### **Moving towards the next generation of food allergen analysis**

#### **Jens Brockmeyer**

*Head of Department Food Chemistry, Institute of Biochemistry and Technical Biochemistry, University of Stuttgart, Germany*

The AllergenScreener solution is an advanced mass spectrometry (MS)-based technology designed to detect a wide range of allergens across various matrices in a single analytical run. This innovative solution encompasses a comprehensive workflow that includes meticulous sample preparation, high-performance liquid chromatography-mass spectrometry (HPLC-MS) analysis, and sophisticated data analysis software. By integrating these components, the AllergenScreener streamlines laboratory workflows, making the process of detecting allergens in food products more efficient and reliable. This holistic approach not only enhances the accuracy of allergen detection but also significantly reduces the time and effort required for food product analysis, thereby offering a robust tool for ensuring food safety and compliance with regulatory standards.

### **Chromatography-free screening and quantification of saffron adulteration with safflower by DART-TQ**

#### **Linda Monaci**

*Research Director at Institute of Sciences of Food Production - National Research Council of Italy*

The high price of saffron has made it a target product for adulteration with other species of lower value, such as safflower. The increasing number of samples to be monitored for authenticity assessment makes it necessary to have at disposal a rapid routine method to test the purity of saffron, thus confirming its value. Direct analysis in real time (DART), a plasma-based ambient ionization technique, permits the effective ionization of a broad range of compounds. It allows to identify, search and quantify the presence of any adulterant species in saffron as case study that will be described in the present communication. This chromatography-free technology allows a rapid automated analysis, perfect for efficient routine work.

This project involves a fast sample extraction without any sample-prep required, the identification and monitoring of potential markers of safflower as an adulterant added to saffron and the evaluation in real samples of the power of DART for quantification purposes. The work has been carried out by a simple fast workflow combining DART ionization coupled to triple quadrupole EVOQ DART-TQ\*.



## VENDOR SEMINARS

### Simultaneous EI and CI in single GC runs for confident unknowns' identification in non-target analysis

**Eliska Ceznerova**

*Application Specialist, Tofwerk Ag, Switzerland*

Non-target analyses in GC-HRMS suffer from unambiguous or insufficient scores in NIST searches of EI data and are prone to false positives. Therefore, often additional GC runs are performed with a CI source to add the molecular ion information for filtering the NIST search results or when appropriate reference standards for compounds are not available. However, that requires mechanical source switching, separate GC runs and generates issues with peak alignments. Presented here is a novel GC-HRMS which simultaneously operates an electron ionization (EI) and a chemical ionization (CI) source. Structural as well as accurate mass molecular ion information are generated in a single GC run which highly improves the confidence for identifying unknown compounds. Various studies will be discussed to prove the potential for non-targeted and suspect screening approaches, including applications in fields such as environmental contaminants, material emissions, food flavor analysis and metabolomic research.

## VENDOR SEMINARS

**November 6, 2024 (13:30-14:15)**



The Power of Precision

### **VENDOR SEMINAR:**

#### **Recent advances in LC-MS/MS techniques for food analysis**

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##### **Recent advances in LC-MS/MS techniques for food analysis**

Recent advances in LC-MS/MS technology have driven major increases in sensitivity and reduced the need for extensive sample preparation and clean-up. However, this approach risks exposing instruments to a higher matrix load and can lead to increased maintenance requirements. In order to maximise instrument uptime and ensure quick and easy user intervention, when necessary, SCIEX has launched several innovations which can help labs with a requirement for high throughput of complex matrix samples to run routine analyses with the utmost confidence.

In this seminar, we will briefly describe the latest technologies and show their performance using examples of real-world applications created with researchers from leading regulatory testing and research laboratories. We aim to demonstrate how such innovations can change the landscape of routine contaminant analysis and bring sensitive, accurate and fast analysis into routine use to meet the increasingly more demanding regulations in food safety.

Main takeaways:

- Introduction to latest LC-MS/MS technologies
- Overview of latest multi-residue mycotoxins workflows
- Examples of new applications developed for quantitative and qualitative analyses

## VENDOR SEMINARS

November 6, 2024 (13:30-14:15)



### VENDOR SEMINAR:

## "In food we trust": a closer look on aroma- and authenticity analysis

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### Can alcohol free beer be tasty?

**Erich Leitner**

*Graz University of Technology, Institute of Analytical Chemistry and Food Chemistry, Stremayrgasse 9/2, 8010 Graz, Austria  
email: erich.leitner@tugraz.at*

First trials about the production of non-alcoholic beer (NAB) is dating back till the early sixties. According to the Austrian, German and Swiss law an alcohol-free beer can have an alcohol concentration of maximum 0,5% by volume. If a beer is labelled as "alcohol free" it actually has to be 0.0%. One of reasons of limited acceptance by consumers is the impact on the flavor interactions due to the removed alcohol. Nevertheless, there is a constant increase in the market share. 2023 the total turnover in Germany for NAB was 1.6 billion € which is approximately 7% of the beer consumption.

One of the reasons for an increasing market share is the use of improved technologies resulting in more palatable products. This talk will focus on various production technologies and the characterization of aroma compounds in various NAB using different chromatographic methods and sensory analysis.

### A novel ion suppression-based MS methodology for identification and authentication of liquid samples; wine as a case study

**Maria Bikaki, Markus Ehrat**

*Genuine-Analytics AG, Solothurnerstrasse 259, 4600 Olten, Switzerland  
email: markus.ehrat@genuine-analytics.ch*

Authentication of complex samples with thousands of compounds is very often a tedious, time consuming and expensive process. To date, LC-MS is a preferred technique for the analysis of such samples due to its high sensitivity, selectivity and identification capability of a plethora of compounds. However, complex samples often require extensive sample preparation and separation before the compounds can be identified by a high-resolution MS. Whereas a simple sample preparation and direct infusions into the MS source shorten time to result, sample matrix can cause unwanted effects such as ion suppression or ion enhancement. We present a novel MS methodology for the authentication of wine. In contrary to existing methods, it is based on ion suppression or enhancement effects. The samples are mixed with a solution comprising multiple chemical compounds, which then are directly measured by ESI-MS in selected positive ion monitoring (SIM) mode. The impact of the wine matrix on the ionization of the added compounds is detected generating a unique MS signal pattern for each sample. For identification, this signal pattern is compared with the reference wine patterns in our data library. This methodology requires a single quadrupole MS, it has a run time of only 1.5 minutes, and it can be used to reliably determine the origin, vintage and producer of the wine.

## VENDOR SEMINARS

**November 6, 2024 (14:45-15:30)**



### **VENDOR SEMINAR:**

#### **SamplePrep - improved analysis of organic pollutants in food products (pesticide residues) sample preparation by optimising bulk sample comminution alongside automated residue extraction equipment**

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**SamplePrep - improved analysis of organic pollutants in food products (pesticide residues) sample preparation by optimising bulk sample comminution alongside automated residue extraction equipment**

**Stephan Siniscalco, Eric Smith, Paul Lynch**

*Cole-Parmer SamplePrep, Metuchen NJ, USA*

Often overlooked the sample preparation step of any analytical method remains one of the most critical parts for achieving 'good' analytical results. We view good analytical results as those which demonstrate both accuracy and precision. Following the global use of the Cole-Parmer Geno/Grinder 2010 for the extraction and clean up step of QuEChERS, Cole-Parmer has gone on to develop a unique and versatile cryogenic bulk homogenizer called the Cryo-Blade.

The Cryo-Blade is an ultra-high-capacity cryogenic grinder for comminution and processing capability up to 1 kg. The homogeneity of the sample is very efficient. Only 1 g is needed as a representative test sample from a 1 kg ground batch. The high-throughput capability saves valuable time for your laboratory and meets Food and Drug Administration (FDA) regulations. The Cryo-Blade automatically dispenses liquid nitrogen to allow the sample to reach cryogenic temperatures and then activates a grinding phase to pulverize samples, resulting in repeatable and reproducible sample preparation. No liquid nitrogen handling required. When liquid nitrogen is not desired or available, the unit can be used with dry ice to achieve desired results.

## VENDOR SEMINARS

**November 6, 2024 (14:45-15:30)**



**EMPOWERING RESULTS**

*Elemental Analysis | GC Mass Spectrometry | Metallography*

### **VENDOR SEMINAR:**

#### **Advanced solutions for complex matrix multi-residues analysis**

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Despite great advances in mass spectrometry and sample preparation some complex food matrices still prove difficult to analyse, such as dried pepper, turmeric, and chilli.

This seminar will address how even the most challenging residue analyses in complex food matrices can be tackled using state of the art GCxGC-TOFMS technology. Learn about how LECO's new industry leading instrumentation and software provide enhanced insights and high results confidence in difficult applications including MOSH/MOAH and pesticides analysis. The renowned sector expertise leaders, Giorgia Purcaro and Michal Stupak will describe their latest developments and approaches in solving complex residue analysis challenges in two insightful presentations:

##### **The role of GCxGC in MOSH & MOAH analysis**

**Giorgia Purcaro, Grègory Bauwens, Aleksandra Gorska, Paula Albendea**

*Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés, 2, Gembloux, B-5030, Belgium*

GCXGC is vital in addressing the limited separation performances of the current 1D LC-GC-FID MOSH MOAH reference method in complex matrices, allowing thorough characterization and resolution of possible coelutions which can otherwise impede accurate MOH quantification. Giorgia will describe current best practices which increase quantification accuracy, whilst ensuring alignment with the analytical criteria established in the most recent European Food Safety Authority opinion.

##### **New strategies for pesticide residue analysis in chilli peppers using GCxGC-TOFMS**

**Michal Stupak, Jingwen Han, Jana Hajslova**

*Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Technicka 3, 16628, Prague, Czech Republic*

Chilli peppers are widely used in many food stuffs and are among imported foods which have recently been reported via the EU rapid alert system for food & feed (RASFF) due to excessive pesticide levels observed from some origins. Chilli peppers present significant analytical challenges for residue analysis due to high matrix complexity, particularly regarding high concentrations of capsaicinoids and alkaloids.

Michal Stupak will present the latest developments in addressing these challenges using the latest ultra-high sensitivity GCxGC-TOFMS technology, the Pegasus BTX 4D, to provide excellent separation and low LOQ detection capabilities and deliver high quality, accurate results.

## VENDOR SEMINARS

November 6, 2024 (14:45-15:30)



### VENDOR SEMINAR:

## **Out of the routine - Automated analysis of contaminants like MOSH/MOAH and Mycotoxins in a contract lab**

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**New automated workflow for the analysis of MOSH/MOAH in different food matrices**

**Sebastian Wißmüller**

*Food Chemist, Institute Burkon, Raudtener Str 19, 90475 Nürnberg, Germany*

The analysis of MOSH/MOAH has become a standard parameter to be analyzed in a lot of food matrices, not only in edible oil and fat. Chemical Institute Burkon is doing MOSH/MOAH analysis for several years now. In routine analysis, manual sample workup and saponification is used to prepare for subsequent LC-GC-FID analysis using epoxidation with mCPBA. As part of a project, the "Automated Workflow" from Trajan Scientific and Medical / AxelSemrau was used to analyse different matrices applying automated sample preparation/saponification an epoxidation with performic acid in chloroform. The parameter setup and the results of the manual method vs automated method are presented. After further improvements to the epoxidation procedure by Marco Nestola from Trajan first validation data are shown for spiked samples using of 1-chlorbutane and hydrogen peroxide 30 % as reagents.

**Validation of an online  $\mu$ SPE system for the analysis of mycotoxins in food**

**Sebastian Wißmüller**

*Food Chemist, Institute Burkon, Raudtener Str 19, 90475 Nürnberg, Germany*

The analysis of mycotoxins is an important parameter in each food lab and belongs to the most requested analytes. Therefore, highly efficient methods are important for type of analysis. To achieve this, Institute Burkon introduced an online coupled  $\mu$ SPE system to analyze these parameters. The lecture presents the key considerations for the use of the automatization for the analysis of the aflatoxins B1, B2, G1 and G2 at Chemical Institut Burkon. Further some validation data for four matrices, in accordance with Commission Regulation (EU) 2023/2782 are reported as well as the experience of the users with the system after half a year in routine analysis.

## VENDOR SEMINARS

November 6, 2024 (14:45-15:30)



### VENDOR SEMINAR:

## The latest advancements in trace elemental analysis and isotope fingerprints in food authenticity

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### ICP-MS workflow for enhanced efficiency and productivity

Matthew Cassap

*Thermo Fisher Scientific, Hemel Hempstead, UK*

The new Thermo Scientific™ iCAP™ MX Series ICP-MS systems consistently produces accurate data with maximized instrument uptime. The systems powerful detection capabilities enable right time results without compromising matrix robustness to simplify your food analysis, even with the most challenging samples. To assess the suitability of the iCAP MX Series ICP-MS systems for the elemental profiling of food, a wide range of samples were analyzed over extended periods of time. We will present the data from these studies and demonstrate how the iCAP MX Series ICP-MS can transform your elemental analysis workflows to consistently deliver accurate and precise results.

### The next generation LC-IRMS for honey authenticity investigation

Mario Tuthorn

*Thermo Fisher Scientific, Bremen, Germany*

The new Thermo Scientific™ LC IsoLink™ II IRMS System delivers a reliable, robust and efficient solution for high precision honey fraud detection using carbon isotope fingerprint. The next generation LC IsoLink II Conversion Interface is now fully integrated in the innovative Thermo Scientific™ Vanquish™ LC platform with modular pull-out design that is saving space and allows easy accessibility of all system parts. The new cartridge-based oxidation reactor minimizes flow path blockage and significantly enhances system uptime and productivity. We have tested the LC IsoLink II IRMS System in analytical food testing laboratories for over 2 years, allowing thorough assessment of long-term stability, system robustness and data reproducibility. Here we report data demonstrating excellent precision and reproducibility of  $\delta^{13}\text{C}$  values for measurements of a laboratory honey standard and commercial honey samples.

## VENDOR SEMINARS

**November 7, 2024 (7:45-8:30)**



### VENDOR SEMINAR:

## **Precision and innovation: Inert columns in mycotoxin and pesticide analysis & automated alkaloid analysis in honey**

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**Honey, we shrunk the analysis! - A case study on the automation and miniaturization of the analysis of pyrrolizidine alkaloids in honey**

**Friedericke Habedank**

*State Office for Agriculture, Food Safety and Fisheries Mecklenburg-Western Pomerania, Thierfelderstraße 18, 18059 Rostock, Germany*

The demand for automation in laboratory processes is growing rapidly due to workforce shortages and significant advancements in robotics. Concurrently, the drive towards miniaturization poses unique challenges. Rising chemical costs and the imperative for green chemistry, such as reducing waste, underscore the need for innovative solutions.

One pertinent example is the detection of pyrrolizidine alkaloids (PAs) in honey. The investigation of PAs in honey is crucial due to their hepatotoxic, genotoxic, and carcinogenic properties, which can pose significant health risks to consumers. As natural contaminants originating from certain plant species, PAs can enter the food supply through honey, necessitating monitoring.

In this seminar we would therefore like to present a case study and a solution for automation and miniaturization for the detection of PAs from honey.

## **Unlocking precision: The power of inert columns in mycotoxin and pesticide analysis**

**Tina Brandscher**

*Restek GmbH, Schaberweg 23, 61348 Bad Homburg v.d.H. Germany*

In Mycotoxin and Pesticide analysis as well as in plant toxin analysis, screening methodologies are normally used. In these multi-compound analyses, the peak form is crucial to identify and quantify your analytes of interest.

Those multi-compound screenings include a variety of chemical classes with different and sometimes difficult behaviour. For example, a lot of metal-sensitive compounds tend to interact with active spots of the metal surface in the analytical pathway of an LC-Instrument or are even forming chelates with these surfaces. Most of these active surfaces are in the column and not in the instrument itself, especially in the frits at the beginning and the end of the column.

In this presentation, we are showing the benefits of a CVD passivation technique to minimize these interactions from the beginning, making priming of the analytical system with either matrix of expensive reference standards obsolete.



## VENDOR SEMINARS

**November 7, 2024 (13:30-14:15)**



### VENDOR SEMINAR:

## Innovative workflows for the multi-residue analysis of organic contaminants

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### **"Pesticide Smart Kit": A new comprehensive approach for multiresidue pesticide analysis**

**Valérie Thibert**

*Thermo Fisher Scientific, Villebon-Sur-Yvette, France*

Pre-configured "out of the box" pesticide workflow methods have been specifically designed and optimized for multi-class pesticides analysis. These solutions include the hardware, software, built-in instrument acquisition methods, and customizable data processing methods including view settings and report templates, along with details of sample extraction and consumables for fast implementation. This new approach, which enables the detection, identification, and quantitation of up to 700 pesticides by GC-MS/MS and LC-MS/MS or LC-HRMS, combines results in a unique software user interface to confirm the identity of residues quickly and accurately, especially those amenable by both techniques. Here we will present analytical strategies related to the use of Thermo Scientific™ TSQ Altis Plus™ (LC-MS/MS) and Orbitrap Exploris™ MX (LC-HRMS).

### **Automated and high throughput PFAS workflows**

**Aristide Ganci**

*Thermo Fisher Scientific, Villebon-Sur-Yvette, France*

The choice between direct injection and the need for (automated) sample preparation is an important consideration in PFAS analysis. This largely depends on the instrument's dynamic range and detection limits compared to the one required by regulation, as well as the complexity of the analyzed matrix.

We will present two innovative workflows:

- High throughput analysis of PFAS in drinking water through direct injection conducted on a high sensitivity Thermo Scientific™ TSQ Altis™ Plus, which allows for rapid analysis meeting very low regulatory limits.
- A new versatile automated sample preparation workflow based on dispersive liquid liquid micro extraction (DLLME), with extraction and pre-concentration conducted on a Thermo Scientific™ TriPlus™ RSH SMART and sample measured on an Orbitrap Exploris™ MX.

## VENDOR SEMINARS

November 7, 2024 (13:30-14:15)

# Waters™

## VENDOR SEMINAR:

### **Tackling separation challenges: Strategies for identifying natural toxin isomers and analyzing Glyphosate & Co. in complex matrices**

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During our lunch seminar, we will explore various technologies that aid in resolving separation issues. You will discover how ion mobility introduces a highly resolved third dimension to your analysis, revealing the composition of plant toxins. Additionally, you will learn how combining modern mixed-mode column chemistries with a traditional 2D-LC approach offers new opportunities for routine food testing labs analyzing Glyphosate & Co. in complex matrices.

#### **Ion mobility mass spectrometry to enhance the determination of natural toxins in food samples**

**Laura Carbonell-Rozas**

*University of Almeria, Spain*

The main challenge related to the determination of some natural toxins such as pyrrolizidine alkaloids and ergot alkaloids (EAs) using LC-MS, is the existence of numerous co-eluting isomers that lead to identical product ions. In this context, ion mobility mass spectrometry (IMS) introduces a third dimension of separation to LC-MS workflows, allowing compounds to be differentiated based on their collision cross section (CCS). This seminar will present several IMS-based strategies aimed at enhancing analytical performance in the determination of EAs and PAs across a variety of food samples, including cereals, spices, herbs, and their derivatives. We evaluated different IMS technologies, such as travelling wave-IM (TWIMS) and cyclic-IMS, in conjunction with LC and high-resolution MS (LC-IM-HRMS), to address the current challenges associated with EAs and PAs determination. We constructed and cross-validated CCS libraries for EAs and PAs across multiple laboratories and additionally, utilized machine learning to support the experimental findings. The incorporation of IMS into LC-MS methodologies helped reduce background noise, enhance the signal-to-noise ratio, and consequently, improve signal sensitivity, yielding higher quality mass spectra for compound identification. This is particularly beneficial in non-targeted analysis and suspect screening. Moreover, cyclic IMS proved to be an effective alternative when a higher resolving power was required, as was the case for some PA epimers that could not be separated by TWIMS. IMS has proven to be a powerful technique for enhancing the performance characteristics of LC-MS methods in the analysis of natural toxins within complex matrices, such as food samples and food-related products.

## VENDOR SEMINARS

### **Bringing the direct analysis of Glyphosate & Co. in complex matrices into routine food testing labs**

**Claudia Rathmann**

*Waters GmbH*

The task of analyzing Glyphosate & Co. in food without the necessity for sample derivatization appeared to be resolved with the numerous methods outlined in the QuPPE document. However, matrices such as dried lentils, lemon concentrate, or black tea challenge this assumption in routine environments. We will present a combined strategy, backed by data from renowned food testing laboratories, that leverages the unique retention capabilities of modern mixed-mode column chemistries and traditional 2D-LC chromatography. This approach effectively separates matrix interferences that suppress highly polar analytes like AMPA, MPPA, glufosinate, and glyphosate.

## VENDOR SEMINARS

November 7, 2024 (14:45-15:30)



### VENDOR SEMINAR:

## Integrated gluten management - Gluten testing along the food production chain

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Ronald Niemeijer

*R-Biopharm AG*

Ensuring the safety and compliance of gluten-free food products is crucial for consumer health, particularly for those with celiac disease or gluten sensitivity. It is estimated around 1% of the population suffers from celiac disease - a gluten-triggered auto-immune disease.

This workshop, "Integrated Gluten Management - Gluten Testing Along the Food Production," will provide a comprehensive overview of the latest advancements in gluten detection methods and integrating digital data management systems to enhance food safety and quality control.

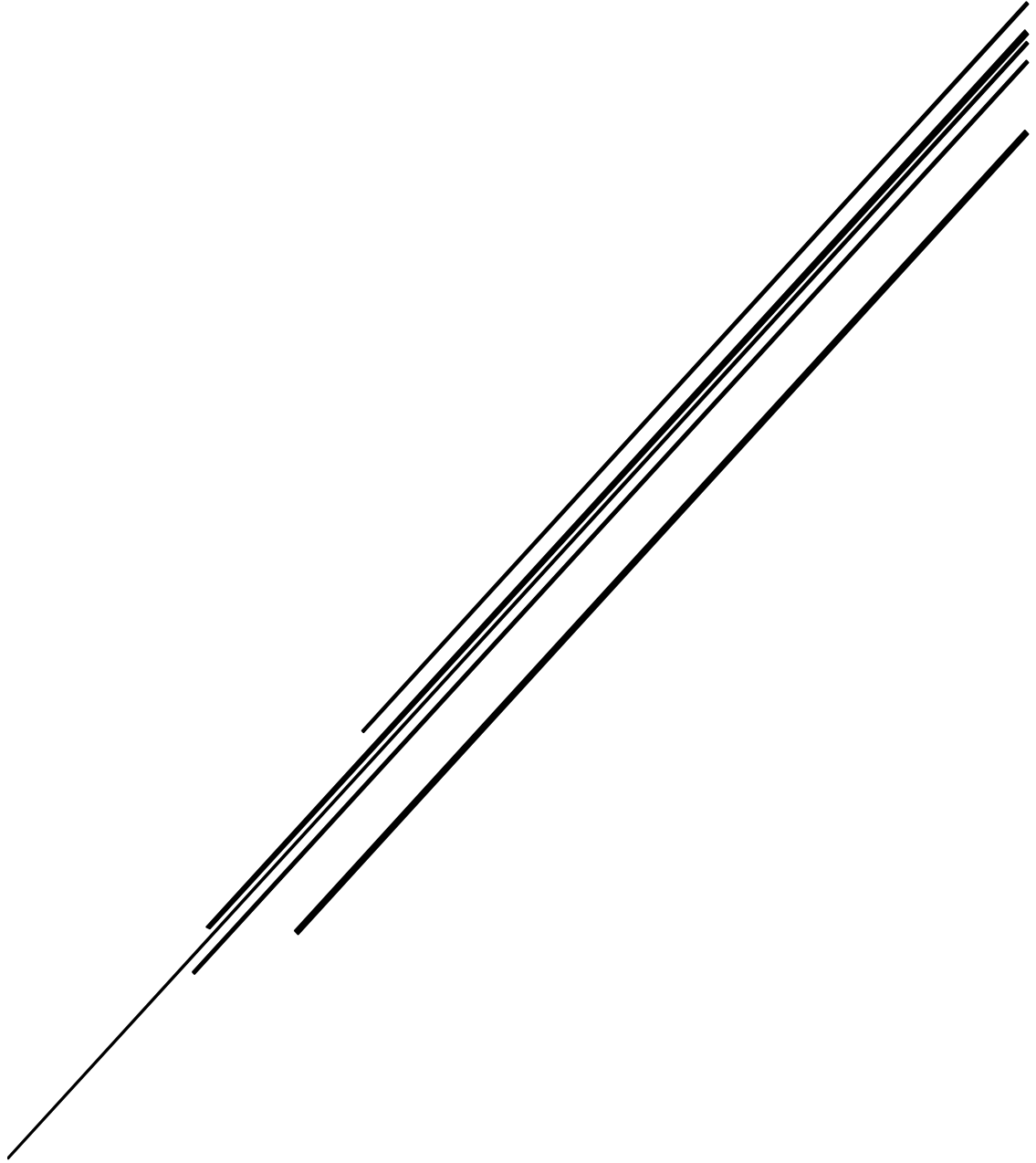
We will delve into three pivotal gluten-testing methodologies:

1. **Quantitative Lateral Flow Tests (LFDs):** These rapid and user-friendly tests offer a quantitative assessment of gluten presence, providing reliable results in a matter of minutes. LFDs are increasingly utilized for their portability and ease of use, making them ideal for on-site testing throughout various stages of food production. With the RIDA® SMART BOX or a selected smartphone results can be interpreted quantitatively using the RIDA® SMART APP.
2. **Enzyme-Linked Immunosorbent Assay (ELISA):** Renowned for its high sensitivity and specificity, ELISA remains a gold standard in gluten detection. This method allows for the precise quantification of gluten proteins in complex food matrices, ensuring rigorous compliance with regulatory standards.
3. **Polymerase Chain Reaction (PCR):** PCR-based techniques are employed to identify gluten-containing cereals at the genetic level, offering exceptional specificity and the ability to distinguish between different gluten sources (wheat, barley, and rye). This method is particularly useful in validating gluten-free claims and ensuring product integrity.

Additionally, the workshop will highlight the integration of digital data management systems (cloud-based platforms) in gluten testing with the RIDA® SMART APP. These systems facilitate real-time data collection, analysis, and sharing, enhancing traceability and compliance across the entire food production chain. This workshop aims to equip food industry professionals with the knowledge and tools necessary to implement effective gluten management strategies, attendees will gain insights into how digital solutions can streamline gluten testing processes, improve data accuracy, and support proactive food safety management.

Join us to explore the cutting-edge technologies and best practices driving the future of gluten testing and food safety. By attending this workshop, participants will gain a deeper understanding of the latest methodologies and digital innovations in gluten testing, ensuring they are well-prepared to meet the evolving demands of gluten-free product assurance.

# LECTURES



## LECTURES

### L1

## AUTHENTICITY & TRACEABILITY CLAIMS IN FOOD: HOW INDUSTRY CAN OBJECTIVATE THEM THROUGH ANALYTICAL STRATEGIES

**Michele Suman**\*<sup>(1)</sup>

<sup>1)</sup> A - Barilla G. R. F.lli SpA, Advanced Laboratory Research, via Mantova 166, 43122, Parma, Italy; B - Department for Sustainable Food Process, Catholic University Sacred Heart, Piacenza, Italy, Italy

\*Corresponding author - E-mail: michele.suman@barilla.com

As global food chains lengthen and grow in complexity, and also the geopolitical scenario is increasingly complicated, it is progressively challenging to ensure that food products remain authentic and of high-quality.

Food and beverage producers must comply with relevant food safety and authenticity regulations to guarantee products will not pose a risk to consumers and avoid food adulteration and fraud risks that may affect brand reputation and threaten financial stability.

In addition, in the digital age, consumers spend large amounts of time reading on social media, trying to understand and assess the narratives and opinions to make informed choices regarding claims.

For instance, geographical origin of ingredients and food products is nowadays a relevant aspect for high-quality food characterization and consumers' needs as well. This is beside the growing interest into variety claims and demonstrable assurances that the label is fully consistent with the content.

Detecting and measuring food fraud is a complicated task for a very wide range of food ingredients may be affected by numerous potential adulterants, many of which are yet unknown.

This scenario could be faced with the use of both rapid screening and confirmatory analytical solutions that can be implemented, within an overall vulnerability assessment and mitigation plan strategy, by food industries.

Several applied research activities, devoted to addressing the corresponding needs and concerning different food chains (e.g. egg products, extra virgin olive oils, wheat...), will be illustrated as case-study examples.

**Keywords:** authenticity, traceability, claims, industry, analytical strategies

## LECTURES

### L2

## AI IN FOOD SAFETY: FROM METHODOLOGY TO APPLICATIONS

**Bas Van der Velden**<sup>\*(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: [bas.vandervelden@wur.nl](mailto:bas.vandervelden@wur.nl)

AI has the potential to revolutionize food safety by enhancing the accuracy, efficiency, and reliability of monitoring and testing processes. This talk will describe the foundational principles of artificial intelligence, including machine learning and deep learning, and will provide specific examples of how AI is being applied to improve food safety. I will explore the use of AI in various aspects of the food supply chain. Examples include the use of AI in measurement devices such as high-resolution mass spectrometry and microscopy. The presentation will also address the technical necessities for deploying AI in a research setting, including data requirements, computational resources, and integration strategies. By leveraging AI, the food industry can better predict and prevent safety hazards, ultimately ensuring a safer food.

**Keywords:** artificial intelligence, deep learning, food safety

## LECTURES

### L3

## HIGH THROUGHPUT EFFECT-DIRECTED ANALYSIS FOR IDENTIFICATION OF CHEMICAL MIXTURES IN ENVIRONMENTAL AND HUMAN SAMPLES

**Marja Lamoree**\*<sup>(1)</sup>

<sup>1)</sup> *Vrije Universiteit Amsterdam, The Netherlands*

\**Corresponding author - E-mail: marja.lamoree@vu.nl*

Human exposure to manmade chemicals occurs continuously, due to the widespread presence of all kinds of chemicals in our everyday living environment (both in- and outdoors), in our water and food and in the air. Traditionally, human exposure was assessed via the targeted analysis of a limited number of chemicals that were a priori selected. Given the multitude of chemicals currently in use, this approach does not provide a realistic, comprehensive picture of human exposure.

With the technological advancements in the field of high-resolution mass spectrometry (HRMS), the feasibility of a more inclusive analysis of all chemicals present in a sample appeared as an attractive goal. The scientific community has created significant momentum in the field of suspect and nontarget screening, with a focus on data processing and evaluation.

To prioritize the mass spectrometric features in the data acquired, several approaches and workflows have developed over the years. In this presentation, I will show the added value of the implementation of in vitro bioassays for effect assessment in the Effect-Directed Analysis (EDA) setup. For identification, the signals that give rise to a biological/toxicological effect are prioritized, focusing on those compounds that indeed may present adverse effects.

In EDA, fractionation using liquid chromatography (LC) is combined with i) HRMS data acquisition and ii) high throughput in vitro bioassays measuring effects such as thyroid hormone disruption and antibiotic activity. The identification efforts in the aligned HRMS data are dedicated to those fractions (time intervals) where a response is measured in the bioassay. At the Vrije Universiteit, a high throughput, high resolution fractionation instrument was developed in-house, for microfractionation into 96/384 well plates that are typically used for the in vitro bioassays.

In this presentation, I will give examples of the use of EDA for the identification of chemicals of concern in samples from the environment-food-human continuum. For these studies, a variety of in vitro bioassays have been used, such as an antibiotic and a mutagenicity assay, assays focusing on endocrine disruption such as thyroid hormone disruption. For HRMS analysis, a quadrupole Time-of-Flight (qToF) instrument was used. Finally, the way forward including the expansion of the application of EDA with regard to matrix, the fractionation approach to expand the chemical space, but also to the type of bioassay used, will be highlighted.

**Keywords:** chemical identification, high throughput, in vitro bioassay, suspect and nontarget screening, effect-directed analysis



## LECTURES

### L4

## FOOD AND THE CHEMICAL EXPOSOME, WHERE DO WE STAND? SUCCESSSES, CHALLENGES, IMPACT ON PUBLIC POLICY

**Bruno Le Bizec**\*<sup>(1)</sup>, **Jean-Philippe Antignac**<sup>(1)</sup>, **Gaud Dervilly**<sup>(1)</sup>

<sup>1)</sup> ONIRIS, INRAE, LABERCA, France

\*Corresponding author - E-mail: [bruno.lebizec@oniris-nantes.fr](mailto:bruno.lebizec@oniris-nantes.fr)

For almost a 100-years now, we have been observing the profound upheavals on our planet, changes of which we humans, nestled in a much larger and more complex ecosystem, are the main victims. This era we are currently living through, which PJ Crutzen (Nobel Prize in Chemistry, 1995) has proposed to call the Anthropocene, exposes us to increasing quantities of chemicals. These molecules follow us throughout our lives, from conception to death. Exposure cocktails are specific to each individual, depending on their environment, lifestyle choices (e.g. diet, professional activity). While mortality linked to infectious diseases is proportionally decreasing, deaths associated with chronic diseases are increasing (Landrigan, Lancet 2015), the risk factors identified in first place being pollution and related chemicals. Biomonitoring studies are used to capture the overall picture of the exposome by exploring different biological compartments (e.g. blood, hair) according to the physicochemical and/or metabolic characteristics of the substances concerned. TK models can be used to trace the exposure routes, specifying the relative proportions of ingestion, inhalation and dermal contact. In the general population, food is the main route of exposure for many substances. Exposure can be reduced by combining annual monitoring plans (risk managers) and total diet studies (risk assessors). Changes in exposure are necessary both to refine the risk characterisation and to assess the effectiveness of the management measures taken. Taking successive pictures of the general population exposure is an obvious limitation of the methodology. Citizens, on the other hand, are waiting for their specific lifetime exposure trajectory. Predictive models based on TK enable these trajectories to be better assessed according to diet, year of birth or socio-professional variables. Risk assessment agencies have not yet incorporated these models into their risk characterisation. Another obvious limitation is that the number of substances screened is limited to those that are regulated, or at the very least have been identified as dangerous; at most, there are 1,000-2,000 of them whereas the reality of the chemical exposome in humans probably reaches 100,000 molecules, and perhaps many more. This is what is at stake in deciphering the exposome and the central role of analytical chemistry and bioinformatics in this field. The scientific community is in full effervescence around this paradigm shift and unprejudiced approaches. The work of scientists specialising in environmental, food and health issues must be based on an interdisciplinary approach. Working in silos should no longer be the rule if the results are to match the ambitions, which are to decipher the chemical exposome and understand the impact on human health and ecosystems, in order to better guide public policies that will reduce exposure and optimally address public health issues in terms of chronic diseases.

**Keywords:** exposome, chemicals, mass spectrometry, risk assessment, exposure

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## LECTURES

### L5

## ANALYTICAL CHALLENGES AND DECISION-MAKING PROCESS IN SCREENING FOR HIGHLY-POLAR POTENTIAL EMERGING CONTAMINANTS RELEVANT FOR THE EFSA'S PROCESS OF IDENTIFICATION OF EMERGING RISKS

Vít Kosek<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: kosekv@vscht.cz

One of the many tasks of the European Food Safety Authority (EFSA) is the continuous assessment of potential emerging risks associated with compounds that may contaminate unprocessed food throughout the primary production chain. Following REACH 1 and REACH 2 projects in which substances were prioritised for their potential to contaminate food and pose a health risk to consumers, in the project OC/EFSA/SCER/2020/02 on "Screening for emerging chemical risks in the food chain" (SCREENER) [1], EFSA called for the development of screening methods for 212 substances, categorised into two groups based on their polarity and, subsequently, the development of quantitative methods for compounds of proven concern.

For the highly polar group of compounds, the developed sample preparation procedure was based on extraction with acetonitrile (QuEChERS) or 50% methanolic solution (QuPPE) and used high-performance liquid chromatography with hydrophilic interactions (HILIC) coupled with tandem mass spectrometry for separation and detection. Some more volatile compounds required capillary column gas chromatography with polar stationary phase coupled with tandem mass spectrometry. The samples were various unprocessed plant and animal foods purchased in the Czech Republic, Germany, the Netherlands and Poland, reflecting an overall European customer basket. A total of 194 samples were analysed.

The required target limits of detection (tLOD) were calculated based on the toxicity of the compounds and the frequency of the food item in the consumer basket. These values served as a guide for method development and comparability of results.

The results of the study were three new analytical methods suitable for monitoring of 27 substances of concern in unprocessed foods as well as data obtained by analysing different types of food. The compounds most frequently detected in the samples were N-methyl-2-pyrrolidone and N-methylacetamide, which in some cases were even present in relatively high concentrations in matrices such as flour and eggs.

[1] EFSA (European Food Safety Authority), Undas AK, Escher S, Hahn S, Hajslova J, Hrbek V, Kosek V, Licht O, Lommen A, Mol H, Pulkrabova J, Stupak M, Zobl W, Hoogenboom R, 2024. Screening for emerging chemical risks in the food chain (SCREENER). EFSA supporting publication 2024: EN-8962. 183 pp. doi: 10.2903/sp.efsa.2024.EN-8962.

**Keywords:** screening, emerging contaminants, liquid chromatography, mass spectrometry

**Acknowledgement:** This work was supported by the project OC/EFSA/SCER/2020/02 on "Screening for emerging chemical risks in the food chain" (SCREENER).

## LECTURES

### L6

#### BRIDGING THE GAP FROM MICRO- TO NANO-PLASTIC ANALYSIS IN FOOD

**Clementina Vitali**<sup>(1)</sup>, **Anna K. Undas**<sup>(1)</sup>, **Hans-Gerd Janssen**<sup>(2)</sup>, **Michel W.F. Nielen**<sup>(1)</sup>, **Francesco Simone Ruggeri**<sup>\*(3)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>2)</sup> Unilever Foods Innovation Centre, The Netherlands

<sup>3)</sup> Wageningen University, Laboratory of Organic Chemistry, The Netherlands

\*Corresponding author - E-mail: simone.ruggeri@wur.nl

The mismanagement of plastic waste and its accumulation in the environment has resulted in the presence of microplastics (MPs) and nanoplastics (NPs) in the food chain and the exposure of consumers (1). A variety of analytical methods has been developed for the analysis of MPs in food, however they lack the spatial resolution and sensitivity required to gather insights into the presence of NP contamination (2). The potential effects of NPs on human health have raised particular concerns as their sub-micrometer size poses an elevated risk of traversing biological membranes and their translocation and accumulation in human tissues can lead to toxic effects on the human body.

In this presentation, we illustrate how infrared nanospectroscopy (AFM-IR) can be leveraged to provide a comprehensive physico-chemical characterization of NPs. Due to the enhanced spectroscopic sensitivity and nano-scale spatial resolution provided by the unique combination of atomic force microscopy and infrared spectroscopy (3,4), the technique enables a thorough multidimensional study of NP contamination, including toxicological relevant insights such as chemical identification, particle size distribution, and 3-dimensional morphology.

We will first give an overview of how - building on our experience in MP analysis - we developed a method for the analysis of NPs in bottled water, also illustrating our strict quality control measures. We will then discuss the characterization of NP contamination and properties as analyzed in a set of commercial bottled water samples. Later, we will display the optimization of a method for the isolation and analysis of NPs from complex lipophilic matrices such as edible vegetable oils. By addressing fatty matrices, we investigate the potential for MP/NP contamination in this overlooked food category and demonstrate how infrared nanospectroscopy can find application in the analysis of complex systems and advance our understanding of the fate and behavior of NPs. The proposed methods, providing a powerful and reliable platform for the analysis of NPs in real samples, contribute to the development of further strategies aiming at the assessment of the impact of NPs on human health and the environment.

(1) C. Vitali, R. J. B. Peters, H. G. Janssen, M. W. F. Nielen, *Trends Anal. Chem.* 159, 116670 (2023).

(2) C. Vitali, R. J. B. Peters, H.-G. Janssen, M. W. F. Nielen, F. S. Ruggeri, *Trends Anal. Chem.* 157, 116819 (2022).

(3) F. S. Ruggeri, B. Mannini, R. Schmid, M. Vendruscolo, T. P. J. Knowles, *Nat. Commun.* 11 (2020).

(4) F. S. Ruggeri et al., *Nat. Commun.* 12 (2021).

**Keywords:** microplastics, nanoplastics, AFM-IR, bottled water

**Acknowledgement:** MONPLAS Project has received funds from the European Union's Horizon 2020 research and Innovation Programme under the Marie Skłodowska Curie Grant Agreement No. 860775.

## LECTURES

### L7

## NATURAL TOXINS IN PLANT-BASED FOOD: DOES A SHIFT IN THE DIET COME WITH A SHIFT IN THE EXPOSURE?

**Chiara Dall'Asta**<sup>(1)</sup>, Raquel Torrijos<sup>(2)</sup>, Octavian Mihalache<sup>(1)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Italy

<sup>2)</sup> University of Valencia, Spain

\*Corresponding author - E-mail: chiara.dallasta@unipr.it

The European market for plant-based foods has experienced substantial growth in the last years, reaching €5.8 billion in sales in 2022, of which 38% came from plant-based beverages (PBBs) and 35% from plant-based meat alternatives (PBMA). This trend can be explained due to an increase in consumers adopting a vegan or vegetarian pattern, as well as by the overall transition towards more sustainable dietary choices such as the flexitarian scheme. Besides ethical concerns and sustainability, health issues like lactose intolerance and allergies to fresh milk have also contributed notably to raising the popularity of plant-based milk.

While the benefits related to shifting towards alternative diets have been largely debated in the recent literature, discussing the impact in terms of sustainability and health, little has been done so far to assess the risk related to extensive consumption of plant-based meat and milk analogues, especially considering that a strong transition to these foods can lead to a substantial change of exposure to natural food contaminants compared to an omnivore diet.

Taking advantage of the large national project ONFOODS as well as of the EU projects PRISMA and FunShield4Med, over the past 2 years we have performed a comprehensive survey from Italy, the UK, and Greece, covering a total of 220 PBMA and 230 PBBs.

Samples have been analyzed for regulated and unregulated mycotoxins, finding up to 19 co-occurring mycotoxins in a single product. Starting from the collected data, exposure assessment scenarios have been drawn and the potential burden of disease has been derived.

Although at rather low concentration levels, the widespread occurrence of mycotoxins - among them aflatoxins - calls for the implementation of proper monitoring schemes and appropriate regulation for plant-protein-based food.

**Keywords:** alternative diets, mycotoxins, exposure, burden of disease, risk assessment

**Acknowledgement:** Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union - NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods".

## LECTURES

### L8

## QUALITY, SAFETY, AUTHENTICITY AND TRACEABILITY OF BOTANICALS AND FOOD SUPPLEMENTS: A NEW HOLISTIC CHALLENGE FOR THE FOOD CHEMISTS?

**Marco Arlorio**\*<sup>(1)</sup>, **Jean Daniel Coisson**<sup>(1)</sup>, **Fabiano Travaglia**<sup>(1)</sup>, **Monica Locatelli**<sup>(1)</sup>, **Matteo Bordiga**<sup>(1)</sup>

<sup>1)</sup> Department of Pharmaceutical Sciences - Food Chemistry, Biotechnology and Nutrition Unit. University of Piemonte Orientale "Amedeo Avogadro", Italy

\*Corresponding author - E-mail: marco.arlorio@uniupo.it

There is an increasing number of products marketed in the European Community as foods containing concentrated sources of nutrients (vitamins, minerals and other bioactive substances), presented for supplementing the intake of those nutrients from the normal diet. Food supplements means "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in "pharmaceutical" dose form (such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles...)" [1].

Despite the popularity and the large use of food supplements, often prepared using botanicals, a large concern about their quality and safety have been raised, especially regarding their authenticity and integrity (e.g. misidentification, adulteration with other/non permitted plant species) or the presence of natural contaminants (e.g. food allergens), potentially triggering adverse reactions [2]. Food supplement-producing Companies must comply with strict EU laws, including the Directive 2006/46/EC, as well as any national laws set by EU member states. The lack of harmonization (especially about botanicals) complicates a European vision. Even if the traceability botanicals is sometimes hard to be clearly defined, the authentication of the plant/fungi ingredients in food has been largely studied in the recent past, applying different analytical and bio-molecular methods. Mass-based chromatographic analyses, spectroscopic methods, DNA analysis (PCR, DNA barcoding, NGS) are usually used to authenticate the composition of the botanicals-based food supplements. Anyway, advanced (and rapid) combined analytical approaches, also including more advanced statistical post-analytical processing of data, are still required to assess the overall quality and safety of these "special foods". The selection of the more performing and functional molecular markers, both considering non targeted and targeted methods, is a key point in food authentication process [3,4]. Moreover, the creation of an "open access repository" of data in EU (at least for the most commonly used ingredients/botanical products) could be of great significance in order to protect the consumers.

All these facts, focusing on some specific case studies on fungi [5], algae and selected plant-derived botanicals [6], will be discussed in this oral communication, highlighting the gaps that need to be overcome to better protect consumers.

[1] Dir. 2006/46/EC.

[2] Palmer, M.E., et al. *The Lancet* 361.9352 (2003): 101-106.

[3] Angelis, E.D., et al. *Foods* 10 (2021): 1238.

[4] Bayen, S., et al. *Trends in Food Science & Technology* 149 (2024): 104550.

[5] Fochi V., et al. *Food Anal Methods* 15(7) (2022): 1803-1815.

[6] Martoccia M., et al. Proceedings of ChimAli XIII (National Congress of Food Chemistry - SCI, 2023, Marsala, IT).

**Keywords:** food supplements, botanicals, quality & safety, authentication/traceability

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## LECTURES

### L9

## A NOVEL MULTI-TECHNIQUE APPROACH FOR ORGANIC FOOD AUTHENTICITY

Lucie Tsamba<sup>\*(1)</sup>, Freddy Thomas<sup>(1)</sup>, Helene Debetencourt<sup>(1)</sup>, Eric Jamin<sup>(1)</sup>

<sup>1)</sup> Eurofins Analytics France, France

\*Corresponding author - E-mail: [lucietsamba@eurofins.com](mailto:lucietsamba@eurofins.com)

In Europe, organic food control relies on both operator's certification and physico-chemical analyses, mostly focused on pesticides residues. However, several studies have shown that these controls might not be sufficient. At the same time, consumers have doubts about the authenticity of organic food. This presentation will focus on the development, validation, and industrial scale transfer of a global multi-technique method for verifying the authenticity of organic apple juice, tomato and UHT milk. These methods have been developed in the framework of the TOFoo (True Organic Food) project, which involves both industrial and academic stakeholders in food quality control.

Thanks to a partnership with several associations, particularly ITAB (French Institute for organic food and agriculture), databases containing an average of 500 samples for 6 different matrices have been built. The sampling strategy aimed to maximize the variability of the samples in accordance with the French market. For apple juice, samples of different production years, locations, varieties, filtered or not were analyzed. For UHT milk, samples from different process, regions, and fat content were collected.

Nuclear magnetic resonance (<sup>1</sup>H-NMR), liquid chromatography coupled with high resolution mass spectrometry (UPLC-HRMS), and isotopic ratios mass spectrometry (IRMS) are preferred techniques for untargeted analysis and food authentication. During method development, emphasis was placed on the reproducibility of the fingerprint. At least two different instruments for each technique were used in the database building. Additionally, correction strategies were implemented, especially for UPLC-HRMS, to overcome the batch effect.

Data pre-processing is crucial for the reliability of the model. Only IRMS data do not require pre-processing. For <sup>1</sup>H-NMR, peaks were realigned with a target spectrum built from the first 200 samples in the database for each matrix. An external reference was used to correct for instrument differences in intensities. Bucketing and logarithmic transformation were applied. For LC-HRMS, pre-processing aimed to correct the batch effect using Quality Control (QC) samples and reduced the data size to keep the processing time for several hundred samples reasonable.

Several classification models were trained on the data from each technique. To keep the cost of testing reasonable, it was decided to retain the two techniques that produced the best results for each matrix. A linear combination of the models scores was calculated to obtain the final classification.

The models were assessed on independent datasets of at least 70 samples analyzed in routine mode. For apple juice, more than 90% of the samples were correctly classified, with the rate reaching 96% among organic samples. For UHT milk, 97% of the test samples were correctly classified.

**Keywords:** organic food, food authenticity, untargeted analysis, chemometrics, sampling strategies

**Acknowledgement:** The authors thank all the members of the TOFoo project, as well as the industrial and academic partners.

## LECTURES

### L10

## LC-TWIMS-HRMS TO ADDRESS BOTANICAL FOOD SUPPLEMENTS QUALITY AND AUTHENTICITY

**Guillem Campmajo**<sup>(1)</sup>, **Marco Gozzi**<sup>(1)</sup>, **Renato Bruni**<sup>(1)</sup>, **Chiara Dall'Asta**<sup>(1)</sup>

<sup>1)</sup> *Department of Food and Drug, University of Parma, Italy*

\*Corresponding author - E-mail: [guillem.campmajogalvan@unipr.it](mailto:guillem.campmajogalvan@unipr.it)

Food supplements, which can be defined as 'foodstuff' according to European legislation, are a source of concentrated substances with nutritional or physiological effects. Their consumption – mainly as pills, tablets, or capsules – has increased in the last years since they have become a potential complement to the regular diet. The European Food Safety Authority (EFSA) has already established the tolerable upper intake level (UL) for vitamins and minerals provided by food supplements. At the same time, food supplements' production with other ingredients, such as botanicals or their derived extracts, has gained particular interest since these can also contain diverse chemical compounds associated with several health-beneficial effects. However, at least within the UE, there is a lack of consistent, harmonised, and unified regulatory guidance regarding allowable plants (as well as their corresponding parts) and well-established ULs, making the control of their quality, safety, and authenticity a challenging task.

In this context, the present study shows the applicability of an analytical workflow based on liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS) to assess the quality and authenticity of several botanical food supplements. In this line, food supplements from three representative botanicals were evaluated – (i) 15 green coffees, (ii) 15 *Rhodiola rosea* L., and (iii) 13 red beetroot supplements – focusing on the standardisation levels of the bioactive compounds related to the purported health claim as well as the botanical authenticity. Therefore, after sample processing consisting of a straightforward solid-liquid extraction (SLE) with a hydroalcoholic extracting mix, samples were analysed through LC-TWIMS-HRMS under common reversed-phase chromatography conditions. Regarding the HRMS detection, each sample was acquired in positive and negative electrospray ionisation (ESI) modes, using a quadrupole-time-of-flight (Q-TOF) mass analyser, working in full-scan MS mode ( $m/z$  range from 100 to 1000).

The profiling analysis (targeted and suspect analysis) through LC-TWIMS-HRMS provided an extensive metabolomic characterisation of the analysed food supplements, including phenolic compounds, phenylpropanoids, and betalains. In general, the obtained results evidenced a clear quantitative and qualitative inhomogeneity and lack of standardisation for each of the tree groups evaluated, exposing consumers to changing content and distribution of bioactive compounds. Furthermore, the proposed method demonstrated its ability to detect green tea in the green coffee supplements and could also suggest the substitution of some *Rhodiola rosea* L. supplements with other botanical varieties (i.e., *Rhodiola crenulata*).

**Keywords:** botanical food supplements, LC-TWIMS-HRMS, green coffee, *Rhodiola rosea* L., red beetroot

**Acknowledgement:** *This work has been carried out in the frame of the ALIFAR project, funded by the Italian Ministry of University through the program 'Dipartimenti di Eccellenza 2023-2027'.*

## LECTURES

### L11

#### CHICKEN GEOGRAPHICAL ORIGIN VERIFICATION VIA MULTI-ELEMENTAL, ISOTOPIC, SPECTROSCOPIC AND METABOLOMIC FINGERPRINTS

**Zora Jandric**<sup>(1)</sup>, **Andreas Zitek**<sup>(2)</sup>, **Rebecca Hood-Nowotny**<sup>(2)</sup>, **Lisa Fischer**<sup>(2)</sup>, **Katharina Schott**<sup>(2)</sup>, **Sara Kornfeind**<sup>(2)</sup>, **Andrea Watzinger**<sup>(2)</sup>, **Christina Troyer**<sup>(2)</sup>, **Stephan Hann**<sup>(3)</sup>

<sup>1)</sup> FFoQSI, Austria

<sup>2)</sup> BOKU, Austria

<sup>3)</sup> University of Natural Resources and Life Sciences (BOKU), Austria

\*Corresponding author - E-mail: zora.jandric@boku.ac.at

Ensuring food authenticity and combating fraud are critical challenges in the global food supply chain. Our research pioneers various methodologies to pinpoint the geographical origin of chicken using a multi-faceted analytical approach, enhancing label verification from Austria and two importing countries, Hungary and Germany. By leveraging multi-elemental, isotopic, spectroscopic, and metabolomic analyses, we achieved a 95% confidence level in distinguishing chickens from different farms. Our findings highlight that stable isotope ratios of nitrogen and carbon are effective in differentiating samples, revealing distinct dietary signatures. Notably, varied carbon isotope values indicate maize-rich diets in Austrian chickens compared to Hungarian and German counterparts. This dietary variation was instrumental in discriminant analysis, where elemental analysis exhibited exceptional classification precision and accuracy (94.6% and 97.7%), surpassing MicroNIR (81.1% and 93.2%) and amino acids analysis (82.3% and 85.9%). Fusing elemental and isotopic data significantly enhanced model performance to 95.7% precision and 98.3% accuracy. Our research challenges the prevailing theory that global isotopic variations in drinking water exclusively impact avian tissue, suggesting instead that feed signature plays a predominant role. The advanced analytical tools developed in this study are pivotal for differentiating Austrian chicken from imports, rationalizing import regulations, identifying fraudulent labelling, and verifying maize-based diets. These methodologies hold substantial promise for administrative controls, supporting meat producers, and safeguarding consumer interests, making them indispensable for food authenticity, fraud detection, and forensic analysis.

**Acknowledgement:** This study was performed within a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence center FFoQSI is funded by the Austrian federal ministries BMK, BMDW and the Austrian provinces (Lower Austria, Upper Austria and Vienna) within the scope of the COMET - Competence Centers for Excellent Technologies.



### L12

## IDENTIFICATION OF MECHANICALLY SEPARATED MEAT IN MEAT PRODUCTS: THE NEW APPROACHES DEVELOPED BY THE "MPSQA" PROJECT IN ITALY

**Marco Iammarino**\*<sup>(1)</sup>, **Oto Miedico**<sup>(1)</sup>, **Michele Tomaiuolo**<sup>(1)</sup>, **Giovanna Berardi**<sup>(1)</sup>, **Monica Casale**<sup>(2)</sup>, **Paolo Oliveri**<sup>(2)</sup>, **Cristina Malegori**<sup>(2)</sup>, **Rogerta Dalipi**<sup>(3)</sup>, **Emanuele Sangiorgi**<sup>(3)</sup>

<sup>1)</sup> *Chemistry Department, Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

<sup>2)</sup> *Department of Pharmacy, University of Genova, Italy*

<sup>3)</sup> *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Italy*

\*Corresponding author - E-mail: marco.iammarino@izspb.it

As reported in a Scientific Opinion released in 2013 by the BIOHAZ Panel of EFSA [1], the identification of a meat product containing mechanically separated meat (MSM) has a significance from a point of view of both meat quality and safety. The same Panel asked "to identify and rank the parameters that may be used to distinguish between the different types of MSM and compare them as well with fresh meat, minced meat and meat preparations, as defined in EU legislation". Consequently, the development of reliable analytical methods, useful to discriminate among foods obtained from those not obtained from MSM is an impelling task for subjects involved in food inspections.

In this study five different analytical approaches, developed within the "MPSQA", a research project financed by the Italian Ministry of Health, are described.

The first approach is based on the simultaneous determination of Sr-90, Sr-88, Ca and ash, coupled to multivariate analysis. The precision of this tool (87%) was higher than the reference method (determination of calcium concentration, also evaluated in this study, 76%) [2].

The second approach is based on the qualitative identification of MSM presence due to the presence of bone fragments that generate ESR signals after irradiation. At least six characteristic signals, identified by specific "g-factors" were observed, identified and characterized. The quantitative approach was also proposed through the validation procedure with good sensitivity (LOD and LOQ equal to 16 and 48 mg/100g (w/w f.w.), respectively), precision (CV%: 12.8%), trueness (mean error percentage: 9%) (n = 18) and measurement uncertainty (14.6%) [3].

The third approach is based on the simultaneous determination of Ca and Mg by cation-exchange ion chromatography with suppressed conductivity detection. The difference in the concentration between 2 ions, named "MSM<sub>index</sub>", was effective for identifying MSM presence in meat products containing MSM percentage higher than 25% [4].

The fourth approach is based on the determination of 43 trace elements by ICP-MS coupled to multivariate analysis. The chemometric classification models were rigorously validated, both internally and externally. A cross-validation strategy with six cancellation groups was applied for an internal validation, achieving a total prediction rate in cross validation of 97.2% regarding MSM presence in meat products [5].

The last approach is based TXRF analysis combined with PCA. This method clearly distinguished different types of meat products containing MSM with a quantification limit of 40% MSM [6].

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**Keywords:** mechanically separated meat, food safety, multivariate analysis, meat quality, novel analytical methods

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## LECTURES

### L13

## NOVEL FOODS IN THE EU - REGULATORY FRAMEWORK AND RISK ASSESSMENT. A CLOSE-UP LOOK AT THE ALLERGENICITY RISK ASSESSMENT CHALLENGES: REQUIREMENTS, KNOWLEDGE GAPS AND RESEARCH NEEDS

Marcello Laganaro<sup>\*(1)</sup>, Estefania Noriega Fernandez<sup>(2)</sup>, Antonio Fernandez Dumont<sup>(1)</sup>, Andrea Germini<sup>(1)</sup>

<sup>1)</sup> European Food Safety Authority (EFSA), Italy

<sup>2)</sup> European Food Safety Authority (EFSA); Nofima, Stavanger, Norway; University of Zaragoza, Zaragoza, Spain, Italy

\*Corresponding author - E-mail: marcello.laganaro@efsa.europa.eu

In the European Union, foods and food ingredients not significantly consumed before May 1997 are considered novel. The European Food Safety Authority (EFSA) is responsible for assessing novel foods' (NFs) safety. Current market developments and EU and national food policy strategies generate increasing interest in alternative protein sources. However, the introduction of foods of proteinaceous nature in the diet also raises concerns about their safety regarding allergenicity.

This work provides outlines the scientific requirements for NFs safety assessment as described in the recently updated EFSA guidance. Emphasis is given to the requirements necessary to establish the potential allergenicity of NFs. The assessment process which still embed the "weight-of-evidence" approach will be illustrated through past examples of scientific opinions.

Different requirements are deemed necessary for NFs according to their complexity, production process, and regulatory status. For those with unknown allergenicity potential, on a case-by-case, the risk assessment can be supported by scientific evidence available in literature, phylogenetic analysis, protein identification and quantification, bioinformatic analysis to predict cross-reactivity, *in vitro* protein stability and digestion, and *in vivo* tests.

Yet, the allergenicity risk assessment is currently facing challenges due to the rapid pace of food innovation and biotechnology advances. The lack of (i) reference standard materials, (ii) targeted and internationally validated investigative means meeting regulatory requirements, and (iii) complete understanding of the mechanisms underlying immune-mediated reactions are some of the major limitations. This highlights an urgent need for development of reference materials and internationally validated food allergen test methods.

Disclaimer: The views expressed in this work are those of the authors and should not be interpreted as representing the official position of EFSA. Thus, the present work is published under the sole responsibility of the authors and may not be considered as an EFSA scientific output. EFSA cannot be held accountable for any errors or inaccuracies that may appear.

**Keywords:** food allergens, allergenicity, risk assessment, novel foods, EFSA

## LECTURES

### L14

## A ROBUST MULTI-ALLERGEN PROTEOTYPIC SCREENING METHOD FOR FOOD CONTROL

Alexander Scherl<sup>(1)</sup>, Alexandre Piletta-Zanin<sup>(2)</sup>, Pierre Zimmerli<sup>(1)</sup>, Didier Ortelli<sup>(1)</sup>, Philippe Eigenmann<sup>(2)</sup>, Patrick Edder<sup>(1)</sup>

<sup>1)</sup> Official Food Control Authority of the State of Geneva, Switzerland

<sup>2)</sup> Geneva University Hospitals, Switzerland

\*Corresponding author - E-mail: alexander.scherl@etat.ge.ch

Five to eight percent of the population suffers from a food allergy, which can lead to a serious reaction in case of accidental consumption. Analyses carried out by food control authorities are important to ensure correct allergen declaration, especially for pre-packaged foods. This report presents a screening method based on the identification of 44 proteotypic peptides corresponding to 12 major food allergens after enzymatic digestion and analysis by liquid chromatography coupled to high-resolution tandem mass spectrometry. The target allergens are cow's milk, crustaceans, eggs, fish, wheat and barley gluten, lupine, molluscs, mustard, nuts (almond, Brazil nut, cashew, hazelnut, pecan, pistachio and walnut), peanut, sesame and soy. The screening method was applied for reliability testing to a wide variety of food matrices, including processed and heat-treated foods. In order to validate the method for food safety control, more than 200 prepacked food samples were collected during official controls, and an additional 69 samples suspected of having triggered an allergic reaction were collected during a clinical study. Comparison with the ingredient list and systematic confirmatory analysis of samples containing undeclared food allergens by ELISA or PCR revealed no false positive results. Ten foodstuffs contained indeed undeclared food allergens, seven among them above the Swiss legal limit of 1g/kg. These seven samples were thus not compliant with the food legislation. Systematic confirmatory analysis on negative samples suspected of having triggered an allergic reaction indicated that the false negative rate was also very low. In summary, the method is robust, specific and sufficiently sensitive for food control. The ability to screen for multiple food allergens in virtually any processed food matrix using a single method represents an alternative to targeted ELISA or PCR tests and contributes to food safety for allergic consumers.

**Keywords:** LC-MS/MS, food allergens, food control

## LECTURES

### L15

## THE CHANGING FOOD ALLERGEN LANDSCAPE IN EUROPE CALLS FOR HARMONISED FOOD MONITORING

**Nathalie Smits**<sup>(1)</sup>, Nicolai Ballin<sup>(2)</sup>, Christine Bruggeman<sup>(1)</sup>, Christiane Fæste<sup>(3)</sup>, Augusto Pastorelli<sup>(4)</sup>, Christof Van Poucke<sup>(5)</sup>, Marleen Voorhuijzen-Harink<sup>(1)</sup>, Yvonne Westphal<sup>(1)</sup>, Michael Walker<sup>(6)</sup>, Matthias Winkel<sup>(7)</sup>, Andries Koops<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>2)</sup> Danish Veterinary and Food Administration (DVFA), Denmark

<sup>3)</sup> Norwegian Veterinary Institute, Norway

<sup>4)</sup> Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Italy

<sup>5)</sup> Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium

<sup>6)</sup> Institute for Global Food Security, School of Biological Sciences, Queen's University, United Kingdom

<sup>7)</sup> GFZ German Research Centre for Geosciences, Germany

\*Corresponding author - E-mail: nathalie.smits@wur.nl

With the changing food allergen landscape in Europe, there is an increasing need to monitor both prepacked and non-prepacked food products to better protect consumers with a food allergy. Although food allergy affects up to 10% of consumers worldwide, a European harmonised approach for managing precautionary allergen labelling, allergen reference doses, analytical methods, or food product monitoring is not in place. This results in limited food choices for consumers with food allergies and can lead to serious allergic incidents. The current state of non-harmonised and unregulated food allergen monitoring in Europe poses risks and considerable costs for individual consumers and society in general. Researchers with expertise in food allergen regulation and measurement joined forces to argue for the introduction of harmonised guidelines for food allergen monitoring at the European level, discussing the wide-ranging implications of their absence. We consider there is a pressing need to update European legislation on food allergens, including the introduction of harmonised monitoring programmes, which will lead to improved overall food safety and better protection and options for consumers with food allergies. This presented work is finalized to be published in a position paper.

**Acknowledgement:** *Special thanks go to Patrick O'Mahony with whom we could discuss and who gave input during the process of preparation of the position paper. Our appreciation and thanks go to Marc de Loose, Isabel Taverniers and Daniela Bartsch, who have helped us during the process of shaping the outline of the position paper. This work was marked as strategically relevant and internally financed by WFSR.*

## LECTURES

### L16

## MULTI-ALLERGEN DETECTION BY UHPLC-MS/MS METHOD IN PROCESSED FOODSTUFFS

**Anne-Catherine Huet**<sup>(1)</sup>, **Jean Henrottin**<sup>(2)</sup>, **Martin Mailleux**<sup>(2)</sup>, **Mathieu Dubois**<sup>(2)</sup>, **Nathalie Gillard**<sup>(1)</sup>

<sup>1)</sup> CER Groupe, Analytical Laboratory, Belgium

<sup>2)</sup> CER Groupe, Belgium

\*Corresponding author - E-mail: [ac.huet@cergroupe.be](mailto:ac.huet@cergroupe.be)

At the moment patients that are diagnosed to suffer from a particular food allergenicity have no other option than avoiding products that contain this ingredient. The EU developed a legislation on labeling of fourteen allergenic ingredients, whenever used in different food formulations, in order to protect the consumers (Regulation EU No 1169/2011).

It is therefore essential to develop sensitive and reliable analytical methods to detect the presence of these allergens in various foodstuffs. They are regularly detected by commercial test kits, based on Enzyme-Linked ImmunoSorbent Assays (ELISA), Lateral Flow ImmunoAssays (LFIA) or on Polymerase Chain Reaction (PCR) but mainly as individual testing. Furthermore, it is known that certain proteins can be difficult to detect using the immunoassays, especially in processed food. As analytical laboratory and National Reference Laboratory, one of our goals is to develop a complementary methodology such as Liquid Chromatography - tandem Mass Spectrometry (UHPLC-MS/MS), able to detect simultaneously multiple food allergens in one sample, even though it is highly processed. This qualitative UHPLC-MS/MS method is able to screen specific proteins from these allergens after their extraction, enzymatic digestion, and purification(s). The interpretation of results is based on signature peptides, carefully selected to give an estimated concentration of the total allergenic protein content.

The presented method is accredited under ISO17025 and is able to detect six allergenic foods: milk, egg, peanut, soybean, lupin and nuts including almond, hazelnut, walnut, cashew nut, pecan nut, pistachio, Brazil nut, and macadamia nut. In this communication, we describe the performance of this qualitative method, in progress developments, and encountered limitations.

**Keywords:** food allergen, peptides, detection, method, mass spectrometry

## LECTURES

### L17

## STANDARDIZATION OF A REFERENCE METHOD FOR MULTIPLE ALLERGEN DETERMINATION IN FOODS, PRECAUTIONARY ALLERGEN LABELLING AND REFERENCE DOSES: THREE ISSUES IN FOOD ALLERGY RESEARCH

**Linda Monaci**\*<sup>(1)</sup>, Elisabetta De Angelis<sup>(1)</sup>, Anna Luparelli<sup>(1)</sup>, Rosa Pilolli<sup>(2)</sup>, Francesca Lambertini<sup>(3)</sup>

<sup>1)</sup> CNR-ISPAA, Italy

<sup>2)</sup> ISPAA-CNR, Italy

<sup>3)</sup> Barilla G. R. F.lli SpA, Analytical Food Science Research, Italy

\*Corresponding author - E-mail: linda.monaci@ispa.cnr.it

Food allergy is considered a major safety issue for allergic consumers. European legislation with regulation 1169/2011 mandated the labelling of 14 allergenic ingredients whenever intentionally introduced into a food, although the risk of detecting allergens in foods expected to be allergen free is likely to exist due to the likelihood of cross-contamination. The risk of accidental cross-contamination, by allergens, has prompted food industries to make excessive use of Precautionary Allergen Labelling (PAL) and the WAO working group on food allergens is currently highlighting the need for a regulated, international framework to underpin application of PAL <sup>(1)</sup>.

Efforts to protect allergic population have been put in place in the last decade at several levels: i) developing sensitive and reproducible analytical methodologies to detect and quantify even minute amounts of allergenic ingredients in foods, to verify compliance with the reference doses recommended ii) regulating the use of precautionary labelling iii) establishing reactivity thresholds for each individual allergen. All these aspects representing crucial issues in food allergy research and efforts in progress at international level to overcome these obstacles will be discussed in this note.

Emphasis will be given to illustrate advantages of analytical methods developed according to the international European guidelines and to illustrate the last results obtained within the ThRAII project <sup>(2)</sup> recently concluded about the development of a MS/MS based reference method for multiple allergens determination in complex food matrices. Finally, application of the method for multiple detection of allergens in two types of bakery products such as cookies and rusks produced at pilot scale will be also presented.

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**Keywords:** analytical methods, mass spectrometry, reference doses, food allergen labelling, food allergy

## LECTURES

### L18

#### SAVING TIME, ORGANIC WASTE, AND MONEY: NEW NEEDS IN MULTI-RESIDUE METHODS FOR DETECTING PESTICIDE RESIDUES IN FOOD

**Amadeo R. Fernandez-Alba**<sup>\*(1)</sup>, **Francisco Garrido**<sup>(2)</sup>

<sup>1)</sup> *European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, Spain*

<sup>2)</sup> *Centro de Innovación Tecnológica COEXPHAL, Spain*

*\*Corresponding author - E-mail: amadeo@ual.es*

Food safety control has significantly improved in recent years, with large-scale monitoring, very low limits of quantification (LOQs), and high analytical performance. Simultaneously, the number of samples tested has increased dramatically, now amounting to millions of samples each year in the EU. These stringent controls are no longer seen as merely restrictive but are understood to be essential for extensive residue monitoring. This is driven not only by concerns for human health but also by the need to protect the environment.

Therefore, this situation has led laboratories to prioritize reducing analysis time, lowering costs per analysis, and minimizing organic waste to increase productivity and provide better service to the community. To meet these new demands, it is essential to revisit multiresidue methods and explore new approaches that can address these objectives effectively. Optimizing calibration procedures and injection volumes, especially considering the latest advances in mass spectrometry. Additionally, automation and miniaturization are critical areas that can support these expanded objectives. In this research, we explore and discuss advancements in analytical process/tools, focusing on both newly introduced technologies and those on the cusp of implementation. This analysis highlights the innovative evolution of analytical methodologies, with a strong emphasis on efficiency and eco-friendly approaches.

We have categorized the examples into three distinct phases. First, we focus on the automation of sample extraction, particularly in dry commodities. Next, we address the importance of calibration and cleanup procedures and the application of Micro-SPE. Lastly, we examine chromatography, with a focus on microflow chromatography. The evaluation of these approaches, whether new or relatively established, shows their potential to enhance sample throughput, reduce laboratory waste, and meet the performance standards required by ISO 17025.

**Keywords:** multi-residue methods, pesticide residues, enhanced sample throughput, reduction laboratory waste

## LECTURES

### L19

## DEALING WITH RESIDUE DEFINITIONS ENTAILING ESTERS AND CONJUGATES

**Michelangelo Anastasiades**\*<sup>(1)</sup>

<sup>1)</sup> CVUA Stuttgart / EURL.SRM, Germany

\*Corresponding author - E-mail: ma@cvuas.bwl.de

Many pesticides/metabolites tend to covalently (but still reversibly) bind to matrix components (e.g. sugars, amino acids) leading to "conjugated residues". These conjugates may potentially break down, e.g. during food processing or digestion releasing the original compound. For this reason, conjugates are sometimes included in the residue definitions (RDs) for enforcement and risk assessment. In rare cases, the RDs name specific conjugates that may be analyzed as such, thus obviating the need for hydrolysis. But in most cases the "conjugates" (and the esters) stated in RDs are unspecified. In such cases the analytical methods employed should involve a generic hydrolysis step to release the analyte. Such methods are more laborious than multiresidue methods and, due to the harsh hydrolysis conditions needed, not suitable for multiclass analysis. For a better effort-to-benefit ratio, most labs apply the methods involving hydrolysis only if the marker (typically the analyte in free form) is detected using a routine method at a level exceeding the trigger. The choice of reasonable trigger-levels, however, requires knowledge about the typical share of conjugated residues in samples. Empirical data on conjugation-rates of acidic pesticides in various samples with incurred residues are currently being collected by the EURL-SRM. A second data collection gives insight on the matrices likely to contain residues of pesticides with relevant conjugates in their enforcement-RDs. Finding generic and mutually recognized hydrolysis conditions for routine monitoring laboratories is quite challenging, as the term "conjugates" is mostly not specified and may include conjugates of very different hydrolysis resistance and extractability. The hydrolysis behavior of conjugates can only be studied on the few model conjugates (mostly glucosides) available as standards. So, little is known about the share of resistant conjugates (e.g. amino acid conjugates of acids) in samples. The lack of standards further impedes proper validation. Esters are commercially available, but their hydrolytic resistance is strongly matrix-depend. No wonder, de-conjugation conditions in methods provided by PPP-applicants vary greatly, involving alkaline, acidic or enzymatic hydrolyses. The EURL-SRM has studied the various hydrolysis approaches in combination with QuEChERS, but the focus of this presentation will be on purely chemical approaches. Using particularly "resistant" esters as model compounds and checking the breakup of incurred conjugates from real samples, generic hydrolysis conditions were elaborated for a wide range of matrices. As not all matrices can be tested, the EURL-SRM proposes the introduction of agreed performance criteria in order to demonstrating that the method used was fit-for-purpose, rather than on striving for achieving exhaustive hydrolysis. Specific "resistant esters" were tested for their suitability as QC-standards, i.e. for verifying hydrolysis efficiency.



## LECTURES

### L20

## EXPERIENCES AND CHALLENGES FROM THE PERSPECTIVE OF THE EURL FOR PESTICIDE RESIDUES IN CEREALS AND FEEDING STUFFS

Ederina Ninga<sup>(1)</sup>, Elena Hakme<sup>(2)</sup>, Julio Cesar Espana Amortegui<sup>(2)</sup>, Mette Ercius Poulsen<sup>\*(2)</sup>

<sup>1)</sup> EURL-CF, National Food Institute, Technical University of Denmark, Denmark

<sup>2)</sup> Technical University of Denmark, National Food Institute, Denmark

\*Corresponding author - E-mail: mpou@food.dtu.dk

The European Union Reference Laboratory on Pesticide Residues in Cereals and Feeding Stuff was appointed in 2006. One of the primary objectives of the EURL is to enhance the performance not only of the National Reference Laboratories (NRLs) but also of the Official Control Laboratories (OfLs). This is achieved through the dissemination of relevant knowledge on analytical methods, method development, workshops, and proficiency tests, as outlined in Commission Regulation 2017/625. In this presentation, I will share examples on the work that has been carried out in 2022-2024.

**Proficiency tests:** The EURL has been accredited according to ISO 17043 since 2010. To date, we have organized 18 proficiency tests covering various cereals such as barley, oat, rice, rye, and wheat, as well as specific feed matrices like composite feed, hay, and straw. For the years 2023 and 2024, the matrices under examination were wheat kernels and wheat straw.

**Method development:** To remain at the forefront of analytical methods in the field of cereals and feeding stuff, we have actively engaged in testing and validating new automatic clean-up systems, specifically on micro-solid-phase extraction ( $\mu$ SPE). Our investigations have included both commercially available cartridges and customized methods. We also work on optimising the extraction of incurred pesticide residues and recent experiments have shown that extended extraction can improve the results with 30-50%. Furthermore, we have explored techniques for analysing pesticides in insects used for feed. The analysis has been used in a feeding study on Black Soldier fly larvae (*Hermetia illucens*) and mealworms (*Tenebrio molitor*). Our findings show that pesticides are transferred from the feed/substrate to the larvae, and that the transfer is dose dependent.

Another important task for the EURLs is to provide scientific advice to the Commission and EFSA. Both entities require information on achievable limits of quantification (LOQs) and analytical feasible residue definitions. Achieving this necessitates the validation of pesticide residues for newly approved pesticides and reevaluated pesticides. Additionally, there is a need to lower the LOQs obtained from previous validations, not only to fulfil the MRLs but to refine intake calculations.

**Keywords:** EURL, pesticide residues, proficiency test, method development

**Acknowledgement:** *This work has been funded by the EU Commission.*

## LECTURES

### L21

## HARMONISATION OF RESIDUE CONTROL IN EUROPE - EXAMPLES OF TOOLS OF THE EURL FOR RESIDUES OF VETERINARY DRUGS

Joachim Polzer<sup>(1)</sup>, Ulrike Mülow-Stollin<sup>(1)</sup>, Ferial Tadjine<sup>(1)</sup>, Katrin Kittler<sup>(1)</sup>, Kai Nieschalke<sup>(1)</sup>, Markus Fifeik<sup>(1)</sup>

<sup>1)</sup> German Federal Office of Consumer Protection and Food Safety - European Reference Laboratory for Residues of Veterinary Drugs, Germany

\*Corresponding author - E-mail: joachim.polzer@bvl.bund.de

REGULATION (EU) 2017/625 defines in Article 94 the tasks of the European Union reference laboratories (EURL). These are among others the provision of suitable residue control methods, the assistance to National Reference Laboratories (NRLs) in the implementation of these methods and the control of the successful implementation by inter-laboratory comparative testing. This includes the provision of pure standard substances for the implementation of methods and the production of incurred reference materials. The key objective of these tasks is to ensure harmonized residue control within the EU. The presentation shows examples of uncommon measures that strengthen this harmonization and helps network laboratories to improve their performance.

Proficiency tests (PTs) with standard solutions

The PTs regularly organized by the EURL are performed with incurred matrix materials. These PTs sometimes led to results where the Horwitz ratio (Horrat) is significantly above the expected outcome (expected Horrat  $\leq 1.5$ ). PTs with standard solutions as test items can provide insight into the uncertainty contributions arising from the applied calibration solutions. The PT findings may represent a starting point for reducing the reproducibility standard deviation in matrix PTs.

Collaborative trials applying experimental design-based validation plans.

Method ring tests usually require a relatively large number of participating laboratories. Experimental design-based validation plans may offer a comparable level of reliability of method performance data whilst reducing the number of participating laboratories. An example of method ring test with European NRLs for validation of an EURL method for coccidiostats in egg is provided and possibilities for additional data evaluation are discussed.

Participation in key comparisons of the BIPM worldwide network of National Metrology Institutes (NMI)

The network of NMIs regularly organises key comparisons with the aim of proving the worldwide traceability of measurement results to SI units. Successful participation in these key comparisons allows the claiming of CMC (calibration and measurement capabilities) entries in the key comparison data base of the BIPM (Bureau International des Poids et Mesure, [www.bipm.org](http://www.bipm.org)). These CMC entries allow calibration of test items and issuing of SI-traceable calibration certificates which are accepted worldwide. By use of such test items in proficiency tests a link between the metrology network and the NRL network can be established, and worldwide comparability of measurement results is promoted.

**Acknowledgement:** The financial support of the European Commission is gratefully acknowledged.

## LECTURES

### L22

## UPDATE ON THE WORK OF THE EURL/NRL NETWORK FOR HALOGENATED POPs IN FEED AND FOOD

Alexander Schaechtele<sup>\*(1)</sup>, Theresa Zwickel<sup>(2)</sup>, Christina Riemenschneider<sup>(1)</sup>, Jakob Hauns<sup>(2)</sup>, Katharina Rund<sup>(1)</sup>

<sup>1)</sup> EURL for POPs, CVUA, Germany

<sup>2)</sup> EURL for halogenated POPs, Germany

\*Corresponding author - E-mail: alexander.schaechtele@cvuafr.bwl.de

The European Union Reference Laboratory (EURL) for halogenated POPs in Feed and Food (EURL POPs) and the network of EURL and National Reference Laboratories (NRLs) of EU member states cover a wide range of halogenated POPs in food and feed. The current focus is, besides polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs), on per- and polyfluoroalkyl substances (PFAS), brominated contaminants like polybrominated diphenylethers (PBDEs), hexabromocyclododecanes (HBCDDs) and emerging brominated flame retardants (eBFRs), polychlorinated alkanes (PCAs, as main constituent of the technical product chlorinated paraffins (CPs)) and polychlorinated naphthalenes (PCNs). For PCDD/Fs, PCBs and PFAS legal limits and criteria for analysis are currently defined in EU legislation. One main task of the EURL is the organization of proficiency tests and interlaboratory studies for NRLs. In the last years, the EURL organized EURL PTs for various food and feedstuffs (e.g. milk powder, bovine meat, fish meal, compound feed) for analysis of PCDD/Fs, PCBs, PBDEs, HBCDDs and PFAS. Additionally, for analytes of interest that are not yet established in most NRLs, interlaboratory studies on the analysis of other brominated contaminants (e.g. eBFRs), PCNs and polychlorinated alkanes were offered.

The EURL is establishing methods for the aforementioned analytes of interest in different food and feed matrices. For further development of analytical criteria and guidance for their analysis, the core working groups formed within the EURL/NRL network together with additional support of invited independent experts, exchange recent analytical information and work on developing guidance documents. These guidance documents are published on the EURL website (eurl-pops.eu) and are regularly discussed and updated.

In addition to the work within the EURL/NRL network and the scientific support of the European Commission regarding analytical issues in halogenated POPs analysis, the four EURLs working in the field of contaminants cooperate and develop guidance documents or recommendations on analytical and regulatory issues of interest to all four EURL/NRL networks. These four EURLs are the EURL for metals and nitrogenous compounds in feed and food (EURL MN), the EURL for mycotoxins and plant toxins in feed and food (EURL M&P), and the EURL for processing contaminants (EURL PC) and the EURL POPs.

**Keywords:** EURL, halogenated POPs, analysis, food, feed

**Acknowledgement:** We would like to thank the European Commission for the support of the work of the European Union Reference Laboratory for halogenated POPs in Feed and Food, Freiburg, Germany, and the network of EURL and NRLs for halogenated POPs in feed and food and invited independent experts for the excellent cooperation and for their scientific contribution.

## LECTURES

### L23

## PROGRESS MADE AND CHALLENGES ON SAMPLING AND ANALYSIS OF MYCOTOXINS AND PLANT TOXINS IN FOOD AND FEED

**Patrick Mulder**<sup>(1)</sup>, **Sylvia Kalli**<sup>(1)</sup>, **Marta Sopol**<sup>(1)</sup>, **Hans Mol**<sup>(1)</sup>, **Josipa Grzetic Martens**<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: [patrick.mulder@wur.nl](mailto:patrick.mulder@wur.nl)

Recently the European Commission has published implementing regulations (EU) 2023/2782 and (EU) 2023/2783 laying down the methods of sampling and analysis for, respectively, mycotoxins and plant toxins in food. These regulations align with the new Commission Regulation (EU) 2023/915 that defines, amongst other contaminants, the maximum levels for mycotoxins and plant toxins in food. For feed the new implementing regulation (EU) 2024/771 describing the methods for sampling and analysis of contaminants is now in place.

One of the challenges often encountered in the analysis of plant toxins is the huge sample inhomogeneity. A few tiny plant parts or seeds can contaminate the commodity at ML or above. To achieve representative test samples, the whole batch (often several kg) needs to be homogenized, requiring labor-intensive sample pre-treatment. To address this issue, studies were undertaken to compare conventional milling approaches with slurry mixing. Three representative cases of contamination were investigated: cumin spice with *Heliotropium* seeds, dried herbs with PA-containing plant parts and maize containing *Datura* seeds. Slurry mixing generally performed better in homogenization of the samples, but there are some important points of attention, in particular regarding the stability of the toxins, that need to be addressed.

Recently two EFSA opinions were published that identified data gaps necessitating the development of (improved) analytical methods. The EURL-MP is currently working on an analytical method for grayanotoxins in honey that will meet the low LOQs indicated in EFSA opinion 2023:7866 (Risks for human health related to the presence of grayanotoxins in certain honey). A particular challenge is the limited availability of analytical standards. Work is also undertaken on a method for tremogens and ergot alkaloids in grass/hay/silage, to address the call for occurrence data expressed in EFSA opinion 2024:8496 (Risks for animal health related to the presence of ergot alkaloids in feed).

To monitor the capabilities of NRLs and their progress made in implementing methods for Official Control yearly a number of proficiency tests are organized. In the past period this covered the analysis of ergot alkaloids in cereals, opium alkaloids in poppy seeds and bakery products, aflatoxins and ochratoxin in maize and cocoa, and hydrocyanic acid in almonds and linseed. The results of these PTs will be discussed.

**Keywords:** EURL-MP mycotoxins & plant toxins, legislation, sampling, analysis, proficiency test

## LECTURES

### L24

## NEW FOOD CHALLENGES AND EXPERIENCES FROM THE EUROPEAN UNION REFERENCE LABORATORY FOR PROCESSING CONTAMINANTS

**Avid Fromberg**<sup>(1)</sup>

<sup>1)</sup> *European Union Reference Laboratory for Processing Contaminants, National Food Institute, Technical University of Denmark, Denmark*

*\*Corresponding author - E-mail: arfr@food.dtu.dk*

When it comes to contaminants formed during the preparation and processing of foods, the European Union Reference Laboratory for Processing Contaminants (EURL-PC) provides analytical methods for this type of processing contaminants. The EURL-PC thereby support the analytical quality for the European National Reference Laboratories (NRLs) and contribute to disseminate relevant knowledge to the NRLs and provides scientific advice to the EU Commission.

The EURL-PC has supported the NRL network with analytical methods for polycyclic aromatic hydrocarbons (PAHs), 3-monochloropropane-1,2-diol (3-MCPD), 3-MCPDE fatty acid esters and glycidyl fatty acid esters to be used for the compliance testing of foods in relation to the maximum limits as described in EU regulation 2023/915. Furthermore, the EURL-PC has established an analytical method for the determination of furan and alkylated furan in foods, which can be used for matrixes mentioned in the EU Recommendation 2022/495 and thereby as basis for establishing data for the further discussion of levels found in foods. For acrylamide Benchmark levels are currently set in Commission Regulation 2017/2158 and the focus has been on improving the limit of quantification for acrylamide and recommendation for troublesome matrixes to be able to meet the low levels set for some of the foodstuffs.

More recently mineral oil contamination of foods has been added to the tasks of the EURL-PC where the MOSH and MOAH determination in foods are in focus. Furthermore, as a consequence of the discussions concerning contaminants in smoke flavours there was a need for development of analytical approaches suitable for determination of 2(5H)-furanone and 1,2-Benzenediol in smoked food including meat, cheese and fish.

The analytical challenges are widely promoted by the EURL-PC throughout supporting the NRLs with hands-on trainings and supporting videos picturing the analytical procedures, which can be used for the processing contaminants.

The presentation will outline the most recent approaches and achievements by the EURL-PC, hosted by the National Food Institute at Technical University of Denmark (DTU Food) within the food area.

**Keywords:** 3-MCPD esters, PAH, MOSH/MOAH, 2(5H)-furanone, 1,2-benzenediol

**Acknowledgement:** *We would like to thank the EU Commission for financial support and the National Reference Laboratories for scientific contributions and excellent cooperation within the network.*

## LECTURES

### L25

#### RECENT ANALYTICAL ADVANCES BY THE EURL-MN

Heidi Amlund<sup>(1)</sup>, Agnieszka Niklas<sup>(1)</sup>, Daniel Clayton-Cuch<sup>(1)</sup>, Doaa Diaaeldin Ahmed M Abouelenein<sup>(1)</sup>, Katrin Löschner<sup>(1)</sup>, Lene Duedahl-Olesen<sup>(1)</sup>, Jens J. Sloth<sup>(1)</sup>

<sup>1)</sup> National Food Institute, Technical University of Denmark, Denmark

\*Corresponding author - E-mail: haml@food.dtu.dk

The area of competence of the European Union reference laboratory for metals and nitrogenous compounds in feed and food (the EURL-MN) include all metals, other elements and nitrogenous compounds in feed and food. As an EURL we contribute to the improvement and harmonisation of analytical methods used for official feed and food control.

In this presentation a brief overview of recent legislative developments in the area of competence of the EURL-MN will be presented. Furthermore, the presentation will include some of the recent analytical advances and give an overview of our latest work on method development. Focus during the last years have been on development of methods for the determination of N-nitrosamines, MeHg (methylmercury) and F (fluorine). We have developed a method for the determination of N-nitrosamines in food (covering both volatile and non-volatile compounds) using QuEChERS extraction and LC-MS/MS. For the determination of MeHg in seafood we have developed and validated a method using acidic extraction and reversed-phase HPLC-ICP-MS. Currently, we are developing a method using IC for the determination of F in feed. The presentation will provide brief overview of the methodological developments.

**Keywords:** EURL, food regulation, maximum levels, metals, nitrosamines

## LECTURES

### L26

#### EMN FOOD: PRIORITIES AND STRATEGIES FOR DRIVING METROLOGY IN SUPPORT OF FOOD SAFETY AND SUSTAINABILITY

Chiara Portesi<sup>(1)</sup>, Nives Ogrinc<sup>(2)</sup>, Andrea Mario Rossi<sup>\*(3)</sup>

<sup>1)</sup> INRIM, Italy

<sup>2)</sup> Jožef Stefan Institute, Slovenia

<sup>3)</sup> National Metrology Institute of Italy - INRiM, Italy

\*Corresponding author - E-mail: a.rossi@inrim.it

The provision of safe, high-quality food is vital for human health, and innovation in the food sector is needed to protect the environment, ensure sustainability, and respond to future needs. EURAMET, the association of National Metrology Institutes (NMI) in Europe, approved in May 2022 the European Metrology Network (EMN) for Safe and Sustainable Food (EMN Food). The EMN Food aims to foster collaboration and coordination in the measurement science community to meet metrology needs along the food chain, working within the European Union's Farm to Fork Strategy.

The network promotes a harmonised approach to food measurements, reference materials and standards, which will allow National Metrology Institutes (NMIs) and Designated Institutes (DIs) across Europe to respond to stakeholders and regulations with confidence and quality. This will afford greater protection to citizens and the environment and accelerate the response to emerging and future metrology needs. The Strategic Research Agenda (SRA) of the Network have been drafted to give as much of a comprehensive overview as possible of the major metrological challenges faced by workers in the food safety and sustainability area, as envisaged by stakeholders of this field and specialists in metrology. Based on the outcomes of dedicated surveys, the stakeholders' needs have been identified and addressed by specific training courses and workshops.

The EMN has also contributed to specific activities in the framework of the World Metrology Day 2023: "Measurements supporting the global food system". It has also supported exchanges of researchers across Europe for sharing knowledge and experience and for promoting interaction with key stakeholders such as European Reference Laboratories. The EMN-Food has also promoted scientific activities related to metrological research in food safety and sustainability, and in the framework of national and European projects, for guaranteeing an adequate economical support of the EMN activities.

A key objective of the EMN is the definition of a common approach for the production of Certified Reference Materials and Reference Materials for food and food-related matrices and analytes. In this presentation, the SRA and the EMN strategy for reference materials will be presented, together with the national and international projects involving the consortium and aligned with the scope of the EMN.

**Keywords:** food safety, food sustainability, metrology, networking

**Acknowledgement:** The project 20NET02 Food-MetNet has received funding from the European Metrology Programme for Innovation and Research (EMPIR), co-financed from the European Union's Horizon Europe Research and Innovation Programme and by the Participating States.

## LECTURES

### L27

## SAFETY ASSESSMENT OF FOOD CONTACT MATERIALS IN THE CONTEXT OF EUROPEAN REGULATIONS

**Erich Leitner**\*<sup>(1)</sup>

<sup>1)</sup> Graz University of Technology, Institute of Analytical Chemistry and Food Chemistry, Austria

\*Corresponding author - E-mail: erich.leitner@tugraz.at

The global food packaging market size was valued at USD 362.9 billion in 2022 and is expected to expand at a compound annual growth rate of 5.7% from 2023 to 2030 [1]. There are different types of packaging materials on the market, one of the largest groups are various polymer materials ("plastic"). Approximately 40% of virgin polymers and 50% of produced paper are used for packaging applications.

Also, in accordance with the European Strategy it is planned preventing packaging waste, boosting reuse and refill, and making all packaging recyclable by 2030 [2].

A new commission regulation released in September 2022 deals with the use of recycled polymers as food contact materials [3].

The European initiative, which stipulates that all packaging materials used must be either reusable or recyclable by 2030, means that new analytical methods are urgently needed to check the safety of products. In addition, the new Regulation (EU) 2022/1616 came into force in October 2022 to create the basis for achieving the ambitious targets: it relies on the development of new recycling technologies ("Novel Technologies", NT). In order to promote this, it enables recycled plastics produced using NT to be placed on the market before an assessment by the European Food Safety Authority (EFSA). In order to guarantee the safety of the products in accordance with Regulation (EC) 1935/2004, "scientific evidence and studies" must be provided when registering the NT. Further data on the technology will be generated during a test period for each batch produced.

These include, for example:

- Detailed data on contamination in the input material and the most likely sources of this contamination
- Remaining contaminants in the recycled product, in particular potentially still present genotoxic substances and endocrine disruptors
- An estimation of the migration of these contaminants into a packaged foodstuff

Paragraph 2 in this document clearly mentions that *"a pre-requisite to any increase in recycled content in food packaging and other food contact materials remains the need to secure a high level of protection of human health."*

So, there is a high demand to develop and use different methods to proof the safety of these materials. In this presentation strategies for safety evaluation by various analytical techniques will be discussed.

[1] <https://www.grandviewresearch.com/industry-analysis/food-packaging-market>.

[2] [https://ec.europa.eu/commission/presscorner/detail/en/ip\\_22\\_7155](https://ec.europa.eu/commission/presscorner/detail/en/ip_22_7155).

[3] <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32022R1616>.

**Keywords:** food contact materials, safety assessment, contaminants, migration



## LECTURES

### L28

## INCREASING THE RELIABILITY OF MOSH & MOAH ANALYSIS IN FOOD BY IMPROVED SAMPLE PREPARATION AND HYPHENATED CHROMATOGRAPHIC TECHNIQUES

**Giorgia Purcaro**\*<sup>(1)</sup>, **Gregory Bauwens**<sup>(1)</sup>, **Aleksandra Gorska**<sup>(1)</sup>, **Paula Albendea**<sup>(1)</sup>

<sup>1)</sup> Gembloux Agro-Bio Tech, University of Liège, Belgium

\*Corresponding author - E-mail: gpurcaro@uliege.be

Mineral oil hydrocarbons, usually referred to as MOSH and MOAH in food, belong to a class of ubiquitous contaminants and represent a long-lasting analytical challenge due to the complexity of their mixture and the difficulty in separating them from the naturally occurring interferences. The routine method involves the use of online LC-GC-FID; nevertheless, it fails to give a more detailed insight into the composition of the contamination. Starting from 2008-2009 the use of GC×GC was suggested, and in 2020, the use of a hyphenated LC-GC×GC-FID/MS system has been proposed and then validated. The use of GC×GC provides more robust data interpretations and a more accurate quantitative insight into the different subclasses of the MOSH and MOAH. Nevertheless, the uncertainty related to the sample preparation step, notably extraction/saponification and epoxidation, still significantly impacts the uncertainty of the determination.

In this presentation, the optimization of an improved saponification method using microwave-assisted saponification and extraction is presented to overpass the uncertainty caused by the recently introduced ISO method, which causes a significant discrepancy in the ratio of the internal standards used for quantification, i.e., TBB/2MN of about 1.25. The optimized procedure achieved a TBB/2MN ratio in the  $1.05 \pm 0.01$  tested in five different fats and oils, namely, sunflower, rapeseed, coconut, palm, and extra virgin olive oils. Furthermore, an alternative to the epoxidation step, which is also a step that significantly contributes to the uncertainty of the determination (causing ~30-40% of MOAH losses), is proposed based on a chromatographic purification using the same LC column used for separating MOSH and MOAH but with a different solvent gradient. The proposed purification allows for ~90% of recovery and good removal of the naturally occurring interferences without any chemical reaction involved.

**Keywords:** mineral oil saturated hydrocarbons, mineral oil aromatic hydrocarbons, LC-GC×GC-FID/MS, sample preparation

**Acknowledgement:** Fonds de la Recherche Scientifique Belgique (FNRS) - PDR projects-ToxAnaMOH T.0187.23.

## LECTURES

### L29

## INVESTIGATION OF SEMI-QUANTIFICATION ACCURACY AND DETECTOR UNIVERSALITY FOR PACKAGING SAFETY ANALYSIS

**Elsa Omer**\*<sup>(1)</sup>, **Marine Ewers**<sup>(1)</sup>, **Fabien Clement**<sup>(1)</sup>, **Yves-Alexis Hammel**<sup>(1)</sup>, **Oriane Bellahoues**<sup>(1)</sup>, **Jesus Varela**<sup>(1)</sup>, **Sander Koster**<sup>(1)</sup>

<sup>1)</sup> *Department of Advanced Packaging Sciences & Technology, Société des Produits Nestlé SA, Research Center., Switzerland*

\*Corresponding author - E-mail: [Elsa.Omer@rd.nestle.com](mailto:Elsa.Omer@rd.nestle.com)

From any packaging materials, chemicals might be transferred into the food and result in consumer exposure. Those migrating substances, both intentionally added substances (IAS) and non-intentionally added substances (NIAS) can represent a multitude of compounds such as plasticizers, antioxidants, biocides, natural substances or oligomers, all exhibiting various physico-chemical properties. Besides the challenge of identification, the ability to detect and accurately estimate the quantity of these migrating chemicals is crucial to avoid any overestimation or, worse still, an underestimation that would bias the conclusions about the packaging food safety assessment. Consequently, to characterize the large number and wide chemical diversity of NIAS, the choice of an appropriate analytical strategy is essential. Targeted approaches are normally employed when dealing with known molecules. However, NIAS comprises both known and unknown molecules requiring non-targeted approaches. Therefore, the use of a complementary approach involving liquid chromatography (LC) and gas chromatography (GC) to screen from non-volatile to volatile substances is relevant. Mass spectrometers are unavoidable for identification and detectors such as Charged Aerosol Detector (CAD) or Flame Ionization Detector (FID) are more suitable for semi-quantification as both are known to be universal, meaning, generate a response independently of the properties/structure of the molecule.

The aim of this study was to investigate the accuracy of the semi-quantification and the universality of the detectors for around 100 analytical standards covering a wide space of physico-chemical properties by using two analytical platforms, LC-CAD/HRMS and GC-FID/MS. Detectors, LC-CAD, GC-FID, LC-HRMS in positive and negative ionization modes and GC-MS were compared amongst themselves. Based on experimental values and on the physico-chemical properties of the studied substances, the ability of these detectors to detect chemicals and their universality in terms of response factor were screened and interpreted. Concerning the detectability, results revealed that LC-HRMS turned out to be the most universal detector in comparison to LC-CAD which presented limitation mainly related to the volatility of substances. To understand the accuracy of semi-quantification, relative response factors (response of analytical standards compared to an external reference) were calculated. CAD and FID appeared to provide the most accurate semi-quantification and results confirmed that external references used were relevant. Optimization of mobile phases composition (pH and salt) enabled a significant increase (~100 times) in the response generated by HRMS in negative ionization mode for some chemicals, especially for bisphenols, without affected the response generated by HRMS in positive ionization mode.

**Keywords:** semi-quantification, non-Intentionally added substances, non-targeted analysis, packaging food safety assessment, complementarity

### L30

## CP-MIMS: A NEW FRONTIER FOR THE REAL-TIME MONITORING OF HAZARDOUS CHEMICAL MIGRATION FROM FOOD CONTACT MATERIALS

**Maurizio Piergiovanni<sup>(1)</sup>, Monica Mattarozzi<sup>(1)</sup>, Federica Bianchi<sup>(1)</sup>, Nicolo Riboni<sup>(1)</sup>, Cristian Maffezzoni<sup>(1)</sup>, Veronica Termopoli<sup>(2)</sup>, Viviana Consonni<sup>(2)</sup>, Davide Ballabio<sup>(2)</sup>, Maria Careri<sup>\*(1)</sup>**

<sup>1)</sup> University of Parma, Italy

<sup>2)</sup> University of Milano Bicocca, Italy

\*Corresponding author - E-mail: maria.careri@unipr.it

Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS) is a promising technique for direct on-line analysis that does not require sample preparation and chromatographic separation [1]. It involves a semi-permeable hollow fiber membrane that is immersed directly into the sample and through which a liquid acceptor phase continuously flows, conveying the permeated compounds to the MS ion source. Since only compounds fulfilling certain physicochemical properties can permeate through the membrane, adequate selectivity can be reached even in untreated complex matrices. These aspects, together with a fast analysis time, make CP-MIMS a suitable strategy for the continuous real-time monitoring of dynamic processes. Despite these advantages, to date the potential of CP-MIMS for food control has been poorly explored.

In the field of food safety assessment, the migration of emerging and re-emerging contaminants from food contact materials (FCMs) into food is an issue that analytical chemistry has to face [2]. In this context, the recent reduction of the tolerable daily intake of bisphenol A (BPA) by EFSA has renewed interest in evaluating its release from FCMs, for which current specific migration limit is set at 50 µg/kg (EU Reg. 2018/213). A safety concern for not yet regulated BPA analogues is also emerging.

In this study, for the first time, a CP-MIMS method was developed and validated for the real-time monitoring and quantification of BPA, BPE, BPF, and BPS released from FCMs. The CP-MIMS probe was coupled to an electrospray source. A full factorial DoE and desirability functions were applied to optimize experimental conditions on the basis of multiple response variables. Method validation was performed in drinking water and food simulants, obtaining LODs at low µg/L; in addition, the reliable applicability of the method in beverage samples was proved. The CP-MIMS method was finally used to monitor in real-time the migration profile of bisphenols over time in the case of plastic FCMs that showed detectable release, thus enabling unexplored levels of material characterization for food safety.

[1] V. Termopoli, et al., *Separations* 2023, 10, 139.

[2] M. Mattarozzi et al., *Anal. Bioanal. Chem.* 2023, 415, 119.

**Keywords:** membrane introduction mass spectrometry, direct mass spectrometry, real-time monitoring, food contact materials, hazardous chemical migration

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## LECTURES

### L31

#### INVESTIGATION OF POTENTIAL MIGRATABLES FROM PAPER AND BOARD FOOD CONTACT MATERIALS INTENDED FOR TAKEAWAY

**Mélanie Di Mario**<sup>(1)</sup>, **Grégory Bauwens**<sup>(2)</sup>, **Florian Peltier**<sup>(1)</sup>, **Pierre-Hugues Stefanuto**<sup>(2)</sup>, **Séverine Goscinny**<sup>(1)</sup>, **Giorgia Purcaro**<sup>(3)</sup>, **Jean-François Focant**<sup>(2)</sup>, **Els Van Hoeck**<sup>(1)</sup>

<sup>1)</sup> *Sciensano, Belgium*

<sup>2)</sup> *University of Liège, Belgium*

<sup>3)</sup> *Gembloux Agro-Bio Tech, University of Liège, Belgium*

*\*Corresponding author - E-mail: melanie.dimario@sciensano.be*

Since the ban on single-use plastic articles in Europe, the food contact material (FCM) industry has been forced to move to more sustainable alternatives. All plastic packaging should be reusable or easily recyclable by 2030 in accordance with the EU strategy for Plastics in the Circular Economy. Various alternatives have been developed, focusing on bioplastics or recycled materials. Alternatively, the applications of other materials have been extended. For example, straws made of single-use plastic have been replaced by paper straws. Paper and board FCM offer a significant advantage, i.e., versatility. Indeed, depending on the coating or treatment of the paper and board material, they can be used for liquids, frozen, fresh, or dry foods. In addition, paper and board is the main material used in fast-food restaurants. Although paper and board FCM are convenient alternatives, they must be safe for consumers. Therefore, it is crucial to evaluate the migration of potentially harmful substances. These substances can be intentionally added, e.g., additives, synthetic fibers, adsorbents, treatment agents, and colorants, or be present unintentionally, like degradation products or substances originating from the recycling process. Furthermore, paper and board FCMs are often coated, glued, printed, composed of several layers, or combined with other materials, so the final FCM contains even more potential. Limited data exist on takeaway articles or straws, although they are gaining popularity, especially since the Covid-19 crisis. Therefore, this study aims to investigate potential migrations of various substances (e.g., plasticizers, photoinitiators, primary aromatic amines, mineral oil, PFAS and bisphenols) from straws and takeaway articles made of paper and board using targeted analysis but also untargeted analysis. Twenty straws and fifty-eight takeaway articles were carefully selected and investigated using liquid and gas chromatography coupled with mass spectrometry or flame ionization detector. Twenty-three substances of all the targeted categories were found in takeaway articles, including seven plasticizers, two photoinitiators, one primary aromatic amine, two bisphenols, nine PFAS and the saturated and aromatic fraction of mineral oil (MOSH and MOAH, respectively). At least one of the target substances was detected in 57% of the samples, demonstrating the importance of further evaluation of these materials. Therefore, in addition to targeted analyses, untargeted analyses using LC-HRMS and GC(xGC)-TOFMS were performed on a panel of samples, highlighting the presence of numerous substances potentially migrating into food.

**Keywords:** targeted analysis, untargeted analysis, paper and board, food contact materials

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### L32

## ACRYLAMIDE: 6 YEARS OF REGULATION (EU) 2158/2017 - RESULTS OF AN OFFICIAL CONTROL LABORATORY (OCL)

**Carmen Breitling-Utzmann**<sup>(1)</sup>, **Nadja Bauer**<sup>(1)</sup>

<sup>1)</sup> *Chemical and Veterinary Investigations Office (CVUA) Stuttgart, Germany*

*\*Corresponding author - E-mail: carmen.breitling-utzmann@cvuas.bwl.de*

Acrylamide (AA) is a process contaminant formed during the Maillard reaction, mostly from the precursors asparagine and reducing sugars [1]. The International Agency for Research on Cancer IARC classified AA as "probably carcinogenic" to humans (Group 2a) and the European Food Safety Authority EFSA stated that acrylamide increases the potential cancer risk for consumers of all age groups [2].

Therefore, numerous mitigation measures have been developed to reduce the acrylamide content in food since AA was first detected in food in 2002. For food stuffs that have shown to be particularly prone to the formation of AA such as gingerbread, coffee, potato chips and French fries, mitigation measures and benchmark levels (BMLs) have been laid down in Regulation (EU) 2017/2158, which has been in force since 11<sup>th</sup> April 2018.

The Chemical and Veterinary Investigation Office (CVUA) Stuttgart is an official control laboratory (OCL) that investigates acrylamide in food samples centrally for southwestern Germany. Between 2018 and 2023, a total of 2426 samples ranging from the usual suspects potato chips, coffee and French fries to matrices like roasted nuts or oxidized olives were analysed for AA. Product groups having BMLs laid down in Regulation (EU) 2017/2158 rarely exceeded the respective indicative values. Furthermore, the average AA content was generally well below the corresponding BML. In contrast, some products that are not yet regulated in (EU) 2017/2158, such as vegetable chips or oxidized olives, were often found to contain very high levels of AA [3]. The acrylamide contents of the investigated food groups will be shown and discussed in the presentation.

[1] Stadler R H, Blank I, Varga N, Robert F, Hau J, Guy P A, Riediker S. 2002. Acrylamide from Maillard reaction products. *Nature* 419:449-450. <https://doi.org/10.1038/419449a>.

[2] EFSA Panel. 2015. Scientific opinion on acrylamide in food. *EFSA Journal*, 13(6):4101-4424. <https://doi.org/10.2903/j.efsa.2015.4104>.

[3] Breitling-Utzmann C M. 2024. "5 Jahre EU-Acrylamid-Verordnung - Alles gut?", internet report by CVUA Stuttgart: [https://www.cvuas.de/pub/beitrag.asp?subid=1&Thema\\_ID=2&ID=3941](https://www.cvuas.de/pub/beitrag.asp?subid=1&Thema_ID=2&ID=3941).

**Keywords:** acrylamide, process contaminant, official control laboratory, regulation (EU) 2158/2017

**Acknowledgement:** *The authors express thanks to the laboratory staff at the CVUA Stuttgart, especially Marion Bord, Simone Goetz, Ragna Gregorius, Cornelia Kobe, and Fiona Schert.*

### L33

## IMPLEMENTING IMAGE DATA ANALYSIS TO PREDICT THE ACRYLAMIDE CONTENT IN CARROT CRISPS PROCESSED BY DIFFERENT FRYING TECHNOLOGIES

**Aristeidis Tsagkaris\***<sup>(1)</sup>, **Beverly Hradecka**<sup>(1)</sup>, **Tomas Kourimsky**<sup>(1)</sup>, **Hana Pirova**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: tsagkara@vscht.cz*

Acrylamide is a thermal processing contaminant commonly found in heat treated foodstuffs (> 120°C) of plant origin, with L-asparagine and reducing sugars play a pivotal role as its precursors. Although acrylamide has been classified as a probable human carcinogen, it is not legislatively regulated and only benchmark levels have been set in the EU (Regulation 2017/2158). In case of benchmark level excess, mitigation measures should be applied, e.g., thermal processing at a lower temperature. The setting of maximum levels for acrylamide is under consideration, following the Commission Recommendation 2019/1888 on the monitoring of the acrylamide presence in certain foods. Considering that the expected sample loads are going to increase by a potential regulatory limit enforcement, rapid methods are highly needed to complement the chromatographic testing. In this study, we achieved non-destructive and cost-effective acrylamide content prediction by using image data analysis acquired by the camera of 2 smartphones and 1 tablet. The prediction is based on the hypothesis that during acrylamide formation a dark colour is being formed on the food surface which is increasing overtime indicating the possibility to correlate the colour formation to acrylamide concentration [1]. Carrot crisps, originating from 7 different varieties, were prepared and processed using conventional, vacuum and air frying. The RGB colour space was used as the analytical signal and the R channel values correlated towards the colour formation. In addition, correlation was noticed towards the acrylamide concentration obtained by an accredited liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Actually, the correlation between acrylamide concentration and R channel values was fitted with a one-phase exponential decay function based on experimental results acquired at 5 endpoints during frying. Importantly, the inter-device result variation was investigated by measuring the same samples with three different devices and not major differences were found. This is a very important finding as there is controversy related to the camera effect on the recorded colour due to the different optical parts used by manufacturers. Overall, the proposed approach has the potential to intensify acrylamide testing and can be applied even at the point-of-need.

[1] R. Sáez-Hernández et al., Determination of acrylamide in toasts using digital image colorimetry by smartphone, 2022, Food Control, 109163.

**Keywords:** smartphones, LC-MS/MS, processing contaminants, food safety, screening method

**Acknowledgement:** *This work was funded by the Technology Agency of the Czech Republic (TACR), grant number TQ03000738 and the Project LUC23140 provided by the Ministry of Education, Youth and Sport of the Czech Republic (MSMT), program INTER-EXCELLENCE II. Also, data/tools/services/facilities were provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>) and supported by MSMT (Project No. LM2023064).*

## LECTURES

### L34

## QUANTIFICATION, EXPLORATION AND MITIGATION OF FURAN AND ITS DERIVATIVES IN INFANT FOODS

Donnelle Sandjong Sayon<sup>(1)</sup>, Aya Fakh<sup>(1)</sup>, Frederic Mercier<sup>(1)</sup>, Nathalie Kondjoyan<sup>(1)</sup>, Berta Torrents<sup>(2)</sup>, Norbert Fuchsbauer<sup>(3)</sup>, Evangelia Kristalli<sup>(4)</sup>, Rallou Thomopoulos<sup>(5)</sup>, Jeremy Ratel<sup>(6)</sup>, **Erwan Engel**\*<sup>(1)</sup>

<sup>1)</sup> French National Research Institute for Agriculture, Food and Environment (INRAE), UR QuaPA, MASS Group, France

<sup>2)</sup> IRTA, Spain

<sup>3)</sup> HIPP GmbH & Co, Germany

<sup>4)</sup> Yiotis Anonimos Emporiki & Viomixaniki Etaireia, Greece

<sup>5)</sup> INRAE, UMR IATE, France

<sup>6)</sup> French National Research Institute for Agriculture, Food and Environment (INRAE), UR QuaPA, France

\*Corresponding author - E-mail: erwan.engel@inrae.fr

Children under the age of 3 are highly vulnerable to chemical hazards because their immune system is immature, and their food absorption capacity is greater than that of adults. Given that food is a major source of exposure, process-induced toxicants like furan and several of its derivatives, formed during heat treatments are of particular concern. Furan is in fact 'possibly carcinogenic to humans' and is also suspected to affect reproductive and nervous systems. In addition to furan, 2-methylfuran (2-MF), 3-methylfuran (3-MF) are also targeted by the European Commission (2022) and the latter is today recommending to expand research to other possible derivatives of furan which could also be at risk for infants.

Due to the ability of furan to be generated by heating processes and its volatility, its quantification in complex matrices like infant foods still represents a real challenge. Although different couplings between the most popular headspace extraction techniques and Gas Chromatography- Mass Spectrometry (GC-MS) systems have been proposed, no study published to date has really made it possible to identify an indisputable method for its quantification [1]. This paper demonstrates the value of the accuracy profile approach for assessment, validation and benchmarking of methods and shows that static headspace -GC-Q Exactive Orbitrap MS is the best choice for the quantification of Furan, 2-MF and 3-MF in infant foods. Moreover, it also points out that GC-Q Exactive Orbitrap MS is also relevant for suspect screening exploration of furan derivatives in infant foods. A broad range of compounds are given, including compounds never reported until now in infant foods.

Given that infants are more exposed to furan than adults mainly via ready-to-eat meals and more particularly via vegetables-based meals, mitigation strategies are needed to reduce infant dietary exposure to furan and to its derivatives in these foods. These mitigation strategies rely on the development of less generating industrial processing but also on recommendations for adapted home practices. Based on the analytical developments described above, the impact of mild processing technologies like High Pressure Thermal Processing and key domestic practices like post-reheating stirring on the level of furan and derivatives in infant food is demonstrated.

[1] Frank, N., Delatour, T., Dubois, M., Novotny, O., Dufossé, T., Mollergues, J., Scholz, G., & Moulin, J. (2024). Do GC/MS methods available for furan and alkylfurans in food provide comparable results? - An interlaboratory study and safety assessment of 2-pentylfuran in food. *Food Additives & Contaminants: Part A*, 41(1), 22-32.

**Keywords:** furan and derivatives, infant food, GC-Q Exactive Orbitrap-MS, home practices, mild processing

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## LECTURES

### L35

#### SEARCH FOR CHLORINATED LIPIDS IN REFINED VEGETABLE OILS

**Tomas Kourimsky**\*<sup>(1)</sup>, **Jakub Tomasko**<sup>(1)</sup>, **Beverly Hradecka**<sup>(1)</sup>, **Vojtech Hrbek**<sup>(1)</sup>, **Jan Kyselka**<sup>(2)</sup>, **Jana Pulkrabova**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> Department of Dairy, Fat and Cosmetics, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: kourimst@vscht.cz

Industrial food processing, especially vegetable oil refining may lead to undesirable chemical changes in the final product. The most important processing contaminants of edible oils include (not only) ester-bound chlorinated contaminants. The largest share of the content of chlorinated processing contaminants in refined oils constitutes of esters of chloropropanediols (MCPD). As shown in recent studies, in addition to chloride, organochlorine compounds can be precursors (chlorine donors) for MCPD esters formation.

In our previous work, focused on chlorinated paraffins (CPs) as potential precursors of MCPD esters during simulated vegetable oil deodorisation / deacidification (heat treatment at 230 °C for 2 hours), a substantial increase in MCPD levels (up to 3.4 times the control levels) was observed in systems spiked with a technical mixture of CPs. During this work, a hypothesis was started regarding the possible chlorination of double bonds in unsaturated fatty acids bound in acylglycerols, by HCl released from CPs during heat treatment, to form (bound) chlorinated fatty acids (CFA). Very little toxicological data is available regarding these substances. Furthermore, commonly used methods of fatty acid analysis involve chemical hydrolysis and derivatisation, which was deemed not suitable for CFA, due to a possible impact of chemical reagents on the compounds of interest.

In this study, a mild alternative procedure, employing enzymatic hydrolysis with non-specific lipase, was successfully developed and optimized and the challenges presented during optimization were discussed. A high throughput method, utilizing ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (UHPLC-HRMS/MS), was optimized for simultaneous targeted screening of released potentially chlorinated fatty acids and a nontargeted 'fingerprinting' of hydrolysed vegetable oils. In-house synthesised standards of CFA, a library of theoretical *m/z* values, and *in silico* fragmentation patterns were utilized for CFA screening. Using lipidomic analysis, multivariate statistical approaches and tentative identification with available spectral libraries, it was possible to discuss changes in CFA profile and origination and content of other lipid species during simulated vegetable oil refining.

**Keywords:** vegetable oil refining, organochlorine compounds, chloropropanediol (MCPD) ester, chlorinated fatty acids, enzymatic hydrolysis

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## LECTURES

### L36

## ANALYSIS OF OXIDIZED AND GLYCATED AMINO ACIDS IN FOOD: WHY MASS SPECTROMETRY IS ESSENTIAL

**Michael Hellwig**\*<sup>(1)</sup>

<sup>1)</sup> Technische Universität Dresden, Chair of Special Food Chemistry, Germany

\*Corresponding author - E-mail: michael.hellwig@tu-dresden.de

Glycation ("Maillard reaction", "non-enzymatic browning") and oxidation are the most important deterioration reactions of dietary proteins [1,2]. The Maillard reaction is responsible for the aroma, taste, and appearance of thermally processed food. Just as protein oxidation, it may induce structural and functional changes in food proteins. Recent studies postulated that dietary glycation compounds, often denoted as "advanced glycation end products" or "AGEs", may pose a nutritional risk as "glycotoxins" due to a possible link to chronic inflammation and other metabolic diseases.

The specific expression of an "AGE content" assessed by ELISA techniques, either in foods or biological samples, is not scientifically justified without giving details about the individual structures targeted by the antibodies [3]. Similarly, quantification of "protein carbonylation" is only targeted on minor protein oxidation structures in food. For a reliable quantification of individual glycation and oxidation compounds, methods measuring structural features such as LC-MS or GC-MS and thoroughly characterized reference materials must be used.

Recent results on structure-based methodology will be presented. Protein oxidation and/or glycation compounds were analyzed in malt, beer, honey, and pasta products by HPLC-MS/MS and by HILIC-MS/MS [4,5]. These data will show the limited applicability of ELISA-based sum methods that are often used in (food) glycation research. In UHT and evaporated milks, up to 8% of methionine was oxidized, whereas up to 33% of methionine was oxidized in milk drinks containing added cocoa or coffee components. Methionine oxidation in brewing malts can be as high as 60%. The concentrations of methionine sulfoxide in milk products or brewing malts are far above the concentrations of protein carbonyls measured by the DNPH method [5,6].

In conclusion, sum methods based on the (immuno)reactivity of more or less characteristic structures of protein oxidation and glycation may have been helpful in gaining first insights into these reactions. However, in order to avoid misinterpretations concerning adverse effects of individual amino acid derivatives on health, chromatography coupled to mass spectrometry is essential [7].

[1] Hellwig M. *Angew Chem Int Ed* 2019, 58, 16742.

[2] Hellwig M, Henle T. *Angew Chem Int Ed* 2014, 53, 10316.

[3] Hellwig M, Humpf HU, Hengstler JG, Mally A, et al. *J Agric Food Chem* 2019, 67, 11307.

[4] Kölpin M, Hellwig M. *J Agric Food Chem* 2019, 67, 8967.

[5] Fleischer K, Hellwig M. *Eur Food Res Technol* 2023, 249, 199.

[6] Scheidegger D, Radici PM, Vergara-Roig VA, Bosio NS, et al. *J Dairy Sci* 2013, 96, 3414.

[7] Hellwig M, Diel P, Eisenbrand G, Grune T, et al. *Arch Tox* 2024, accepted.

**Keywords:** glycation, protein oxidation

## LECTURES

### L37

## STATISTICAL APPROACHES TO METABOLOMICS DATA HANDLING

Vit Kosek<sup>\*(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: kosekv@vscht.cz*

Although metabolomics is a relatively established analytical strategy, understanding some aspects of data processing and handling can still be complicated, especially for beginners. However, the correct handling of data is crucial for the outcome of the study and can lead to unstable statistical models that are unable to correctly classify samples or produce a misleading list of potential marker compounds, to name just a few. This seminar aims to provide an overview of the typical workflow in a metabolomics study, including classical and novel approaches that deliver robust results.

**Keywords:** metabolomics, statistics, chemometrics

### L38

## DATA FUSION AND ARTIFICIAL INTELLIGENCE BASED NOVEL TESTING SYSTEM FOR TEA GEOGRAPHICAL ORIGIN AUTHENTICATION

**Yicong Li<sup>(1)</sup>, Chris Elliott<sup>(2)</sup>, Natasha Logan<sup>(1)</sup>, Awanwee Petchkongkaew<sup>(3)</sup>, Nicholas Birse<sup>(1)</sup>, Terry F. McGrath<sup>(4)</sup>, Di Wu<sup>\*(1)</sup>**

<sup>1)</sup> National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gard, United Kingdom

<sup>2)</sup> International Joint Research Center on Food Security (IJC-FOODSEC), 111 Thailand Science Park, Thailand School of Food Science and Technology, Faculty of Science and Technology, Thammasat University, United Kingdom

<sup>3)</sup> International Joint Research Center on Food Security (IJC-FOODSEC), Thailand

<sup>4)</sup> Bia Analytical Ltd, 63 University Road, Belfast, Northern Ireland, UK, United Kingdom

\*Corresponding author - E-mail: d.wu@qub.ac.uk

Tea is the world's most widely consumed beverage. Its global supply chain is complex and lacks transparency, making it extremely vulnerable to fraud, especially for geographical indication (GI) products. This is because the market value and important tea characters, such as flavour and taste, are largely determined by their geographical origin. Therefore, the easy-to-use and sustainable tools must be developed to test fraud and uncover products with false declaration of geographical origin. This work presents the development of tea geographical origin authenticity testing system based on Fourier Transform Infrared (FTIR), Near Infrared (NIR), and X-ray Fluorescence (XRF) spectroscopy, data fusion strategies and artificial intelligence algorithms. A total of 532 authentic black tea samples originating from four world-leading tea producing GI regions, namely Assam, Darjeeling, Keemun, and Sri Lanka, were collected and analysed by FTIR, NIR, and XRF spectroscopy with three replicates per sample.

The whole datasets were divided into training set and two independent test sets to ensure the unbiased analysis. Different data pre-treatment and transmission methods were analysed for each data type individually. The hyper-parameters of each machine learning and deep learning models were fine tuned through training set before modelling.

IR vibration-based spectroscopy, such as NIR and FTIR, can detect hundreds of molecular features simultaneously and provide a rapid, high throughput, unbiased, and non-destructive test, which is fit for on-site testing and effective management of fast-paced global food networks. XRF spectroscopy can measure multi-elemental contents with advantages of being rapid, non-destructive, cost efficient, environmental-friendly.

Additionally, the advanced different data fusion strategies, including low-level fusion, mid-level fusion, and high-level fusion, enhanced the applicability of these techniques. The application of different machine learning and deep learning algorithms, such as random forest, k-nearest neighbour, support vector machine, and multilayer perceptron, increased the accuracy of decision-making results. The sufficient model validation system, including internal cross validation and independent external validation make sure the reliability of the final predictions.

The results showed that the corroboration of the information provided by these three analytical techniques enhance the discrimination capability of the developed recognition models (accuracy up to 100%) as compared to the case when only one technique is used. Moreover, this study highlights the importance of using advanced artificial intelligence algorithms to capture key information, learn recognition patterns, and make accurate predictions.

**Keywords:** data fusion, artificial intelligence, rapid testing, geographical origin, tea authenticity

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## L39

**MACHINE LEARNING ALGORITHMS AT THE BRIGHT SIDE OF FTIR-ATR ANALYSIS IN LATHYRUS SATIVUS L.**

**Elsa Mecha**<sup>(1)</sup>, **Pedro R. Brandao**<sup>(2)</sup>, **Letice Gonçalves**<sup>(3)</sup>, **Elsa Brito**<sup>(4)</sup>, **Andreia Bento da Silva**<sup>(5)</sup>, **Maria Carlota Vaz Patto**<sup>(3)</sup>, **Maria Rosario Bronze**<sup>(6)</sup>

<sup>1)</sup> Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal, Portugal

<sup>2)</sup> Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; LAQV-REQUIMTE, Department of Chemistry, NOVA School of Science and Technology, FCT NOVA, Universidade NOVA de Lisboa, Caparica, Port, Portugal

<sup>3)</sup> Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal, Portugal

<sup>4)</sup> Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal, Portugal

<sup>5)</sup> Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal, Portugal

<sup>6)</sup> Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal; Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal; Faculdade de Farmácia, Universidade de Lisboa, Lisboa,, Portugal

\*Corresponding author - E-mail: emecha@ibet.pt

*Lathyrus sativus* L., also called grass pea, possesses favourable nutritional characteristics as well as adaptative traits for handling stress [1]. Its consumption associated to a very restrictive non-diversified diet, has been linked to the onset of lathyrism. The discovery of a neurotoxin  $\beta$ -N-oxalyl-L- $\alpha$ ,  $\beta$ -diaminopropionic acid ( $\beta$ -ODAP), tarnished the image of grass pea as a valuable food source [2]. Nevertheless, grass pea has undeniable nutritional value, being a precious source of L-homoarginine, L-hArg, a promising cardio protector compound [3].

Advanced vibrational spectroscopy to characterize legumes nutritional quality has been applied for different legumes [4,5], but its use to characterize grass pea accessions is still very limited. In this study, we applied ATR-FTIR spectroscopy supported by machine learning methods focusing on the development of robust, and cost-effective models for ODAP, homoarginine and phenolic compounds content in grass pea.

For that, we cropped more than 200 different accessions of grass pea over three years under similar conditions in Alvaiázere (Portugal). The aqueous extracts were analysed by HPLC-ESI-MS/MS to determine ODAP and homoarginine contents [6]. The ethanolic extracts were analysed by spectrophotometry to determine the total phenolic content by Folin-Ciocalteu's method, and the *in vitro* antioxidant activity by Oxygen radical absorbance capacity (ORAC) [7]. We screened the samples applying the Attenuated Total Reflectance - Fourier Transformed Infrared Spectroscopy, ATR-FTIR (Thermo Nicolet 6700 spectrometer).

Different algorithms were applied to more than 2000 collected spectra based on partial least square regression multivariate analysis, kernelized Support Vector Machine (SVMs), 1D convolutional neural nets e random forests, combined with variable selection methods to restrict the spectrum to relevant areas were applied using Python interface programming language to recognize patterns and explore the potential correlations between ATR-FTIR spectral data and the quantified quality parameters.

Following the numerous combinations of pre-processing methods and hyperparameters, 9600 pipelines were tuned by random search cross-validation. Data suggested the adequacy of ATR-FTIR for predicting quality parameters only after specific pre-processing methods (e.g., detrend, Savitzky-Golay derivatives, and multiplicative scatter correction, MSC). ATR-FTIR was able to derive alpha-ODAP ( $R^2=0.73$ ), beta-ODAP ( $R^2=0.86$ ), and Homoarginine ( $R^2=0.78$ ), considering the spectral region between 1806 and 892  $\text{cm}^{-1}$ . Other algorithms are currently being tested.

The results of the present work suggest that ATR-FTIR spectral data can represent fundamental tools to support future breeding programs dedicated to the improvement of grass pea's quality.

[1] 10.3389/fnut.2021.826208.

[2] 10.1007/s00425-018-03084-0.

[3] 10.1111/fcp.12858.

[4] 10.1002/leg3.40.

[5] 10.3153/FH18008.

[6] 10.3390/molecules24173043.

## LECTURES

[7] 10.3390/foods8080296.

**Keywords:** grass pea, beta-ODAP, homoarginine, spectroscopy, machine learning

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## LECTURES

### L40

## UNLOCKING TOMATO QUALITY ATTRIBUTES IRRIGATED WITH TREATED WASTEWATER USING MACHINE LEARNING

**Anja Vehar**<sup>(1)</sup>, Jan Drole<sup>(2)</sup>, Doris Potocnik<sup>(3)</sup>, Ana Kovacic<sup>(3)</sup>, Lidija Strojnik<sup>(3)</sup>, Marta Hudobivnik<sup>(3)</sup>, Marina Pintar<sup>(4)</sup>, Nina Kacjan Marsic<sup>(4)</sup>, David Heath<sup>(1)</sup>, Ester Heath<sup>(1)</sup>, Nives Ogrinc<sup>\*(1)</sup>

<sup>1)</sup> Jožef Stefan Institute, Department of environmental sciences and Jožef Stefan Postgraduate School, Slovenia

<sup>2)</sup> Faculty of Computer and Information Science, University of Ljubljana, Slovenia

<sup>3)</sup> Jožef Stefan Institute, Department of environmental sciences, Slovenia

<sup>4)</sup> Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia

\*Corresponding author - E-mail: nives.ogrinc@ijs.si

The increasing competition for freshwater resources, especially in drought-prone regions, is exacerbating water scarcity globally. One solution lies in the increased utilisation of treated wastewater for irrigation, with the potential to reduce the demand on river and groundwater. It is important to note that while treated wastewater is a source of fresh water and nutrients, it also contains many anthropogenic chemicals and elements, which could affect not only safety, but also the quality of the crops. This study aimed to assess the effects on tomato fruit quality by comparing tomatoes irrigated with potable and treated wastewater, grown in soil (lysimeters) and hydroponically. Soil-grown tomatoes were irrigated with: (S1) potable water containing commercial fertilizer, (S2) wastewater effluent, (S3) wastewater effluent spiked with 14 model contaminants of emerging concern (CEC) at 0.1 mg L<sup>-1</sup>. Hydroponically grown tomatoes were irrigated with: (H1) potable water containing fertiliser (H2) potable water containing fertilizer and spiked with CEC at 0.1 mg L<sup>-1</sup>. The model CEC included industrial chemicals (bisphenols), pharmaceuticals (non-steroidal anti-inflammatory drugs, estrogens), and common stimulants (caffeine).

The quality of the tomato fruits was assessed in terms of the amino acids, fatty acids, carotenoids, polyphenols, volatile organic compounds and elemental composition, using optimized and validated methods based on GC-MS, GC-FID, LC-MS, HS-SPME GC-MS, ICP-MS. These quality parameters were chosen since they influence flavour, texture, structure, mouthfeel, the colour of tomatoes, and the content of bioactive compounds and specific elements that promote good health. To investigate whether fruit quality was influenced by growing and irrigation media, we employed explainable machine learning. We trained classifiers with quality parameters to predict five different treatments for tomatoes grown in soil and hydroponic systems. Robust benchmarking demonstrated that the classifiers achieved 90-95% accuracy in detecting the treatment. Using the explainable SHAP method, we identified the most important quality parameters associated with each treatment.

**Keywords:** tomato, wastewater reuse, quality attributes, explainable machine learning

**Acknowledgement:** The authors acknowledge the Slovenian Research and Innovation Agency for the Young researcher grant, program group P1-0143, project L7-4422 and TunTWIN project GA N° 952306.

## LECTURES

### L41

## SAFEGUARDING OUR FOOD CHAIN: NOVEL STRATEGIES FOR THE CONTROL AND ANALYSIS OF MYCOTOXINS AND OTHER EMERGING CONTAMINANTS

**Rudolf Krska**<sup>(1)</sup>, **Stephan Freitag**<sup>(2)</sup>, **Michele Suman**<sup>(3)</sup>, **Eleonara Rollo**<sup>(3)</sup>, **Alexandra Schamann**<sup>(4)</sup>, **Martin Wagner**<sup>(5)</sup>

<sup>1)</sup> A - BOKU University, Vienna, Department IFA-Tulln; B - Queen's University Belfast, Institute for Global Food Security, Belfast, NI, UK; C - Austrian Competence Centre for Feed and Food, Quality, Safety, Austria

<sup>2)</sup> BOKU University, Vienna, Department IFA-Tulln, Austria

<sup>3)</sup> BARILLA S.p.A., Food Chemistry and Safety Research, Barilla Research Labs, Italy

<sup>4)</sup> Austrian Competence Centre for Feed and Food, Quality, Safety & Innovation (FFoQSI), Austria

<sup>5)</sup> A - Austrian Competence Centre for Feed and Food, Quality, Safety & Innovation (FFoQSI); B - Institute of Food Safety, Food Technology and Veterinary Public Health Unit of Food Microbiology, Unive, Austria

\*Corresponding author - E-mail: [rudolf.krska@boku.ac.at](mailto:rudolf.krska@boku.ac.at)

Food safety and security are pressurized by multiple threats including climate change, increased food intolerance, and dietary changes, to mention just a few. Consequently, next level measures are required to adequately respond to these threats. In particular, global warming accelerates the (co-) emergence of foodborne pathogens, natural toxins and toxic elements in food crops and food systems. The major goal of the European Commission funded project FoodSafeR, which was kicked off in October 2022, is to identify, assess, and manage emerging chemical and microbial food safety risks.

Climate-related impacts, such as heavy rainfall, are expected to increase the contamination rate of plant food sources and farm animals kept in extensive housing. Unintentional chemical contaminants in food, such as environmental and food process contaminants (e.g., furans) and natural toxins (especially mycotoxins and plant toxins), are posing growing public health concerns. To address emerging chemical contaminants and associated risks, several advanced analytical approaches need to be advanced and explored. These include improved prediction tools for mycotoxin and plant toxin occurrence in major food commodities using big data approaches, the development of novel (bio)analytical sensing and diagnostic tools, possibly integrated into smartphones to facilitate the detection of plant toxins in products like herbal teas and buckwheat. There is also a need for horizon scanning approaches, combining targeted LC-MS/MS and untargeted LC-HRMS metabolomics (liquid chromatography-high resolution mass spectrometry) to study the influence of climate change on the presence of emerging (toxic) secondary metabolites and agrochemicals.

This presentation will focus on advancing innovations to combat selected (emerging) contaminants in the context of climate change and globalization, utilizing cutting-edge management approaches and novel tools in analytical chemistry.

[1] Krska R, Elliott C, McNerney O, Wagner M. Emerging global food safety challenges and how to address them. *New Food Magazine*. 2022;25(4):6-9.

**Keywords:** emerging contaminants, mycotoxins, food safety, climate change

**Acknowledgement:** The authors express gratitude to the European Union for the funding provided within FoodSafeR (grant agreement n°101060698) and PHOTONFOOD (no. 101016444).

## LECTURES

### L42

#### RECENT ADVANCES IN UNDERSTANDING OF MYCOTOXINS IN BEER

**Milena Stranska**<sup>(1)</sup>, **Nela Prusova**<sup>(1)</sup>, **Tereza Dolezalova**<sup>(1)</sup>, **Adam Behner**<sup>(1)</sup>, **Zbynek Dzuman**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: milena.stranska@vscht.cz*

Beer is the second most consumed alcoholic beverage worldwide, and mycotoxin contamination of beer is a significant and unresolved food safety issue. Besides deoxynivalenol and its glycosides as the most common mycotoxins in beer, other mycotoxins with even higher toxicity such as T2 toxin, HT2 toxin, fumonisins and ochratoxin A may occur. The main source of mycotoxins and their glycosides in beer is malt, but its control before the brewing process is complicated - for the majority of mono-/oligoglycosides, no analytical standards are available, and they cannot be targeted by control laboratories. Therefore, rapid deglycosylation methods that allow easy quantification of the complex mycotoxin pool are very important to enable rapid control of malt quality in routine brewing laboratories. The achievements in this field will be discussed.

Regarding the reasons for the presence of high levels of mycotoxins in malt, extensive research has been carried out so far and grain germination has been identified as the most critical phase. Within the lecture, the recent technological progress in minimizing fungal contamination and mycotoxin production during malting, represented specifically by the pulsed electric field technology (PEF), will be presented. Important results of the research include the demonstration of the reduction potential of PEF for fungal pathogens and mycotoxins, especially for T2/HT2-producing species, under optimal PEF treatment conditions that inhibit fungal development during malting without affecting the enzymatic activity and technological quality of the final malt. Especially the latter is of paramount importance to maintain the best sensory properties of the final beer with minimized mycotoxin content. Finally, recent methodological and data-processing advances related to the description of the germinating grain physiology and the 'grain-pathogen' crosstalk for a complex understanding of the mutual interactions influencing malt/beer safety at the metabolomics and transcriptomics level will be discussed. The open-access software platforms and workflows for effective data mining, feature annotation and multi-omics data integration with 3D visualization will be shown. The use of artificial intelligence and machine learning approaches for beer quality and safety prediction will be outlined as a future perspective.

**Keywords:** mycotoxins, malt, beer, metabolomics, multiomics



## LECTURES

### L43

## NAVIGATING COMPLEX MIXTURES: NON-TARGETED APPROACHES IN DETECTING EMERGING NATURAL TOXINS

Laura Righetti\*<sup>(1)</sup>

<sup>1)</sup> Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: [laura.righetti@wur.nl](mailto:laura.righetti@wur.nl)

Natural toxins have incredibly complex chemical structures, and factors like climate change, circularity, and reduced pesticide use will alter them even further. Therefore, we need innovative methods to resolve this complex mixture and improve our ability to confidently identify and annotate emerging toxins.

In recent years, the scientific community has increasingly adopted non-targeted methods, particularly those based on high-resolution mass spectrometry (HRMS), to capture a broader spectrum of compounds. Additionally, ion mobility mass spectrometry (IMS) has been employed to enhance the separation of isomeric species. This is crucial because natural products often contain numerous isomers, reflecting the unparalleled chemical diversity that nature produces. These isomeric species are challenging to separate using traditional chromatographic techniques. For example, in regulatory contexts such as with pyrrolizidine and ergot alkaloids, isomers are often reported as a sum of their epimers due to these difficulties.

However, in certain cases, different isomers exhibit distinct properties. For instance, CBD and THC are chemically similar, but only THC has psychoactive effects, making their resolution critical. In our recent publication [1], we demonstrated the power of advanced ion mobility technologies, specifically cyclic IMS, to resolve the isomeric complexity in cannabis-derived products. By leveraging the unique adduct-forming properties of silver ions, we developed a milliseconds-separation method for 14 cannabinoids using cyclic IMS–mass spectrometry.

This talk will focus on novel approaches, such as cyclic IMS, for enhancing isomeric separation of plant toxins and mycotoxins, as well as the necessity of using advanced computational tools and data processing strategies to deconvolute the complexity of the data generated in non-targeted studies.

[1] Huang, S. et al. (2024) *Anal. Chem.* 96, 10170–10181.

**Keywords:** natural toxins, ion mobility mass spectrometry

**Acknowledgement:** Several colleagues from the laboratory of Organic Chemistry (Wageningen University) and from Wageningen Food Safety Research that contributed to this work are gratefully acknowledged.

## LECTURES

### L44

#### THE ANALYTICAL CHARACTERIZATION OF THE CIGUATERA POISONING AS AN EMERGING RISK IN EUROPE THROUGH THE EFFORTS CARRIED OUT DURING THE TWO EDITIONS OF THE EUROCIQUA PROJECT

Ana Gago Martínez\*<sup>(1)</sup>, David Castro<sup>(1)</sup>, Jorge Giraldez<sup>(1)</sup>, Alejandro Penin<sup>(1)</sup>, Jose Manuel Leao<sup>(1)</sup>

<sup>1)</sup> University of Vigo (CINBIO), Spain

\*Corresponding author - E-mail: anagago@uvigo.gal

The incidence of Ciguatera poisoning (CP) in certain subtropical areas of Europe, as Canary islands (Spain) and Madeira (Portugal) as a result of climate change, and other factors which favour the migration of microalgae responsible for this natural contamination of the marine environment and consequently on seafood from the affected coastal areas, has become an emerging risk of particular interest for the European Food Safety Authority (EFSA). This interest was reflected on the EFSA efforts to establish a Framework Partnership Agreement with several EU Institutions to characterize the Risk CP in Europe. Among the Institutions involved on this FPA, is the University of Vigo responsible for a Specific Grant focused on the analytical characterization of the CP risk by identifying the Ciguatera toxins (CTXs) involved in this risk. This analytical characterization is a major analytical challenge, not only because of the complexity of the chemical structures of the toxins involved, but also due to the complexity of the biological matrix in which CTXs are present at trace level. On top of that and as a very critical limitation, is the lack of reference materials commercially available, which hamper the characterization of the different CTX analogues involved, as well as compromise the advances on the development of efficient analytical methods for their control. On the other hand a critical limitation as a consequence of the lack of reference materials is the lack of toxicological data on the toxicity of the different CTX analogues, which also compromise the establishment of regulatory levels due to the lack of information about toxicity equivalent factors, which are essential for an adequate quantitation. The work carried out over the last seven years under EuroCigua projects cofinanced by EFSA has been very successful in terms of analytical characterization of the CP risk in Europe. These results are going to be summarized and a revision on the main analytical challenges, as well as the future perspectives will be also presented and discussed.

**Keywords:** algal toxins, ciguatoxins, characterization, LC/MS

**Acknowledgement:** EuroCigua project (EFSA FPA): GP/EFSA/KNOW/2022-23

## LECTURES

### L45

#### **FATE OF MYCOTOXINS DURING GLUTEN-FREE PASTA PROCESSING: UNTARGETED <sup>13</sup>C-LABELLING LC-HRMS BASED APPROACH**

**Eleonora Rollo<sup>(1)</sup>, Alexandra Schamann<sup>(2)</sup>, Alexandra Malachova<sup>(3)</sup>, Michele Suman<sup>\*(4)</sup>, Rudolf Krska<sup>(5)</sup>**

<sup>1)</sup> Department of Food and Drug, University of Parma, Viale delle Scienze 27/A-43124 Parma, Italy. Barilla G. R. F.lli SpA, Analytical Food Science, via Mantova 166, 43122 Parma, Italy., Italy

<sup>2)</sup> FFoQSI - Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, FFoQSI GmbH, Technopark 1C, 3430 Tulln, Austria, Austria

<sup>3)</sup> FFoQSI - Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, FFoQSI GmbH, Technopark 1C, 3430 Tulln, Austria, Austria

<sup>4)</sup> Barilla G. R. F.lli SpA, Analytical Food Science, via Mantova 166. Department for Sustainable Food Process, Catholic University Sacred Heart, Piacenza, Italy. 43122 Parma, Italy., Italy

<sup>5)</sup> University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria., Austria

\*Corresponding author - E-mail: michele.suman@barilla.com

Our multidisciplinary academic-industrial research companies started several years ago to conduct in-depth research in order to investigate and understand how the fungal toxins behave during various food production chains. Based on the previous experience and current knowledge in this area, it is assumed that the toxins could degrade or be modified due to exposure to energetic and mechanical conditions during the process, and thus form novel modified mycotoxins.

However, reduction of mycotoxin level does not have to necessarily result in a mitigation of toxicological effects. Nowadays, most of the information on the novel degradation products of mycotoxins is still missing identification and investigation of their structure and toxicity potential is of a high interest for risk assessment strategies.

To study the fate of mycotoxins during food processing is a very challenging task for several reasons: (i) characterization and structural identification of degradation products in complex matrix is difficult, (ii) matrix composition varies over the production steps, (iii) availability of analytical standards is limited, (iv) preparation of "in-house" analytical standards in larger amounts needed for structural confirmation and for toxicological assessment is costly and time-consuming. Therefore, most of the published studies were mainly focused on the detection of the parent mycotoxins and calculation of the rate of decline over the production.

In this study, the untargeted stable isotope labelling (SIL)-LC-HRMS approach was applied. Material used for food production is treated with a mixture of non-labelled and <sup>13</sup>C-labelled standard of mycotoxin, intermediate products and final pasta are then analyzed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). Detected pair of signals originated from non-labelled and isotopically labelled compounds are extracted from the full-scan chromatogram.

Results on aflatoxins and fumonisins fates along this industrial process will be presented.

**Keywords:** gluten-free pasta, aflatoxins, fumonisins, degradation products, untargeted stable isotope labelling (SIL)-LC-HRMS

**Acknowledgement:** FoodSafeR - European Union's Horizon Europe Research and Innovation Programme Under Grant Agreement No.101060698. NextGenerationEU - National Resistance and Resilience Plan (PNRR) - Mission 4 Component 2 Investment 1.3 - Notice No. 341 of 15 March 2022 of the Ministry of Universities and Research; Project Code PE00000003, MUR Directive Decree No. 1550 of 11 October 2022 granting of funding, CUP D93C22000890001, Project Title "ON Foods - Research and innovation network on nutrition and nutrition Sustainability, Safety - Working ON Foods".

## LECTURES

### L46

## NMR SPECTROSCOPY - A VERSATILE TOOL IN FOOD CONTROL

**Carsten Fauhl-Hassek**\*<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: carsten.fauhl-hassek@bfr.bund.de

Nuclear Magnetic Resonance (NMR) spectroscopy is an established analytical technique in food science, with many applications in research, process and quality control. Quantitative deuterium NMR spectroscopy, also called SNIF-NMR (Site Specific Isotopic Fractionation) spectroscopy, has been implemented as standard method for official control purposes already 30 years ago and allows, for example, the detection of chaptalization and water addition to wine. In recent years, proton NMR (<sup>1</sup>H-NMR) spectroscopy has become increasingly important in food control due to technical improvements, particularly in automation and analytical reproducibility. The main applications are linked to authenticity testing as sensitivity of <sup>1</sup>H-NMR spectroscopy allows the detection of food ingredients with high reproducibility. In this regard, <sup>1</sup>H-NMR spectroscopy is applied to characterize food using targeted and non-targeted approaches. Nowadays, detection of key ingredients and their quantification in a few minutes is possible using <sup>1</sup>H-NMR spectroscopy. Furthermore, the non-targeted application of <sup>1</sup>H-NMR enables to detect anomalies in food samples and the verification of product properties that make this technique of utmost interest for food surveillance.

The presentation will include state of the art NMR applications in official food control including the quantification of ingredients, determination of standard purity, non-targeted analysis, detection of unknown adulterants, jointly usable spectral databases, and comparison to mass spectrometry in terms of sensitivity.

**Keywords:** food analysis, adulteration, quantification, databanks, detection of unknowns

## LECTURES

### L47

#### EVALUATION OF LOW-FIELD VERSUS HIGH-FIELD PROTON NMR SPECTROSCOPY FOR THE AUTHENTICITY TESTING OF PEPPER

Dalal Werner<sup>(1)</sup>, Horacio Heinzen<sup>(2)</sup>, Marivil Islam<sup>(2)</sup>, Paul Rentz<sup>(1)</sup>, Alina Mihailova<sup>(2)</sup>, Morane Lointier<sup>(1)</sup>, Simon Kelly<sup>(2)</sup>

<sup>1)</sup> AERIAL, France

<sup>2)</sup> Food Safety and Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, Austria

\*Corresponding author - E-mail: da.werner@aerial-crt.com

Economically motivated adulteration (EMA) is an increasing concern for the food industry, driven by the globalization of trade, the complexity of supply chains and high demand. Spices and seasonings are an important commodity for global trade. Pepper is economically one of the most important and the most widely used spice crops. Having a high added value and being a part of a complex supply chain, pepper has become a frequent target for EMA requiring fast and efficient authenticity testing. High-field NMR spectroscopy combined with multivariate statistical analysis is a robust analytical tool to verify food authenticity, determine origin, and detect fraud. However, the high cost of supercooled magnets, associated infrastructure and cryogenics, hampers the wider application of NMR in food authentication. The advent of bench top, Fourier Transform low-field permanent NMR magnets opens up the opportunity to incorporate them in the daily routine work of the laboratory to verify food authenticity and detect EMA; however, their usefulness must be assessed on a case-by-case basis.

In the present communication the comparison between proton ( $^1\text{H}$ ) NMR spectra of the deuterated methanol and chloroform extracts collected at 400 and 60 MHz for pepper authenticity assessment is presented. As a proof of concept, a set of authentic *Piper nigrum* samples and a wide range of commonly used adulterants were measured, and the data were elaborated using multivariate statistical analysis. Furthermore, the limit of detection of adulteration was assessed and compared. The spectral data from both high and low-field NMR equipment showed consistently similar ability to discriminate authentic and adulterated *Piper nigrum* and enabled the classification of the peppers studied according to their degree of ripeness. Data-driven soft independent modelling of class analogy (DD-SIMCA) models built using high-field  $^1\text{H}$  NMR data of the deuterated methanol extracts achieved 100% sensitivity and 95% specificity while low-field  $^1\text{H}$  NMR data of the deuterated methanol extracts resulted in sensitivity and specificity of 96%. These results highlight the promising potential of 60 MHz benchtop NMR as a relatively inexpensive and useful tool for rapid screening, and routine control of pepper authenticity.

**Acknowledgement:** This study was supported by the UMT ACTIA "Authenticity Markers and Analytical Innovations" (Joint Technological Unit - French Ministry responsible for Food) and by the French Region Grand Est.

## LECTURES

### L48

## 1H-NMR AND RAMAN DATA FUSION: A NEW STRATEGY FOR THE DEVELOPMENT OF RELIABLE WINE AUTHENTICATION MODELS

**Ariana Raluca Hategan**\*<sup>(1)</sup>, **Maria David**<sup>(1)</sup>, **Adrian Pirnau**<sup>(2)</sup>, **Bogdan Cozar**<sup>(2)</sup>, **Simona Cinta-Pinzaru**<sup>(3)</sup>, **Francois Guyon**<sup>(4)</sup>, **Dana Alina Magdas**<sup>(1)</sup>

<sup>1)</sup> Faculty of Physics, Babeş-Bolyai University & National Institute for Research and Development of Isotopic and Molecular Technologies, Romania

<sup>2)</sup> National Institute for Research and Development of Isotopic and Molecular Technologies, Romania

<sup>3)</sup> Faculty of Physics, Babeş-Bolyai University, Romania

<sup>4)</sup> Service Commun des Laboratoires, France

\*Corresponding author - E-mail: ariana.hategan@ubbcluj.ro

Wine, being recognized as one of the European Union's premier agricultural commodities, holds an important economic value and is therefore a target for fraud on the worldwide market. In this regard, a prevalent type of falsification is the commercialization of wine with a false declaration of its origin. In response to this issue, significant efforts are being undertaken by the research community and control authorities to develop and implement reliable methods for detecting and preventing subtle forms of wine adulteration. Recently, to mitigate the drawbacks given by the employment of acknowledged approaches such as stable isotope ratios determination, in the field of wine traceability assessment, a special interest has been manifested in the development of new wine recognition tools relying on analytical techniques that are non-invasive, non-destructive, more environmentally friendly and easy-to-use.

The present study aimed to test the potential given by the fusion of two spectroscopic techniques, <sup>1</sup>H-NMR and Raman, for the development of new wine recognition models based on machine learning (ML) in regard to the cultivar, geographical origin, and vintage. For this purpose, a data set consisting of 50 authentic white wine samples, belonging to four cultivars (i.e. Chardonnay, Pinot Gris, Riesling, and Sauvignon) and produced in the most representative viticultural regions of Romania (i.e. Transylvania, Muntenia, and Moldova) during five consecutive years (i.e. 2012 - 2016), has been employed.

Due to the high amount of information generated by the fusion of two spectroscopic data sources (i.e. more than 20,000 variables), special attention had to be given to the data dimensionality reduction step for the construction of reliable classification models. Therefore, prior to machine learning application, a supervised feature selection algorithm relying on Partial Least Squares regression has been employed to identify, for each investigated classification criterion, the most discriminant <sup>1</sup>H-NMR and Raman markers. The effectiveness of the proposed data fusion strategy for constructing wine classification models was demonstrated by the obtained performance metrics, reaching 100% accuracy in both cross-validation and on an external test set. Nonetheless, in the case of the cultivar recognition, the corroboration of the relevant variables found from the <sup>1</sup>H-NMR and Raman spectra has significantly outperformed the differentiation capabilities of the models developed on a single-source input data, emphasizing the suitability of integrating these two spectroscopies for attaining a stronger recognition capacity concerning wine traceability assessment.

**Keywords:** data fusion, wine authentication, <sup>1</sup>H-NMR, Raman, machine learning

**Acknowledgement:** This work was supported by the MCID through the "Nucleu" Program within the National Plan for Research, Development, and Innovation 2022-2027, contract no. 27 N/2023, PN 23 24 03 01.

## LECTURES

### L49

## INTEGRATED LIBS-RAMAN SYSTEM FOR FOOD AUTHENTICATION AND SAFETY ANALYSIS

**Bartek Rajwa**<sup>\*<sup>(1)</sup></sup>, **Sungho Shin**<sup>(2)</sup>, **Euiwon Bae**<sup>(1)</sup>, **Kemal Eseller**<sup>(1)</sup>, **J. Paul Robinson**<sup>(1)</sup>

<sup>1)</sup> *Purdue University, United States of America*

<sup>2)</sup> *Gwangju Institute of Science and Technology., South Korea*

*\*Corresponding author - E-mail: brajwa@purdue.edu*

Researching food fraud threats is crucial due to their growing prevalence and adverse impacts on public health, consumer trust, and economic activity. Developing effective models, theories, frameworks, methods, techniques, and tools for combating food fraud remains critically important. Our work explores the potential of combining Laser-Induced Breakdown Spectroscopy (LIBS) and Raman spectroscopy for food authentication and fraud prevention. We plan to enhance the accuracy and reliability of product classification, thereby improving authentication efforts and addressing a key aspect of food fraud. Our project involved designing and testing an early prototype of a hybrid Raman and LIBS system, which offers both elemental and molecular analysis capabilities.

LIBS is an analytical technique that uses a high-intensity laser pulse to ablate a small amount of a sample, creating a plasma. The emitted light from this plasma is analyzed to determine the sample's elemental composition. LIBS is valued for its rapid in situ analysis with minimal sample preparation, making it suitable for various applications, including recent advancements in food analysis. Conversely, Raman spectroscopy studies vibrational, rotational, and other low-frequency modes in a system through inelastic scattering of monochromatic light, usually from a laser. This technique is commonly used in analytical chemistry to provide a molecular fingerprint for identifying molecules. Our research aimed to leverage the strengths of both LIBS and Raman spectroscopy by combining the elemental analysis capabilities of LIBS with the molecular identification strengths of Raman. This combination was intended to enhance food authentication and fraud prevention. We developed and evaluated a custom-built hybrid Raman/LIBS system (Hy-R-LIBS) and compared its performance in food analysis to conventional single-spectroscopy systems. Spectral analyses and classification tasks were performed using actual food samples, assessing the performance of multiple classifiers through a multivariate feature selection approach, which included two different data fusion methods. A substantial component of our project focused on enhancing the system's performance and refining the measurement protocols of the hybrid benchtop instrument. We leveraged machine learning to develop feature-selection algorithms for LIBS/Raman fingerprinting, significantly improving the evaluation of food products using our hybrid technology. Furthermore, we designed and developed the first simple prototype of a portable instrument capable of performing Hy-R-LIBS measurements directly in the field, eliminating the need for laboratory-based analysis.

**Keywords:** LIBS, Raman, food fraud, food authentication

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## LECTURES

### L50

#### A HARMONIZED APPROACH FOR FOOD AUTHENTICITY MARKER VALIDATION AND ACCREDITATION

**Stephane Bayen**<sup>\*(1)</sup>, Chris Elliott<sup>(2)</sup>, Marco Arlorio<sup>(3)</sup>, Nicolai Zederkopff Ballin<sup>(4)</sup>, Nicholas Birse<sup>(2)</sup>, Jens Brockmeyer<sup>(5)</sup>, Shawninder Chahal<sup>(1)</sup>, Lei Tian<sup>(1)</sup>, Jianguo Xia<sup>(1)</sup>, Maria G. Corradini<sup>(6)</sup>, Robert Hanner<sup>(6)</sup>, Stephan Hann<sup>(7)</sup>, Teresa Steininger-Mairinger<sup>(7)</sup>, Kristian Holst Laursen<sup>(8)</sup>, Alina Mihailova<sup>(9)</sup>, Michele Suman<sup>(10)</sup>, Saskia Van Ruth<sup>(11)</sup>

<sup>1)</sup> McGill University, Canada

<sup>2)</sup> Queen's University Belfast, United Kingdom

<sup>3)</sup> University of Piemonte Orientale "Amedeo Avogadro", Italy

<sup>4)</sup> Danish Veterinary and Food Administration (DVFA), Denmark

<sup>5)</sup> University of Stuttgart, Germany

<sup>6)</sup> University of Guelph, Canada

<sup>7)</sup> University of Natural Resources and Life Sciences (BOKU), Austria

<sup>8)</sup> University of Copenhagen, Denmark

<sup>9)</sup> International Atomic Energy Agency, Austria

<sup>10)</sup> Barilla G. e R. Fratelli S.p.A, Italy

<sup>11)</sup> University College Dublin, Ireland

\*Corresponding author - E-mail: [stephane.bayen@mcgill.ca](mailto:stephane.bayen@mcgill.ca)

Significant technological advances over the past decade have changed food authentication. A wide range of analytical strategies can now be applied to measure intricate changes in sample composition resulting from the presence of adulterants, different production methods, or varying geographical origins of food. While the relative 'explosion' of applications of marker-based approaches is to be welcomed in the complex fight against food fraud, there is a need for a systematic and harmonized approach to describe, discover and validate such markers. In this presentation, we introduce our recommendations for terminologies and definitions that should be applied in the field, including the terms "primary" and "secondary" markers, "single" and "dual" authenticity markers, and authentic "profiles" and "fingerprints". We also advocate for harmonization in marker discovery approaches and propose guidelines for the analytical community relating to the approaches taken to validate markers. We believe the adoption of these approaches will prove to be a major step forward in achieving the ultimate goal of providing a broad range of fully validated and accredited methodologies that can be implemented in a forensic capacity in food authenticity monitoring and control programmes.

The complete publication can be found at: <https://doi.org/10.1016/j.tifs.2024.104550>.

**Keywords:** accreditation, classification algorithm, food fraud, foodomics, machine learning



## LECTURES

### L51

## EXPLOITING THE POTENTIAL OF DART-HRMS FOR RAPID AUTHENTICITY TESTING AND FOOD FRAUD DETECTION: INTRODUCING AN INTEGRATED WORKFLOW IN THE CASE STUDY OF EXTRA VIRGIN OLIVE OIL

Dimitrios A. Papelis<sup>(1)</sup>, Sofia K. Drakopoulou<sup>(2)</sup>, Ilona Nordhorn<sup>(3)</sup>, Carsten Baessmann<sup>(3)</sup>, Nikolaos S. Thomaidis<sup>\*(2)</sup>

<sup>1)</sup> National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771, Greece

<sup>2)</sup> National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771 / Track4Value - NKUA spinoff, Greece

<sup>3)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: ntho@chem.uoa.gr

Authenticity assessment has been emerged as a critical issue in today's globalized food market. Incidences of food fraud of any kind (e.g. adulteration, mislabelling) are commonly reported, thus posing significant threats to public health and consumer's trust. One of the food products most affected by these kinds of bad practices is extra virgin olive oil (EVOO). EVOO is highly ranked in the global market, as a landmark food of the Mediterranean diet with several beneficial properties associated to its consumption. Therefore, economically motivated adulteration often takes place across EVOOs' supply chain, with one of the most frequent profit-driven fraudulent procedures being its substitution with cheaper alternatives, such as other vegetables oils or olive oils of lower quality.

Chromatographic techniques prevailed so far, often coupled to mass spectrometry, while effective, are often time-consuming and labour-intensive. The main limiting factor is the chromatography, which requires a significant amount of time, as well as a large volume of analytes that end up as toxic waste to the environment. Moreover, specific sample treatment step is required to extract the fraction of interest, which increases the time and the cost of analysis. Thus, there is a growing need for more rapid, yet efficient, and eco-friendly approaches.

In the present study, the potential of direct analysis in real time (DART) coupled to quadrupole-time-of-flight mass spectrometry (QTOF-MS) was exploited for food authenticity assessment. In this context, a holistic methodology was developed and optimized for the case study of EVOO, taking into account its total profile (no sample treatment step was applied). Aiming to detect potential adulteration, vegetables oils commonly used as adulterants (corn, sunflower, canola, soybean, sesame and linseed oils) and olive oils of lower-quality (olive pomace, refined oils and mixed olive oils), were analysed along with EVOO samples. Noteworthy differentiations in their profiles were recorded, and robust prediction models were built, successfully discriminating authentic EVOOs from adulterated ones. Advanced chemometrics were also employed to predict adulteration and assess EVOO's authenticity, introducing a rapid, integrated workflow, highly applicable in routine analysis. Finally, authenticity markers of each oil category were highlighted and successfully identified, utilizing high-resolution mass spectrometry (HRMS) potential. It is worth mentioning that this is the first study, to the best of our knowledge, which achieves the discrimination of 10 different oil categories in one comprehensive methodology. Key point of this study and main advantage is the introduction of a holistic approach, applicable in different cases of food authenticity assessment, which incorporates nicely the reliability of HRMS workflows and the fast screening of DART.

**Keywords:** DART, authenticity, extra virgin olive oil, HRMS, adulteration

## LECTURES

### L52

## FIGHTING DEFORESTATION THROUGH ADVANCES IN ANALYTICAL CHEMISTRY

Yuhne Hong<sup>(1)</sup>, Maria Aparicio Muriana<sup>(1)</sup>, Nicholas Birse<sup>(1)</sup>, **Chris Elliott**\*<sup>(2)</sup>

<sup>1)</sup> Institute for Global Food Security, Queen's University, Belfast, United Kingdom

<sup>2)</sup> Institute for Global Food Security, Queen's University, Belfast & International Joint Research Center on Food Security, Thailand & Thammasat University, Thailand, United Kingdom

\*Corresponding author - E-mail: Chris.Elliott@qub.ac.uk

Deforestation in the rainforest is a significant environmental issue, particularly in the Amazon, which is the largest rainforest in the world. There are many reasons why this has occurred, but the main drivers have been clearing land for cattle ranching, soy plantations, and other crops and large-scale logging. Deforestation in the Amazon has severe impacts on biodiversity, indigenous communities, and globally climate change.

The European Union (EU) has implemented various legislative measures to combat deforestation and promote sustainable land use practices. These are part of the EU's broader environmental and climate policies aimed at reducing the environmental footprint of its consumption patterns. The EU Deforestation Regulation (EUDR) which came into force in 2023 with the aim of preventing the import and sale of products linked to deforestation and forest degradation in the EU market. This includes important food and feed commodities like soy, palm oil, cocoa, coffee, and beef (the big 5), and all products derived from these commodities.

While such legislative initiatives must be welcomed as with all legislation the enforcement will be a major challenge. The collective world trade in the big 5 estimated to be over \$1.3 trillion. There are multiple stakeholders involved ranging from many million smallholders to large multinational companies involved along with highly complex and often opaque supply chains. Vast sums of money changes hands and many in industry have expressed major concerns about the unintended consequences of the legislation in terms of impact on smallholders, availability and price rises for all the big 5 globally.

Another unintended consequence will be the massive opportunity for fraud i.e. false claims about where the big 5 were farmed in terms of links to deforested land. There is a clear and important role for analytical science to help identify where the big 5 originated from, to determine links to deforestation and to combat food fraud.

The presentation will give an overview of the scale of the challenge and how science and technology can be exploited to support the fight against deforestation.

**Keywords:** deforestation, food and feed commodities, food fraud

## LECTURES

### L53

## THE KNOWN AND THE UNKNOWN: THE ANALYTICAL TOOLBOX TO TACKLE THE PFAS CHALLENGE

Stefan Van Leeuwen\*<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: [Stefan.vanLeeuwen@wur.nl](mailto:Stefan.vanLeeuwen@wur.nl)

The PFASs problem has evolved to an unprecedented societal challenge. PFASs are found everywhere in our environment and foods, the number of compounds is continuously expanding, and already at low levels exposure may be exceeding the safe levels. Food and drinking water are important vectors for human exposure, and its therefore of utmost importance that analytical tools become available to unravel the PFASs problem in food. In this presentation, a comprehensive toolbox will be presented that we have developed, that allow investigation of the following:

- Mass balance analysis, i.e. the determination of the total PFASs amount in foods
- Targeted analysis, i.e. the (sub) pg/g level determination of PFASs in foods
- Unknowns identification, i.e. the screening and identification of (yet) unknown PFASs in foods

These methods are applied to a wide range of foods and abiotic samples (food contact materials, water). Highly sensitive targeted approaches for reliable, ultrasensitive (low/sub pg/g level) are developed for carboxylic acids, sulfonates, telomer sulfonates, telomer alcohols, sulfonamides, ultrashort-chain PFAS etc. The mass balance approach was applied on freshwater fish samples, showing that over 50% of the extracted organic fluorine could not be attributed to the know PFAS (as determined by targeted approaches). This means that a substantial amount of yet unknown PFAS are present in these samples. A Thermo Scientific Orbitrap IQ-X Tribrid HRMS is used to identify the unknown PFAS causing this missing proportion of extracted organic fluorine. For that purpose, we have developed a workflow to screen for suspects in sample extracts. In addition, advanced acquisition modes were developed combined with specific data filtering tools (i.e. Kaufman approach, Kedrick mass defect) are applied to elucidate unknown PFAS signals in food extracts, which the need further confirmation by (ideally) Level 1 identification using reference standards.

Altogether, this toolbox allows for battling the PFAS challenge in foods and other samples, as it provides insights into PFAS contamination from different angles. Next to current state-of-the-art, analytical challenges will be addressed, as well as the needs to advance this field further in the future.

**Keywords:** PFAS, mass balance, ultra-sensitive targeted analysis, HRMS suspect screening and identification, extractable organic fluorine (EOF)

**Acknowledgement:** *Several colleagues of WFSR that contributed to this work are gratefully acknowledged. Moreover, funding was received from various sources, including the Ministry of Agriculture, Nature and Food quality. Support of TE Instruments with the use and optimisation of the CIC instrument is greatly appreciated.*

## LECTURES

### L54

## **SIMULTANEOUS ACTIVATION OF 3 LEVERS TO EXTEND THE SPECTRUM OF PFAS STUDIED IN FOOD MATRICES, THE APPROACH THAT MAKES ALL THE DIFFERENCE**

**Cassandre Jeannot**<sup>(1)</sup>, **Bruno Le Bizec**<sup>(2)</sup>, **Julien Parinet**<sup>(3)</sup>, **Gaud Dervilly**<sup>\*(4)</sup>

<sup>1)</sup> LABERCA - Oniris / Laboratory of Food Safety - ANSES, France

<sup>2)</sup> LABERCA - Oniris, France

<sup>3)</sup> ANSES, Laboratory of Food Safety, France

<sup>4)</sup> ONIRIS, INRAE, LABERCA, France

\*Corresponding author - E-mail: [gaud.dervilly@oniris-nantes.fr](mailto:gaud.dervilly@oniris-nantes.fr)

Chronic exposure to chemical hazards through the environment and diet can contribute to the development of certain chronic diseases. From a public health perspective, it is essential to characterize as many as possible of the chemical substances to which humans are exposed throughout their lives, in order to implement effective health prevention strategies.

PFAS have been produced since 1950s and are used in various types of industrial and consumer products, due to their water- and oil-resistant properties. Popularised as "forever chemicals", they are considered as contaminants of emerging concern (CECs), listed for a few of them in the Stockholm Convention as persistent organic pollutants (POPs) while four have recently been regulated in food. However, these chemicals exist in several thousand chemical structures, which need to be monitored to provide a more complete picture of environmental contamination and human exposure.

Recent non-targeted profiling technologies are making it possible to assess the chemical exposome in an increasingly global way, offering access to knowledge of a wider spectrum of chemicals. As food, particularly eggs and fish, is the main route of exposure to PFAS, our research focused on developing an alternative, suspect and non-targeted approach for identifying PFAS in addition to those traditionally monitored in foodstuffs. We have activated three levers to extend PFAS chemical space: sample preparation (optimised QuEChERS), data acquisition (LC-HRMS) and data processing (prioritisation of fluorinated signals). The ability to detect novel PFAS compounds was assessed against a traditional ISO17025 targeted approach currently in routine use involving SPE and MS/MS acquisition (QqQ). This new non-specific approach proved conclusive, as applied to a wide range of food samples it enabled other PFASs to be detected in addition to the twenty or so substances regularly monitored. For example, perfluoropropanoic acid (PFPrA), which is not usually monitored but is known to be present in the environment, was detected and identified (level 1) in several foods. In the interests of better risk assessment, this methodology breaks with conventional approaches and paves the way for broader characterisation of consumer exposure to these compounds of concern.

**Keywords:** PFAS, non-targeted screening, sample preparation, QuEChERS

## LECTURES

### L55

## PFAS ANALYSIS IN FOOD: A MULTI-TECHNIQUE APPROACH FOR REGULATORY COMPLIANCE AND CONSUMER PROTECTION

Xanthippi Theurillat\*<sup>(1)</sup>

<sup>1)</sup> *Société des Produits Nestlé SA, Nestlé Research Lausanne, Vers-chez-les-Blanc, Switzerland*

*\*Corresponding author - E-mail: Xanthippi.Theurillat@rdls.nestle.com*

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals that have been widely used in various industrial and consumer products due to their water and grease resistance properties. However, they have been found to be persistent in the environment and can accumulate in the food chain, posing potential human health risks.

The European Food Safety Authority (EFSA) has set a Total Weekly Intake (TWI) at 4.4 ng/kg body weight for four main PFAS, namely perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS) [1]. As a response to health risks, the European Commission issued recommendations and regulations ((EU) 2022/1431 and (EU) 2022/2388) [2, 3] requesting Member States to monitor PFAS levels in foodstuffs. Strong emphasis is put on the four PFAS with targeted LOQs, indicative levels, and Maximum Levels (MLs) established in a selection of foods. Apart from these four PFAS, the EU Recommendation requests the monitoring of 23 other PFAS, such as fluorotelomer alcohols and sulfonates. Targeted limits of quantification (LOQs) requested for the four main compounds, depending on the matrix, are in range from 0.001 µg/kg for fruits, vegetables, tubers and infant foods to 0.5 µg/kg for offal and fish oil.

This presentation will highlight the need of a combination of analytical techniques, for being able to meet the EU requirements. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is commonly used for the quantitative determination of PFAS with MLs and targeted LOQs. However, for certain PFAS such as fluorotelomer alcohols, gas chromatography (GC) is more preferred. This technique is more suitable for the analysis of volatile PFAS, which may be present in food matrices. Furthermore, for the analysis of a broader range of PFAS, including those that may be added on the list of monitored PFAS in future, liquid chromatography-high resolution mass spectrometry (LC-HRMS) is another alternative to be considered.

This presentation will share our experience and technical choices made in our facilities to provide the best respect to EU requirements.

[1] EFSA, European Food Safety Authority - Risk to human health related to the presence of perfluoroalkyl substances in food. 2020: p. <https://www.efsa.europa.eu/fr/efsajournal/pub/6223>.

[2] EU, COMMISSION RECOMMENDATION (EU) 2022/1431 on the monitoring of perfluoroalkyl substances in food. 2022.

[3] EU, Commission Regulation (EU) 2022/2388 of 7 December 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs. 2022.

## LECTURES

### L56

#### PFAS IN FRUITS AND VEGETABLES: AN INTERLABORATORY VALIDATION STUDY ON THE ACHIEVABILITY OF EU TARGETED LOQS

Consolato Schiavone<sup>\*(1)</sup>, Chiara Portesi<sup>(1)</sup>, Francesco Romaniello<sup>(1)</sup>, Christina Riemenschneider<sup>(2)</sup>, Katharina Rund<sup>(2)</sup>, Alexander Schaechtele<sup>(2)</sup>

<sup>1)</sup> *Istituto Nazionale di Ricerca Metrologica, Italy*

<sup>2)</sup> *EURL for POPs, CVUA, Germany*

*\*Corresponding author - E-mail: c.schiavone@inrim.it*

More than 7,000 synthetic chemicals, known as per- and polyfluoroalkyl substances (PFAS), are used in food packaging and other materials to provide resistance to fat, fire, and water. Industrial waste emissions of PFAS contaminate water, air, and soil. PFAS are likely to contribute to human carcinogenesis and reprotoxicity, as well as vaccine resistance and immune system depression. People may be exposed to PFAS daily through the environment, food, or water. The European Food Safety Authority recommends acceptable weekly intakes for PFOA, PFOS, PFHxS, and PFNA. The EU has advanced in the fight against PFAS contamination by releasing guidelines, recommendations, and legislation to limit their presence in food and by seeking new data to better estimate the extent of human exposure. In this framework, there is an urgent need for validated analytical methods for assessing the level of PFAS listed in Commission Recommendation (EU) 2022/1431. This will support control and regulatory bodies to undertake suitable actions and decisions related to food safety and the protection of human health. The aim of this study was to validate, according to EURL-POPs guidance, an analytical method for assessing PFAS levels in vegetable and fruit samples using an interlaboratory approach. The study was conducted involving at two different laboratories (INRIM and CVUA-FR) to achieve interlaboratory validation, including all the steps from the sample preparation (exchanging extracts prepared in both laboratories) to the instrumental analysis by liquid chromatography coupled with mass spectrometry. Different analyzer types were selected: HRMS and TQ-MS, aiming to determine the highest grade of variance and to develop a robust and accurate methodology. Also, in the individual laboratories the analysis was performed by at least two different operators for intralaboratory validation of the method and the spectrometers chosen were from different manufacturers (such as Waters and Thermo) keeping an eye on different MS analyzer types to better evaluate the targeted LOQs to be achieved. The PFAS selected were extracted using an ion pair approach and quantified, including, besides the four EU regulated ones, new generation and branched PFAS, with a focus on PFCAs and PFSAAs that can most likely occur in fruit and vegetables. Chromatographic separation was performed using mixed-mode chromatography with a pH gradient. The conclusions of the study aim to produce a robust, accurate, and validated method for quantifying PFAS and to evaluate the possibility to achieve the EU target levels specified by the latest recommendations in fruit and vegetable matrices. The finding will help develop a standard analysis approach for quantifying PFAS in samples of plant origin in order to support the development of EU legislation in this area.

**Keywords:** PFAS, HRMS, food safety, fruit and vegetables, TQ-MS

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## LECTURES

### L57

## NON-TARGETED SCREENING FOR PFAS IN COMPLEX FOOD MATRICES: FOCUS ON THE KEYS TO DATA PRIORITIZATION

**Nicolas Macorps**<sup>(1)</sup>, **Elysa Oliveira**<sup>(1)</sup>, **Bruno Le Bizec**<sup>(1)</sup>, **Gaud Dervilly**<sup>(1)</sup>

<sup>1)</sup> ONIRIS, INRAE, LABERCA, France

\*Corresponding author - E-mail: nicolas.macorps@oniris-nantes.fr

Human exposure to per- and polyfluoroalkyl substances (PFAS) is mainly dietary (EFSA, 2020). They can enter foods through environmental contamination [1] or through migration from food packaging. In consequence, maximum levels for four PFAS (i.e. PFOA, PFNA, PFHxS and PFOS) have been set for foodstuffs in 2022 [2]. However, occurrence data for larger number of compounds are required. Yet, the considerable number of PFAS renders almost impossible to use authentic reference standards to monitor them all in conventional targeted analysis. As such, non-targeted screening approaches (NTS) based on high-resolution mass spectrometry (HRMS) coupled to efficient data reduction and prioritization have become essential to broaden the scope. In this context, this work focused on developing a specific PFAS NTS workflow to be applied in complex food matrices based on HRMS (Thermo Q-Exactive). MS1 and MS2 data were acquired in fullscan and data independent acquisition (DIA) mode respectively. Raw files were then processed using Compound Discoverer SP2 (Thermo Fisher). Data reduction and prioritization on PFAS suspect features was made possible after estimation of the carbon number using isotopic peaks, calculation of mass to carbon (m/C) and mass defect to carbon (md/C) ratios [3]. Finally, features were tentatively identified using Kendrick mass defect homologue series, databases and suspect list matching along with the investigation of fragmentation pattern and profiles. The results of the proof of concept of this method, with validation parameters and the identification workflow of suspect PFASs will be presented. The potential of ion-mobility mass spectrometry to focus on PFAS chemical domain and to provide additional identification criteria will be introduced as an improvement of NTS strategies. These results will be illustrated by the elucidation of unsuspected PFAS in food matrices.

[1] EFSA, 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFS2 18. <https://doi.org/10.2903/j.efsa.2020.6223>.

[2] European Commission, 2022. Commission Regulation (EU) 2022/2388 of 7 December 2022 amending Regulation (EC) No 1881/2006 as regards maximum levels of perfluoroalkyl substances in certain foodstuffs.

[3] Kaufmann, A., Butcher, P., Maden, K., Walker, S., Widmer, M., 2022. Simplifying Nontargeted Analysis of PFAS in Complex Food Matrixes. *Journal of AOAC INTERNATIONAL* 105, 1280-1287. <https://doi.org/10.1093/jaoacint/qsac071>.

**Keywords:** PFAS, HRMS, suspect-screening, prioritization, ion-mobility mass-spectrometry

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## LECTURES

### L58

## MAKING POLYCHLORINATED ALKANE ANALYSIS IN FOOD MORE ACCESSIBLE: EXPLORING LOW-RESOLUTION LC-MS/MS AS A SUITABLE ALTERNATIVE TO LC-HRMS AND GC-HRMS

**Ingus Perkons**\*<sup>(1)</sup>, **Anna Skrastiņa**<sup>(2)</sup>, **Laura Lazdiņa**<sup>(2)</sup>

<sup>1)</sup> Institute of Food Safety, Animal Health and Environment & BIOR, Latvia

<sup>2)</sup> Institute of Food Safety, Animal Health and Environment & BIOR; Faculty of Medicine and Life Sciences, University of Latvia, Latvia, Latvia

\*Corresponding author - E-mail: [ingus.perkons@bior.lv](mailto:ingus.perkons@bior.lv)

Polychlorinated alkanes (PCAs) are a group of high-production volume chemicals with a general formula  $C_nH_{2n+2-x}Cl_x$ . They are the main ingredient of chlorinated paraffin (CP) technical mixtures, widely used in various industries as machining fluids, plasticisers, and flame retardants. Current evidence suggests that PCAs are persistent and bioaccumulative, with a toxicological profile showing mammalian and environmental toxicity, posing potential risks to human health. PCAs are virtually ubiquitous in food samples, especially products of animal origin and high lipid content. However, the recent human health risk assessment of PCAs conducted by the European Food Safety Authority was inconclusive, partially due to the lack of supporting food occurrence data. Such an outcome may seem surprising, considering that CP technical mixtures have been manufactured since the 1920s, and the first studies on their occurrence date back more than 50 years. Nevertheless, the enormous complexity of PCAs renders them challenging to study by conventional instrumental workflows employed for POP analysis.

Most recent studies rely on high-resolution mass spectrometry (HRMS), a long-sought antidote that enables comprehensive characterisation of PCAs. By contrast, low-resolution MS (LRMS) applications are becoming scarce. This trend limits the availability of PCA analysis because HRMS is a more costly instrumentation that may not always be accessible in food analytical chemistry laboratories. Therefore, in this study, we developed a simplified LC tandem MS (LC-MS/MS) method for quantifying PCAs- $C_{10-17}Cl_{5-8}$  in food, which could serve as a feasible alternative to more complex HRMS methods. The LC-MS/MS method was validated and compared to an LC-Orbitrap-MS method regarding analytical performance. The results show that the simplified approach can accurately quantify PCAs in food samples and provides consistent results with the LC-HRMS method.

This presentation will focus on method development. Specifically, it will cover (i) the selection of mobile phase additives (i.e. ammonia chloride and ammonia acetate) to improve PCA ionisation and fragmentation, (ii) the choice of reversed-phase stationary phases for better resolution between critical PCA homologue groups that are isobaric under low-resolution MS conditions, and (iii) the optimization of LC gradient conditions to maximise separation efficiency.

**Keywords:** polychlorinated alkanes, chlorinated paraffins, tandem mass spectrometry, liquid chromatography, environmental contaminants

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## LECTURES

### L59

## STUDY OF MINERAL OIL HYDROCARBONS IN DIFFERENT TYPES OF UNPROCESSED MEAT BY LC-GC×GC-FID/MS

Paula Albendea<sup>(1)</sup>, Giorgia Purcaro<sup>\*(1)</sup>

<sup>1)</sup> Gembloux Agro-Bio Tech, University of Liège, Belgium

\*Corresponding author - E-mail: gpurcaro@uliege.be

Mineral oil hydrocarbons (MOH) are a complex mixture of liposoluble environmental and processing contaminants of petrogenic origin. They may pose different toxicological risks to humans depending on their structure (i.e., saturated (MOSH) or aromatic (MOAH)) [1]. The increasing interest in more detailed information on the composition of MOH is driving a shift from LC-GC-FID, considered the routine technique, to more advanced techniques, notably LC-GC×GC-FID [2]. Moreover, due to the complexity of MOH and food matrices, an efficient sample preparation method must be performed. This work aimed to optimize a method to determine MOH in meat and to characterize in more detail the MOSH profile (i.e. linear and cyclic), as requested by the European Food Safety Authority Opinion published in 2023 [1].

For the sample preparation, two microwave-assisted saponification and extraction (MASE) procedures were evaluated in terms of recovery and internal standards distribution. Indeed, it was recently shown that the saponification procedure strongly impacts the internal standard partition, affecting the reliability of the results [3]. The first method referred to a recently published procedure using KOH saturated solution in methanol [4], while the other was adapted from [3], using KOH 2 M in EtOH/H<sub>2</sub>O (1/1 v/v). The optimization was performed using three different types of meat (raw bacon, pig rib, and beef rib). The most accurate internal standard distribution and the highest recoveries were obtained with the later method.

Meat samples coming from various types of animals (mammals, poultry, and ruminants) were purchased from different local supermarkets. They were subjected to the MASE with KOH 2M in EtOH/H<sub>2</sub>O (1/1 v/v) before their analysis in LC-GC×GC-FID. The use of GC×GC together with the information obtained by the MS allowed for a detailed investigation and to differentiate linear from cyclic MOSH when present.

[1] EFSA, Update of the risk assessment of mineral oil hydrocarbons in food, J. EFSA 21 (2023) 1-143.

[2] G. Bauwens, A. Gorska, G. Purcaro. The role of comprehensive two-dimensional gas chromatography in mineral oil determination; ABC 415 (2023) 5067-5082.

[3] G. Bauwens, G. Purcaro, Improved microwave-assisted saponification to reduce the variability of MOAH determination in edible oils, Anal. Chim. Acta 1312 (2024) 34278.

[4] P. Albendea, C. Conchione, L. Menegoz Ursol, S. Moret. A study on mineral oil hydrocarbons (MOH) contamination in pig diets and its transfer to back fat and loin tissues; Animals 14 (2024) 1450.

**Keywords:** LC-GC×GC-FID/MS, meat, mineral oil hydrocarbons, LC-GC-FID, microwave-assisted saponification

**Acknowledgement:** The authors thank Milestone, LECO, and Restek for their support.

### L60

## SHORT- AND MEDIUM-CHAIN CHLORINATED PARAFFINS IN INSECT-BASED FOODS

**Jakub Tomasko**\*<sup>(1)</sup>, **Michaela Freyova**<sup>(2)</sup>, **Katerina Sebelova**<sup>(1)</sup>, **Jana Pulkrabova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

<sup>2)</sup> *University of Chemistry and Technology Prague, Czech Republic*

\*Corresponding author - E-mail: tomaskoj@vscht.cz

Short- and medium-chain chlorinated paraffins (SCCPs and MCCPs, respectively) are emerging and ubiquitous groups of environmental pollutants associated with adverse effects on human health (such as endocrine disruption and possible carcinogenicity of SCCPs). They are classified based on their carbon chain lengths (SCCPs with C<sub>10</sub>-C<sub>13</sub> and MCCPs with C<sub>14</sub>-C<sub>17</sub>). The analytical chemistry of CPs is a very challenging task, mainly due to the nature of the compounds (their technical mixtures contain thousands of isomers and homologues, which are practically inseparable by conventional chromatographic techniques) and the lack of suitable commercially available internal standards.

This study evaluated the risk assessment of SCCPs and MCCPs in a novel food category - edible insects. CPs were extracted from insect-based food from the Czech market (n=67) and samples obtained directly from farms (n=22) by ethyl acetate partitioning. The CPs were then isolated from co-extracting lipids using solid phase extraction on a silica gel (deactivated by 2% of water) and then analysed by gas chromatography coupled to high-resolution mass spectrometry operated in negative chemical ionisation (GC-NCI-HRMS; Agilent 7890B GC with coupled with Agilent 7200B quadrupole-time of flight mass spectrometer - GC/Q-TOF system; the CPs were separated on HP-5MS UI column, 15 m × 0.25 mm × 0.25 μm, all Agilent Technologies, USA). The method was validated, with recoveries in the 70 - 100% range and repeatabilities (expressed as relative standard deviations) <17%. The limits of quantification (LOQs) were 0.01 and 0.03 μg/g lipids (SCCPs and MCCPs, respectively).

The CP concentrations in samples from the Czech market were in the range of <0.01 - 21.36 (median 0.08) μg/g lipids and <0.03 - 7.74 (median 0.09) μg/g lipids (SCCPs and MCCPs, respectively). The consumption of analysed samples would not exceed the tolerable daily intakes (30 and 4 μg/kg body weight, SCCPs and MCCPs, respectively). Nevertheless, the CPs dietary exposure is expected to be low in the Czech Republic, therefore the consumption of insect-based foodstuff would be a significant source of exposure to CPs, with a possible increase of the share in the total dietary intake of insect-based food, due to the approval of edible insects being safe for human consumption in the EU.

To find the possible source of contamination in the edible insects, samples obtained directly from farms were also analysed. The CP concentrations were in the range of <0.09 - 2.16 (median 0.24) μg/g lipids and <0.05 - 1.81 (median 0.44) μg/g lipids (SCCPs and MCCPs, respectively). The samples were more contaminated than samples from the market (by medians), with lower extremes.

**Keywords:** chlorinated paraffins, gas chromatography, high resolution mass spectrometry, edible insect

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## LECTURES

### L61

## ANALYTICAL STRATEGIES TO DETERMINE 3-7 RING MOAH IN FOOD AND FOOD CONTACT MATERIALS: DACC-HPLC-GC AND GCXGC

**Martin Lommatzsch**\*<sup>(1)</sup>

<sup>1)</sup> *Laboratory Lommatzsch & Säger GmbH, Germany*

\**Corresponding author - E-mail: lommatzsch@mosh-moah.de*

The contamination of food with different types of hydrocarbons is an on-going topic. The introduction pathways are wide-ranging and can occur at all stages of the production chain from farm to fork. The main focus is remaining on mineral oil hydrocarbons (MOH), which can be subgrouped into mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH). MOSH & MOAH can enter the food in substantial amounts during processing or via migration from food contact materials (e.g. cardboard & jute bags). These contaminants are routinely measured via online HPLC-GC-FID, which is enabling a matrix clean-up as well as a separation into MOSH and MOAH fraction.

From a human health perspective, the MOAH fraction is considered of major concern by the European Food Safety Authority (EFSA) [1]. However, an evaluation of MOAH is subdivided. On the one hand, MOAH containing 3 or more aromatic rings (usually up to 7 rings are considered), are associated with genotoxicity and carcinogenicity, due to their structural similarity to non-alkylated polycyclic aromatic hydrocarbons (PAH). On the other hand, currently no toxicological information is available for 1-2 ring MOAH. Consequently, a human health concern regarding 1-2 ring MOAH cannot be excluded by the EFSA [1]. In order to obtain a better data base for an exposure calculation and risk assessment, a routine analytical differentiation of the MOAH subgroups is highly demanded [1].

Two analytical strategies are presented to enable a quantification of 1-2 ring and 3-7 ring MOAH. Firstly, a comprehensive GC (GCxGC) analysis of the MOAH fraction is shown, which is providing an optimized 2D GC separation for 1-2 ring and 3-7 ring MOAH [2]. Secondly, an online HPLC-GC-FID method involving an additional HPLC separation step via a donor-acceptor-complex chromatography column is discussed, which is enabling a further HPLC subdivision of the MOAH fraction into mono-/diaromatic fraction (MDAF) and tri-/polyaromatic fraction (TPAF) for quantification [3].

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM), Update of the risk assessment of mineral oil hydrocarbons in food, EFSA J. 21 (9) (2023).

[2] M. Biedermann, A. Eicher, T. Altherr, G. McCombie, Quantification of mineral oil aromatic hydrocarbons by number of aromatic rings via comprehensive two-dimensional gas chromatography: First results in food, J. Chromatogr. Open 2 (100072) (2022).

[3] M. Lommatzsch, M. Eckardt, J. Holzappel, S. Säger, T. Simat, Advanced separation of mineral oil aromatic hydrocarbons by number of aromatic rings using donor-acceptor-complex chromatography to extend on-line coupled liquid chromatography-gas chromatography, J. Chromatogr. A 1715 (464600) (2024).

**Keywords:** MOAH, TPAF, PAH, MDAF

## LECTURES

### L62

#### **INTERACTIVE SEMINAR: STEP BY STEP STRATEGIES FOR FAST DEVELOPMENT OF SMART ANALYTICAL METHODS**

**Katerina Mastovska**<sup>\*(1)</sup>, **Hans Mol**<sup>(2)</sup>, **Michal Stupak**<sup>(3)</sup>, **Octavian Augustin Mihalache**<sup>(4)</sup>

<sup>1)</sup> AOAC International, United States of America

<sup>2)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, Wageningen, The Netherlands

<sup>3)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>4)</sup> University of Parma, Italy

\*Corresponding author - E-mail: [monika.tomaniova@vscht.cz](mailto:monika.tomaniova@vscht.cz)

This educational seminar is intended not only for young scientists, all other RAFA attendees are also welcome!

Interactive demonstration of general approaches to fast development and troubleshooting in laboratory focused on food quality and safety control will be provided. The moderators will introduce interesting real-life case studies with various conceivable scenarios for each step in the method development (including both sample preparation and instrumental analysis) and/or for each troubleshooting problem. Attendees will be invited to identify the most suitable solution using an anonymous online voting; discussion about each presented option will follow. Attendees will have a possibility to check their knowledge, present their experience and, last but not least, win special prizes by participating in a short quiz.

You are invited on the board and enjoy the special atmosphere of this informal seminar, which has become a popular and well-received event at previous RAFAs.

Join the discussion, share your vision, learn something new and have some fun!

**Keywords:** troubleshooting, analytical methods development, sample preparation, instrumental analysis

## LECTURES

### L63

## SENSORY EVALUATION AS ESSENTIAL TECHNIQUE FOR THE DETERMINATION OF OFF-FLAVOURS IN FOOD

**Barbara Siegmund**\*<sup>(1)</sup>

<sup>1)</sup> Graz University of Technology, Institute of Analytical Chemistry and Food Chemistry, Austria

\*Corresponding author - E-mail: barbara.siegmund@tugraz.at

The flavour of food is one of the most important quality criteria as it is directly perceived by the consumers. In general, consumers are very sensitive to changes or alterations of a product's flavour which they are familiar with. Changes in flavour may be caused by various reasons, such as changes in the formulation, raw materials or conditions during production. Furthermore, off-flavour, which is the occurrence of sensory characteristics that are not usually associated with the product and therefore have a negative impact on the food, may be perceived. The occurrence of off-flavour is of serious concern for the food industry as it may cause significant economic damage.

The reasons for off-flavour formation are manifold, for example (i) chemical reactions of food components such as oxidative reactions (e.g. lipid autoxidation in edible oils), Maillard reaction during processing and storage, light-induced radical reactions (e.g. the formation of sunstruck flavour in beer) or acid-catalysed rearrangement reactions in citrus juices; (ii) microbial contamination of the food leading to the formation of odour-active secondary metabolites; (iii) genetically determined factors such as the formation of androsterone leading to the formation of boar-taint in pork; (iv) formation of off-flavour in eggs, meat or milk products based on odour-active components in the feedstock, or (v) migration of odour-active compounds from the packaging material into the food as well as permeation of odour-active compounds from the surrounding through the packaging material into the packaged food. For the producers it is of utmost importance to identify the compounds which are responsible for the off-flavour formation, in order to quickly set measures to avoid the occurrence of off-flavour in further batches.

For the identification of off-flavour, sensory evaluation is of particular importance. Many compounds which may cause off-flavour show very low odour thresholds, which implies that even though the compounds cause a sensory defect in the product, they are present ultra-trace concentrations only. This requires targeted analytical methods for the identification and quantification of the compounds of interest. When performing sensory evaluation of tainted food commodities, a panellist's statement "this is stinky" is not sufficient. The panellists must be well-trained to be able to specifically address the quality of the perceived off-odour (in the best case to address the compound class which is responsible for the taint). With this information at hand, the methods for targeted analysis of the samples can be developed. The use and the importance of sensory evaluation for the identification of off-flavour will be demonstrated with several case studies.

**Keywords:** sensory evaluation, off-flavour, odour threshold, targeted analysis

**Acknowledgement:** The author would like to acknowledge all panellists who participated voluntarily in the sensory evaluation of tainted products.

### L64

## RESPONSE SURFACE METHODOLOGY (RSM) AND UNTARGETED-TARGETED METABOLOMIC ANALYSIS AS TOOL TO OBTAIN POLYPHENOLS-ENRICHED EXTRACT FROM CITRUS MEDICA L. WITH PROMISING USE IN NUTRACEUTICAL FIELD

**Vittorio Calucci**<sup>(1)</sup>, Ludovica Lela<sup>(1)</sup>, Nadia Benedetto<sup>(1)</sup>, Tereza Jaegerova<sup>(2)</sup>, Luigi Milella<sup>\*(1)</sup>, Jana Hajslova<sup>(2)</sup>

<sup>1)</sup> Department of Sciences, University of Basilicata, Via dell'Ateneo Lucano, 10, 85100 Potenza, Italy, Italy

<sup>2)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: luigi.milella@unibas.it

*Citrus medica* L. (citron) cultivar Diamante Liscia is a typical fruit cultivated in Italy (Calabria region). It is currently underutilized in the nutraceutical field; however, it has been used in traditional medicine for centuries for its carminative and anti-inflammatory properties [1]. This latter might be due to the presence of antioxidant bioactive compounds that have been reported in *C. medica* [2]. In recent years growing demand for natural products and extracts is leading to the development of appropriate or alternative extraction methods to reduce the use of organic solvents, raw materials and time for increasing health promoting molecule extraction yield [3].

For this purpose, in this work, the Response Surface Methodology (RSM) was built using three independent variables (temperature, solvent, and time) and as dependent variables the antioxidant activity was evaluated by Total Polyphenol Content (TPC), 2,2-diphenyl-1-picryl hydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) [4]. In order to investigate the impact of the three independent variables, the untargeted-target screening of bioactive compounds was performed using ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS). Moreover, the antioxidant activity of the optimized extract was evaluated in an intestinal cell line (Caco-2).

Optimal conditions obtained by RSM for the extraction of compounds with high antioxidant activity were 47°C, 60 min and 82% EtOH. The optimized extract (yield 3.56±0.13%) reported the following data: 3.79±0.09 mgGAE/g, 4.45±0.03 mgTE/g and 10.62±0.26 mgTE/g for TPC, DPPH and FRAP, respectively. The results revealed that the optimized extract returned from the RSM is a rich source of bioactive compounds like polyphenols followed by limonoids and phenolic acids. Furthermore, the optimized extract reported no toxic effect at tested doses (10-400µg/mL) in Caco-2 cells after 2 and 24 h of treatment measured by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay and exerted a protective effect against oxidative stress by reducing reactive oxygen species and restoring the expression of cytoprotective enzymes, such as superoxide dismutase (SOD), catalase, and NADPH-quinone oxidase NQO1.

In this framework, the RSM represented a valid method in our study to predict the best extraction condition to obtain an extract rich in bioactive compounds paying particular attention on phenolic compounds. Moreover, a comprehensive profiling ensured by untargeted-targeted metabolomics on the whole fresh fruit was provided.

[1] Pieroni, A., et al. *Journal of Ethnopharmacology*, 91(2-3), 331-344.

[2] Carlucci, V., et al. *Plants*, 12(12), 2267.

[3] Yolmeh, M., et al. *Food and bioprocess technology*, 10(3), 413-433.

[4] Kleijnen, J. P. In *Handbook of simulation optimization* (pp. 81-104).

**Keywords:** *Citrus medica* L., response surface methodology, antioxidant activity, polyphenols

### L65

## USING HRMS TO IDENTIFY POTENTIAL MIGRANTS IN BIOBASED AND BIODEGRADABLE FOOD CONTACT MATERIALS: POLYESTER AND POLYLACTIC ACID BASED MATERIALS AS A STUDY CASE

**Patricia Vazquez-Loureiro**\*<sup>(1)</sup>, **Ronan Cariou**<sup>(2)</sup>, **Antía Lestido-Cardama**<sup>(3)</sup>, **Letricia Barbosa-Pereira**<sup>(4)</sup>, **Raquel Sendon**<sup>(4)</sup>, **Juana Bustos**<sup>(5)</sup>, **Ana Gasco**<sup>(5)</sup>, **Perfecto Paseiro-Losada**<sup>(3)</sup>, **Ana Rodriguez Bernaldo de Quiros**<sup>(4)</sup>

<sup>1)</sup> Oniris, INRAE, LABERCA, 44300 Nantes, France & FoodChemPack research group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Spain

<sup>2)</sup> ONIRIS, INRAE, LABERCA, France

<sup>3)</sup> FoodChemPack research group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Spain, Spain

<sup>4)</sup> FoodChemPack research group, Department of Analytical Chemistry, Nutrition and Food Science, Faculty of Pharmacy, University of Santiago de Compostela, Spain & Instituto de Materiales (iMATUS), Univ, Spain

<sup>5)</sup> National Food Centre, Spanish Agency of Food Safety and Nutrition, Majadahonda, Spain., Spain

\*Corresponding author - E-mail: patriciavazquez.loureiro@usc.es

Biobased and biodegradable food contact materials (FCMs) are replacing conventional plastics derived from petroleum sources to create more environmentally friendly polymers. However, from the food safety perspective, there are still huge knowledge gaps such as the migration of low molecular weight compounds that can be present and susceptible to migrate to the food, as well as the risk assessment that is associated.

In this work, acetonitrile extracts of polyester and polylactic acid (PLA) type FCMs, mainly bags for fruit and vegetables, were investigated using non-targeted strategies. For the analysis of non-volatile compounds, Liquid chromatography coupled to high resolution mass spectrometry (HRMS) with electrospray as ionization source (LC-ESI-Q-Exactive) was employed using a C18-like column (100 mm × 2.1 mm, 1.9 μm), while semivolatile compounds were analysed by gas chromatography coupled to HRMS using electron impact as ionization mode (GC-EI-Q-Exactive) with a DB-5MS (30 m × 0.25 mm, 0.25 μm) column. Structural elucidation was based on MS<sup>1</sup> and MS<sup>2</sup> experiments as well as scientific literature, mass spectral libraries (NIST libraries) and "in-house" databases.

Intentionally added substances (IAS) including a great variety of additives such as antioxidants, plasticizers or slip agents, and non-intentionally added substances (NIAS) including reaction and degradation products and oligomers were identified. It should be noted that numerous combinations of oligoesters were identified in polyester samples, including linear and cyclic combinations arising from diols and diacids used as monomers such as ethylene glycol, 1,3-butanediol and/or neopentyl glycol with adipic acid and/or phthalic acid. Previous works support these results [1,2,3,4]. PLA oligomer series were also newly identified in PLA samples. The toxicity of these substances has not been evaluated and many of the detected compounds remain to be elucidated.

[1] Omer et al., 2018, 10.1007/s00216-018-0968-z.

[2] Lestido-Cardama et al., 2022, 10.3390/polym1403048.

[3] Cariou et al., 2022, 10.1016/j.jhazmat.2022.129026.

[4] Vázquez-Loureiro et al., 2023, 10.1016/j.fpsl.2023.101183.

**Keywords:** LC and GC-HRMS, biobased, biodegradable, food packaging, NIAS

**Acknowledgement:** (i) Ministerio de Ciencia e Innovación, Agencia Estatal de Investigación and Fondo Europeo de Desarrollo Regional (FEDER), PID2021-124729NB-I00 "MIGRABIOQUANT" (MCIN/AEI/10.13039/501100011033/FEDER, UE), (ii) Ministerio de Ciencia, Innovación y Universidades (PRE2019-088195) and Xunta de Galicia for the predoctoral and postdoctoral fellowships awarded to PVL.

## LECTURES

### L66

## SIMULTANEOUS ANALYSIS OF 17 ANTIVIRAL SUBSTANCES IN POULTRY MUSCLE BY UHPLC-MS/MS

**Christelle Robert**<sup>(1)</sup>, **Gilles Pierret**<sup>(1)</sup>, **Nathalie Gillard**<sup>(2)</sup>

<sup>1)</sup> CER Groupe, Belgium

<sup>2)</sup> CER Groupe, Analytical Laboratory, Belgium

\*Corresponding author - E-mail: c.robert@cergroupe.be

Although antiviral drugs are not licensed for the treatment of influenza in food-producing animals, there have been reports of their illegal use in poultry. In consequence, these compounds are now considered as Prohibited or unauthorised pharmacologically active substances in food-producing animals regarding the regulation (EU) 2022/1644.

The study aimed to detect 17 antiviral drug residues in poultry muscle (chicken and turkey) using liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). This included 9 influenza-related drugs and associated metabolites (amantadine, rimantadine, oseltamivir, oseltamivir carboxylate, memantine, arbidol, and moroxydine), anti-herpes drugs (acyclovir, ganciclovir, famciclovir, penciclovir), an immunomodulator (imiquimod), the pro drug of Ribavirin viramidine as a multipurpose drug and others.

The method was based on Douillet et al (2022) involved chromatographic separation using a hydrophilic interaction chromatographic (HILIC) BEH amide column to retain the highly polar compounds. Detection was achieved with a triple quadrupole mass spectrometer operating in positive electrospray ionization mode. The determination of the target compounds was conducted in less than 12.0 min, and specificity was ensured by the use of multiple reaction monitoring (MRM) positive acquisition mode.

Various sample preparation protocols were evaluated to accommodate polar compounds, with the most effective procedure involving acid/acetonitrile-based protein precipitation followed by a defatting step with hexane. The sample was further pretreated using a modified QuEChERS method. The method was validated according to the EU 2021/808 guidelines for qualitative confirmatory method across different poultry species, including chicken and turkey. As no target value or minimum method performance requirement has yet been set, a target concentration of 2.5 µg/kg was used. Decision limits varied from 2.7 µg/kg to 3.7 µg/kg. Ribavirine, zanamivir and favipiravir did not pass the validation criteria due to a lack of sensitivity. The method can be now implemented to official controls to control the illegal use of antiviral drugs in poultry.

**Keywords:** antiviral drugs, chicken muscle, LC-MS/MS method



### L67

## DEVELOPMENT OF A DNA METABARCODING METHOD FOR PLANT SPECIES IDENTIFICATION IN FOOD

**Andrea Nicole Aigner**\*<sup>(1)</sup>, **Stefanie Dobrovlny**<sup>(1)</sup>, **Rupert Hochegger**<sup>(1)</sup>, **Konrad J. Domig**<sup>(2)</sup>, **Margit Cichna-Markl**<sup>(3)</sup>

<sup>1)</sup> Austrian Agency for Health and Food Safety (AGES), Austria

<sup>2)</sup> University of Natural Resources and Life Sciences, Vienna (BOKU), Austria

<sup>3)</sup> University of Vienna, Austria

\*Corresponding author - E-mail: andrea.aigner@ages.at

Plant materials make up the base of human and animal nutrition around the world, from staple grains to side dishes, drinks, and spices. In accordance with frequent use, several of the common targets for food fraud are plant-based: vegetable oils (olive in particular), fruit juices, honey, spices, and herbs. Recurring types of fraud include substitution with cheaper alternatives, waste diversion, and adulteration [Haji et al., 2023, Food Science and Nutrition]. To enable consumer choice and ensure the proper labelling of food as per EU Regulation No. 1169/2011, efficient identification methods need to be established. Screening methods such as DNA metabarcoding offer a significant advantage because of the application of universal primers. The next generation sequencing (NGS) technology employed allows efficient large-scale parallel sequencing, as well as analysis of samples with mixed composition.

Expanding from the *Codex Alimentarius Austriacus*, a list of 900 food-relevant plant species was compiled. Of these, 694 ITS2 reference sequences were available in the NCBI database. *In silico* analysis of the ~270 bp barcodes was performed using the CLC genomics workbench software, assessing the theoretical capability to differentiate plant species. DNA was extracted using a commercially available kit (Maxwell®), followed by amplicon PCR. Primers were optimized to achieve better PCR amplification. DNA library preparation was performed according to a protocol from Illumina [Dobrovlny et al., 2019, Food Chemistry]. The samples were sequenced on the MiSeq® instrument. The resulting FastQ files were processed with a Galaxy analysis pipeline and the results compared to those of a DADA2 analysis pipeline [Callahan et. al., 2016, Nature Methods]. BLASTn was used to identify the generated DNA sequences.

We have developed an ITS2 primer system for DNA metabarcoding of plants. Reference materials tested with this method include fruits, grains, honey, juices, leaves, nuts, oils, spices and vegetables. Of the sequences generated, 94 % could be identified on genus level, 65 % on species level. As expected, differentiation between closely related species, such as *Brassica spp.*, *Citrus spp.*, or *Triticum spp.*, turned out to be the most challenging. Testing will be expanded to include more processed food samples. Proficiency test samples of known composition will be used to validate the method. When comparing the results of data analysis by Galaxy pipeline, it was observed that roughly 31 % of the total number of reads could be used for analysis, whereas the application of the DADA2 pipeline resulted in 66 % of usable reads after data processing.

In conclusion, ITS2 DNA metabarcoding seems promising for differentiating plants in food on genus level, and further capable of identifying many plants on species level. Furthermore, the DADA2 pipeline is more suitable for our data analysis.

**Keywords:** DNA metabarcoding, Next Generation Sequencing (NGS), plant species identification, food authenticity

**Acknowledgement:** This research was funded by the Austrian Agency for Health and Food Safety (AGES), Institute for Food Safety Vienna, Department for Molecular Biology and Microbiology. The project is a cooperation with the University of Natural Resources and Life Sciences, Vienna (BOKU) and the University of Vienna.

### L68

## PAPER-IMMOBILIZED LIQUID PHASE MICROEXTRACTION FOR DIRECT PAPER SPRAY MASS SPECTROMETRY AND IMMUNO-DETECTION OF ATROPINE IN BABY FOOD, BUCKWHEAT CEREALS, AND EDIBLE OILS AT REGULATORY LEVELS

**Ids Lemmink**<sup>(1)</sup>, **Sipke Bosma**<sup>(1)</sup>, **Erik Beij**<sup>(1)</sup>, **Han Zuilhof**<sup>(1)</sup>, **Toine Bovee**<sup>(1)</sup>, **Gert IJsbrand Salentijn**<sup>(1)</sup>

<sup>1)</sup> *Wageningen University & Research, The Netherlands*

\*Corresponding author - E-mail: [ids.lemmink@wur.nl](mailto:ids.lemmink@wur.nl)

Contamination with tropane alkaloids (TAs), such as atropine (ATR), in different food commodities is increasing globally, but frequent TA-analysis is expensive and time-consuming. Moreover, the range of relevant matrices, as well as corresponding differences in required detection limits are wide, further complicating the matter. Therefore, the objective of the current work has been to develop a more rapid and affordable paper-based workflow, combining immuno-detection and mass spectrometry to detect ATR in buckwheat, baby cereals, and canola oil at relevant levels (0.5-15 µg/kg). In this workflow, ATR is extracted from the food commodities with a dilution or solid-liquid extraction, followed by a selective enrichment using a dual paper-immobilized liquid phase microextraction (PI-LPME; enrichment factor of ±144). One of the two PI-LPME papers can be directly coupled to a validated indirect competitive lateral flow immunoassay (icLFIA; IC50-value = 0.56 ng/mL) for initial screening at the point-of-need. During validation of this icLFIA, a cut-off value was established at a false negative rate of 1% resulting in an estimated false positive rate of 0.7%. In case of a suspect sample, the second PI-LPME paper can be transported to the laboratory, where it can be stored at room temperature (stability of ATR > 90% up to at least 10 days). In the laboratory, the PI-LPME paper can be rapidly analyzed with paper spray-high resolution mass spectrometry (PS-HRMS). The developed PS-HRMS method could reach detection limits in matrix below regulatory levels (0.8 - 2.7 µg/kg). Furthermore, the PS-HRMS exhibited good performance compared to HPLC-HRMS (after PI-LPME sample preparation) in terms of precision (1% - 12% vs. 6.3% - 10%) and accuracy (0.2% - 15% vs. 0.9% - 17%). In conclusion, our paper-based workflow reduces the average sample-to-result time, required storage space, and the amount of samples requiring HPLC-MS/MS analysis in the laboratory. Therefore, it has the potential to aid in the fast and inexpensive monitoring of ATR. Importantly, the method's suitability is demonstrated for very diverse matrices, and it is expected that it can be easily adapted to monitor for other food safety hazards - given the wide applicability of liquid-liquid extractions.

**Keywords:** tropane alkaloids, immunoassay, on-site sample preparation, ambient ionization mass spectrometry

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### L69

## ELEMENTOMICS, METABOLOMICS, AND CHEMOMETRIC APPROACHES AS TOOLS IN EXPLORING BLACK PEPPER IDENTITY

**Cynthia Adaku Chilaka**<sup>\*(1)</sup>, **Nicholas Birse**<sup>(1)</sup>, **Chris Elliott**<sup>(1)</sup>

<sup>1)</sup> Queen's University Belfast, United Kingdom

\*Corresponding author - E-mail: c.chilaka@qub.ac.uk

Black pepper (*Piper nigrum* L.) is one of the most used spices worldwide because of its flavouring and taste enhancement potential. Its use in traditional medicine is attributed to the antimicrobial and therapeutic properties of the bioactive compounds. The sensory and chemical characteristics of black pepper are highly influenced by the environment encompassing geographical origin, soil quality, and handling processes. In recent years, consumers have placed high economic importance on product geographical indication, as a way of preventing fraud through protecting the origin of a product and its characteristics. This trend has increased the demand for detailed information regarding the geographical source of food products. Verifying the geographical origin of black pepper is relevant to protect value chain actors while upholding food integrity and well-being.

The present study aims to assess the geographical traceability of black pepper using elementomics, untargeted metabolomics, and chemometrics tools. A total of 150 black pepper samples collected from 5 countries (Vietnam, Indonesia, India, Brazil, and Cambodia) were analysed using inductively coupled plasma mass spectrometry (ICP-MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS) to obtain elemental and chemical fingerprints of the samples, respectively. Chemometric technique was used for the multivariate statistical analysis of the generated elemental and chemical fingerprints, specifically the unsupervised model principal components analysis (PCA) and the supervised model partial least squares-discriminant analysis (PLS-DA) for the discrimination and classification of the samples. Our models demonstrated a classification accuracy rate of above 90% regardless of the instrumentation used. Application of data fusion technique on the data improves the classification of the geographical origin to above 95%. Our findings provide empirical evidence for combined elementomics, metabolomics and chemometrics techniques as a tool for black pepper authentication and possible application to other food products for fraud prevention while ensuring the quality and safety of end users.

**Keywords:** black pepper, authenticity, elementomics, metabolomics, chemometrics

**Acknowledgement:** This research was funded by Agilent Thought Leader Award.

## LECTURES

### L70

## EMERGING CONTAMINANTS EXPOSED: COMBINING EFFECT-BASED TESTING AND ANALYTICAL CHEMISTRY FOR FOOD AND FEED SAFETY

**Arjen Gerssen**<sup>(1)</sup>, **Milou Van de Schans**<sup>(1)</sup>, **Toine Bovee**<sup>(1)</sup>, **Caroline Dirks**<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: arjen.gerssen@wur.nl

Food authorities strive to ensure the safety of our food, necessitating highly sensitive analyses to detect both banned and regulated substances at low concentrations. Additionally, comprehensive screening methods are essential to identify new and emerging risks. Effect-based bioassays, when combined with mass spectrometric analyses, provide a significant advantage in achieving these goals.

Utilizing both effect-based bioassays and conventional mass spectrometric techniques in routine monitoring may reveal discrepancies. These discrepancies can be further investigated with analytical methods to identify and confirm the presence of emerging substances that exhibit similar biological effects to already regulated substances.

For example, during routine monitoring of dioxins in agricultural products, samples are initially screened using the DR CALUX (Dioxin-Responsive Chemical Activated Luciferase Gene Expression) bioassay. Suspect samples are then analyzed for dioxins using the confirmatory gas chromatographic high-resolution mass spectrometric (GC-HRMS) method. A discrepancy was observed in egg and broiler fat samples between the results of the DR CALUX bioassay and the GC-HRMS analysis. The bioassay indicated high responses, suggesting a significant exceedance of the maximum dioxin limits, yet regulated dioxins or dl-PCBs were not detected by the GC-HRMS analysis.

Ultimately, a comprehensive screening analysis using GC-HRMS led to the identification of 2,3,7,8-tetrabromo-dibenzofuran (2,3,7,8-TBDF) in both egg and broiler fat samples. This finding was confirmed by comparison with a commercial standard solution, and the bioactivity of this compound was verified using the DR CALUX bioassay.

The source of the contamination remained unknown, prompting the analysis of various samples, including bedding material, poultry feed, feed additives (choline chloride and L-lysine), and seaweed. Both the poultry feed and feed additives contained 2,3,7,8-TBDF. By employing a feed-to-food transfer model, it became evident that the poultry feed was likely the source of 2,3,7,8-TBDF in broilers and eggs, originating from a feed additive like L-lysine or choline chloride [1].

During the presentation, this example, along with others, will be discussed to highlight the importance of combining effect-based screening assays with sensitive analytical methods in detecting potential new and emerging risks.

[1] Dirks, C.; Gerssen, A.; Weide, Y.; Meijer, T.; van der Weg, G.; van de Schans, M.G.M.; Bovee, T.F.H. Brominated Dioxins in Egg, Broiler, and Feed Additives: Significance of Bioassay-Directed Screening for Identification of Emerging Risks in Food. *Foods* 2024, 13, 931. <https://doi.org/10.3390/foods13060931>

**Keywords:** food and feed safety, effect-based bioassays, mass spectrometry, emerging contaminants

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## LECTURES

### L71

#### **EXPLORING MASS SPECTROMETER LIMITS: UHPLC-MS/MS METHOD FOR DETERMINATION OF 1,000 TOXINS IN 10 MINUTES**

**Lidija Kenjeric**<sup>(1)</sup>, **Alexandra Malachova**<sup>(2)</sup>, **Rudolf Krska**<sup>(3)</sup>, **Michael Sulyok**\*<sup>(3)</sup>

<sup>1)</sup> Austrian Competence Centre for Feed and Food Quality, Safety and Innovation FFoQSI GmbH, Austria

<sup>2)</sup> FFoQSI GmbH, FFoQSI Austrian Competence Centre for Feed & Food Quality, Safety and Innovation, Tulln, Aust, Austria

<sup>3)</sup> University of Natural Resource and Life Sciences, Vienna, Department of Agrobiotechnology, IFA - Tulln, Institute of Bioanalytics and Agro-Metabolomics, Konrad-Lorenz-Strasse 20, 3430 Tulln an der Dona, Austria

\*Corresponding author - E-mail: michael.sulyok@boku.ac.at

Our in-house method is fully validated and capable of quantifying over 1000 fungal metabolites and plant toxins in 40 minutes, with 2 injections in positive/negative ionization modes, 20 minutes each. To explore the extent to which the method can be accelerated without compromising accuracy and precision, we applied fast polarity switching (FPSW) alongside with scheduled multiple reaction monitoring (sMRM).

Nine (9) different setups were tested to compare the applicability of HPLC & UHPLC column, to explore variations in method performance between FPSW and positive/negative polarity as well as the impact of accelerated gradients, ranging from 20 minutes measurement time to 10 minutes measurement time.

To determine whether the implementation of these accelerated methods is feasible without significant deterioration of data quality, all 9 methods were validated according to SANTE 11312/2021(V2). Validation was carried out on 5 distinct samples of oats and muesli. Samples spiked on a high concentration level (pre- and post-extraction) were tested to determine both repeatability as well as intermediate precision, matrix effects and recoveries of the extraction. Additionally, three low concentration levels were employed to determine the limits of detection (LOD) and quantification (LOQ).

Final analysis of the validation data is pending due to the extensive number of validated setups. Results retrieved so far indicate that matrix effects are not significantly affected by FPSW, while repeatability and intermediate precision still comply to official criteria. However, applying a fast UHPLC-gradient on top of FPSW severely compromises data quality especially on lower concentration levels.

### L72

## INVESTIGATION OF DIFFERENT SEPARATION STRATEGIES IN COMPREHENSIVE TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY FOR CHARACTERIZATION OF SECONDARY METABOLITES IN COMPLEX FOOD MATRICES

**Roberto Lagana Vinci**<sup>(1)</sup>, **Katia Arena**\*<sup>(1)</sup>, **Francesco Cacciola**<sup>(2)</sup>, **Paola Dugo**<sup>(3)</sup>, **Luigi Mondello**<sup>(3)</sup>

<sup>1)</sup> Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Palatucci snc 98168, Italy

<sup>2)</sup> Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Via Consolare Valeria 98125, Italy

<sup>3)</sup> Messina Institute of Technology and Chromaleont s.r.l. c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Viale G. Pala, Italy

\*Corresponding author - E-mail: arenak@unime.it

In the last decade, the increasing demand for healthy and sustainable foods enhanced the interest on bioactive molecules from natural sources. Among these, secondary metabolites, with more than 8000 molecules currently identified in several plant and food matrices, are well known for their antioxidant activities and therapeutic powers. In this context, achieving a complete characterization of complex food samples often requires the use of advanced chromatographic platforms. Among these, comprehensive two-dimensional liquid chromatography hyphenated with PDA and MS or MS/MS detectors is one of the most used approaches to obtain high-quality data for unequivocal identification.

The determination of secondary metabolites in food analysis is generally carried out by reversed-phase liquid chromatography (RP) and hydrophilic interaction liquid chromatography (HILIC) separation mode. The object of the current contribute is to compare different platforms (HILIC × RP, RP × HILIC, RP × RP) employing a fixed solvent modulation for the analysis of complex matrices.

Their performances were evaluated in terms of peak capacity, orthogonality and maximum number of identified compounds.

In particular, this contribution was focused on food products naturally derived from herbs and spices, such as sumac, herbal liqueur and juice; these under study platform enabled the identification of a huge number of compounds, higher compared to conventional monodimensional separations. The increase in separation power allowed for a more accurate and robust quantification of the analytes, due to the possibility to solve many coelutions and reduce matrix effects related to interfering compounds.

**Acknowledgement:** The authors are thankful to Shimadzu and Merck Life Science Corporations for the continuous support. The researches were performed within the framework of the Research Project PRIN PNRR - MISSIONE 4, COMPONENTE 2, INVESTIMENTO 1.1 - BANDO PRIN 2022 PNRR - DECRETO DIRETTORIALE N. 1409 DEL 14-09-2022: Sumac nutraceutical Potential through Innovative and Comprehensive investigation (SPICE), supported by the Italian Ministry of University and Scientific Research, CODICE IDENTIFICATIVO: P20224B5BE, CODICE CUP: J53D23014460001.

## LECTURES

### L73

#### **ANALYTICAL DEVELOPMENTS AND CHALLENGES FOR MICRO-/NANOPLASTICS ANALYSIS IN FOOD**

**Haiyan Li**\*<sup>(1)</sup>, Lin Min Lee<sup>(1)</sup>, Joanna Khoo<sup>(1)</sup>, Sheot Harn Chan<sup>(1)</sup>, Dingyi Yu<sup>(1)</sup>

<sup>1)</sup> National Centre for Food Science, Singapore Food Agency, Singapore

\*Corresponding author - E-mail: Li\_Haiyan@sfa.gov.sg

Micro-/Nanoplastics (MPs/NPs) are emerging environmental pollutants of concern, with scientific evidence on potential exposures and health risks still rapidly evolving. Contamination of the food chain is considered as one of the pathways of human exposure to MPs/NPs. While there are a number of monitoring studies that reported occurrence of MPs/NPs in food products, the interpretation of reported results is challenging due to the lack of a harmonised method, resulting in highly variable results that may not be directly comparable. There is an urgent need to develop a set of validated methods for reliable identification, characterization and quantification of MPs/NPs in food.

We have developed and validated an analytical method for MPs that can be used to simultaneously identify, size characterize and quantitate MPs that are present in table salts and seafood by both number and mass. The analytical method was subsequently deployed for a retail survey of MPs in table salts and seafood to provide occurrence data to aid in risk assessment. For NPs, we have built a multi-technique based analytical platform to identify, characterize and quantify NPs in water samples. Starting from a simple matrix, it has the potential to be applied to complex food types. The established analytical platform can fill an analytical gap by offering a solution for quantifying size-resolved mass concentrations of NPs.

Currently, there is no legislation for MPs/NPs as contaminants in food. More exposure and toxicity data are needed to fill the gaps in scientific knowledge and risk assessment of MPs/NPs to human health, and to facilitate the final translation of data into policy decisions.

**Keywords:** microplastics, nanoplastics, identification and quantification, food matrix, multi-technique-based analysis

## LECTURES

### L74

#### THE FRACTIONATION PROCESSING AFFECTS THE AMOUNT OF ANTINUTRIENTS IN DIFFERENT PLANT-BASED FOOD INGREDIENTS

**Giovanni D'Auria**<sup>\*(1)</sup>, Chiara Nitride<sup>(1)</sup>, Alfonso Piscitelli<sup>(1)</sup>, Ever Hernandez Olivas<sup>(2)</sup>, Andre Brodkorb<sup>(2)</sup>, Pasquale Ferranti<sup>(1)</sup>

<sup>1)</sup> University of Naples Federico II, Italy

<sup>2)</sup> Teagasc Food Research Centre, Ireland

\*Corresponding author - E-mail: giovanni.dauria@unina.it

The food production system contributes to one-third of global greenhouse gas emissions, with a significant portion associated with animal farming. Sustainable alternative protein ingredients, particularly whole flour or protein fractions from cereals and pulses, are being introduced to the market as substitutes for animal proteins due to their functional properties. These ingredients require a thorough nutritional assessment and evaluating anti-nutritional factors (ANFs) is a critical part of this process. This investigation is focused on commercial ingredients selected to be comprehensively studied in the context of the Horizon Europe project GIANT LEAPS.

We developed an analytical pipeline to investigate how protein fractionation impacts key ANFs—phenolics, phytic acid, lectins, and protease inhibitors—in high-protein food ingredients derived from cereals, pulses, and press cake. Phenolics were included as ANFs for their ability to bind proteins, thereby interfering with their digestibility and bioavailability. The combination of quantitative spectrophotometric determinations, electrophoresis, and high-resolution mass spectrometry allowed the discrimination of ingredients based on the amount of ANFs. The activity of proteinaceous ANFs, identified by mass spectrometry, was confirmed with biological assays. Active lectins were examined via an in vitro hemagglutination test, and trypsin inhibition tests assessed the presence of active serine protease inhibitors.

Our analyses revealed that protein fractionation affects both non-proteinaceous and proteinaceous ANFs, influencing the levels of these factors in the final ingredient. We found a connection between mass spectrometry semi-quantitative determinations and confirmatory biological activity assays. Depending on the type of ANFs, the fractionation process does not necessarily increase ANFs activity, with wet and dry extraction leading to lower and higher activity, respectively. On the other hand, both wet and dry protein fractionation led to a concentration of phytic acid, especially in pulses.

The collected evidence strongly suggests the need for a communicative bridge between ingredient producers and food product designers to adequately inform them about the type and quantity of ANFs present in each ingredient.

**Keywords:** protease inhibitors, lectins, phytic acid, mass spectrometry, hemagglutination

**Acknowledgement:** The authors thank the European Union for funding the Giant Leaps project, which supported this research [grant agreement No 101059632].



## LECTURES

### L75

#### PROTEOMICS TO ANSWER PROTEIN-RELATED FOOD SAFETY QUESTIONS

**Yvonne Westphal**<sup>\*(1)</sup>, Floor Leurs<sup>(1)</sup>, Twan America<sup>(2)</sup>, Aard De Jong<sup>(3)</sup>, Nathalie Smits<sup>(1)</sup>, Marco Blokland<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>2)</sup> Wageningen Plant Research, The Netherlands

<sup>3)</sup> Wageningen Food Biobased Research, The Netherlands

\*Corresponding author - E-mail: yvonne.westphal@wur.nl

In recent years, many questions related to proteins within food and feed safety were raised, including “which allergens are present in (processed) foods, and in what concentrations?” and “what are the new food and feed safety risks of the protein transition?”.

Mass spectrometry techniques are increasingly used to answer these questions as other techniques (alone) do not achieve the specificity and/or sensitivity needed. Within WFSR, we developed a non-targeted proteomics workflow based on liquid chromatography coupled to high-resolution mass spectrometry (LC-hrMS/MS) in data-independent acquisition (DIA) mode. In short, proteins are extracted from food or animal feed and enzymatically cut into specific peptides by protease. The resulting peptides are then analyzed with LC-hrMS/MS in DIA mode. Resulting peptides are identified and mapped to corresponding proteins by applying neural network-based software such as FragPipe and DIA-NN. Afterwards, a uniqueness test (UniPept) is performed to quickly evaluate which peptides are taxonomically unique for a given species and/or tissue (e.g. specific to soy, pea or other plant ingredients).

Recently, we applied this non-targeted proteomics workflow to a selection of heat-processed legumes with or without adding sugar and/or salt. Legumes are increasingly used in our nutrition, unprocessed or processed, or are entering the food chain unintendedly, resulting in potential allergic reactions of consumers when not labelled correctly. Therefore, in both cases, qualitative and (semi)-quantitative analysis of the legume proteins is relevant but occurs to be difficult in processed foods as proteins tend to react and change upon processing (e.g. Maillard reaction). Due to these chemical changes, proteins and thus corresponding signature peptides might escape targeted analysis by applying standard settings. In our research, we applied our workflow to investigate the (in)stability of legume proteins (and subsequent signature peptides) during processing. We further evaluated the possibility to accurately quantify legume proteins in heated matrices by LC-hrMS/MS. We will present our findings on the application of this non-targeted LC-hrMS/MS proteomics workflow to heat-processed legumes, revealing both the identification of process-stable peptides and the challenges ahead in quantifying legume proteins in complex food matrices.

**Keywords:** proteomics, safety, quantification, processing, legumes

**Acknowledgement:** This work was supported by the Dutch Ministry of Agriculture, Nature and Food Quality through the Knowledge development program ‘Healthy and safe food systems’ (projects KB-37-001-021 & KB-37-002-037).

## LECTURES

### L76

## PROTEOMIC IDENTIFICATION OF INSECT SPECIES IN FOOD PRODUCTS USING MASS SPECTROMETRY

Stepanka Kuckova<sup>(1)</sup>, Tatiana Smirnova<sup>(1)</sup>, David Straka<sup>(1)</sup>, Alena Meledina<sup>(1)</sup>, Jiri Santrucek<sup>(1)</sup>

<sup>1)</sup> University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: kuckovas@vscht.cz

Since the last decade, proteomics has an increasing role to play in food analyses and control. The food labels information must be validated, and therefore precise and reliable analytical methods are required in order to check the seller's declaration and confirm that the provided information is accurate with regard to product safety, composition and quality expected by the consumers. The proteomic methods are suitable to authenticate the protein origin in food products.

In our Laboratory of Applied Proteomics at the University of Chemistry and Technology in Prague, we employed two mass spectrometric techniques (matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) and liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-Q-TOF)) to the analyses of four approved edible insect species (*Acheta domesticus*, *Alphitobius diaperinus*, *Locusta migratoria*, and *Tenebrio molitor*) and six not yet approved insect species (*Blaptica dubia*, *Gryllus assimilis*, *Hermetia Illucens*, *Schelfordella tartara*, *Schistocerca gregaria*, and *Zophobas morio*). We looked for optimal conditions (e.g. weight of sample, time of enzyme digestion, matrix to sample ratio) for samples preparation and then for *m/z* values and amino acid sequences characteristic for each analysed insect species to be able to distinguish them.

Using PostgreSQL database system, which was accessed using the pgAdmin tool, we found tens to hundreds of the characteristic *m/z* values and amino acid sequences for each insect species. We verified the applicability of these markers on model samples on selected commercial products from edible insects. Using MALDI-TOF, the insect origin of the model samples was determined, which were like blank samples prepared by mixing two or three different insect species. It was also possible to determine the insect origin of selected commercial products. The results obtained from LC-ESI-Q-TOF show also a great potential in the authentication of insect species in food products.

In the lecture, we would like to point out the critical points of sample preparation and the possibilities and limitations of the applicability of proteomic methods in the determination of insect species in food.

**Keywords:** proteomics, mass spectrometric techniques, edible insects, insect species identification, food analyses and control

**Acknowledgement:** *The work was supported by the project: The comprehensive laboratory strategy for identification of insect species intended for human consumption and the production of processed animal protein, authentication of insect-based foods. (NAZV (QK23020101), Ministry of Agriculture, Czech Republic).*

### L77

## QUANTITATIVE PEPTIDE PROFILING OF HYDROLYSED INFANT FORMULA BY AN UNTARGETED DIAPASEF APPROACH ON A MICROLC-IM-QTOF-MS/MS

**Alina Kupfer<sup>(1)</sup>, Joachim Stützer<sup>(1)</sup>, Sabrina Gensberger-Reigl<sup>(2)</sup>, Monika Pischetsrieder<sup>\*(2)</sup>**

<sup>1)</sup> Chair of Food Chemistry, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

<sup>2)</sup> A) Chair of Food Chemistry, Department of Chemistry and Pharmacy, Friedrich-Alexander-Universität Erlangen-Nürnberg B) FAU NeW - Research Center New Bioactive Compounds, Friedrich-Alexander-Universität, Germany

\*Corresponding author - E-mail: monika.pischetsrieder@fau.de

Most infant formulae use cow's milk as the only protein source. As cow's milk allergy is one of the most common food allergies for infants, the proteins can be hydrolysed into smaller peptides, thus destroying epitopes recognised by the immune system and reducing the allergic potential. These hypoallergenic (HA) formulae are used for prevention or therapy of cow's milk allergy. The physiological evaluation of these products is particularly important since infants are a highly sensitive target group. Peptides have a direct impact on the nutritional quality but have mainly been investigated qualitatively. Therefore, quantitative peptide profiling is necessary as it provides further information on the peptide composition to give a comprehensive overview of the profile and ensure adequate quality control of infant formula.

An untargeted diaPASEF (data independent acquisition parallel accumulation serial fragmentation) approach on a microLC-IM-QTOF-MS/MS system was applied in the present study for a relative quantification of the peptide profiles. As DIA performs an unbiased selection of precursor ions, a spectral library is required to deconvolute the resulting mixed MS/MS spectra. Therefore, a DDA (data dependent acquisition) approach was used to generate a sample- and device-specific spectral library containing the information required for peptide identification. The data was evaluated with the software PEAKS® Online.

The DDA library, generated from 16 different commercial HA formulae, consists of 583 proteins and 5546 peptides. It was used for the subsequent optimisation of the diaPASEF method. For this purpose, py\_diAID, a freely available Python package, was applied. It generates diaPASEF methods with variable isolation widths adjusted to the precursor density, thus overcoming the limitations of an original method with equidistant widths. The optimisation process increased the number of 17 equidistant to 30 variable ion mobility windows and reduced the cycle time from 1.8 to 1.7 sec. The method was then further optimised using one specific sample and individualised for infant formula. Comparison of the original and final method revealed a 20.3% increase in identification rate and a 29.2% improvement in quantitative reproducibility. 628 peptides were identified with a CV of 0.8% and quantified with a CV of 17.2%.

This diaPASEF method was used to analyse the 16 HA formulae from the spectral library. Depending on the sample, between 275 and 955 peptides were identified using an LFQ (label free quantification) approach. This data was then used to compare the quality of the commercial products on an extended basis. In this way, not only the length or origin of the peptides can be considered, but also their relative abundance and composition. The results showed that the peptide profile of HA formula differs greatly between manufacturers, which highlights the need for a quantitative approach to not only differentiate but also evaluate the products.

**Keywords:** hydrolysed infant formula, peptide profiling, diaPASEF, spectral library, quantitative proteomics

## L78

**INNOVATIONS AND CHALLENGES IN QUANTITATIVE VOLATILOMICS: THE ROLE OF FID/MS CHROMATOGRAM FUSION IN ENHANCING PATTERN RECOGNITION**

**Andrea Caratti**<sup>(1)</sup>, **Angelica Fina**<sup>(2)</sup>, **Fulvia Trapani**<sup>(2)</sup>, **Carlo Bicchi**<sup>(2)</sup>, **Stephen E. Reichenbach**<sup>(3)</sup>, **Qingping Tao**<sup>(4)</sup>, **Daniel Geschwender**<sup>(4)</sup>, **Chiara Cordero**<sup>(1)</sup>

<sup>1)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>2)</sup> *University of Turin, Italy*

<sup>3)</sup> *University of Nebraska, United States of America*

<sup>4)</sup> *GC Image LLC, United States of America*

*\*Corresponding author - E-mail: andrea.caratti@unito.it*

Volatilomics is an emerging discipline aimed at characterizing volatile metabolites in various samples. Recently, two-dimensional gas chromatography (GC×GC) with parallel flame ionization detector (FID) and mass spectrometry (MS) has gained attention for combining compound identification (via MS) with accurate quantification (via FID). Typically, FID and MS traces are processed separately. This study focuses on merging MS and FID chromatograms to improve pattern recognition during template matching and enable quantitative volatilomics on a large set of features. Features matching is guided by MS spectral similarity, reducing mismatches and extracting FID responses for accurate quantification.

This approach was tested for characterizing the aromatic identity of hazelnuts, a premium confectionery ingredient. GC×GC-FID/MS traces of hazelnuts highlight molecules that differentiate cultivars, geographical origin, post-harvest treatments, bacterial/mold contamination, oxidative stability, and sensory quality [1]. This strategy aligns with the Sensomics-Based Expert System (SEBES), which act as an AI-Smelling predicting food aromas without human olfaction [2].

The dataset included raw hazelnut samples analyzed over four years of production, in which MS response variability requires normalization and internal standards correction for consistent analysis. Chromatographic misalignments over time can cause 2D peak pattern inconsistencies [3]. Data fusion, guided by MS spectral similarity, reduces false negatives by about 80% on 441 detectable features in raw hazelnuts compared to FID alone and minimizes false positives, enhancing method specificity and selectivity. Data fusion also halves processing time and facilitates metadata transfer. After pattern recognition step, FID signals are extracted for quantitation based on calibration or predicted FID response factors.

Quantitative volatilomics with parallel detector signal fusion reliably tracks aroma changes over crops and shelf-life, enabling robust marker discovery for industrial quality assessment.

[1] Squara S., ... Cordero C. 2023. Artificial Intelligence decision-making tools based on comprehensive two-dimensional gas chromatography data: the challenge of quantitative volatilomics in food quality assessment. *J. of Chromatography A*, 1700, 464041. <https://doi.org/10.1016/j.chroma.2023.464041>.

[2] Nicolotti L, ... Schieberle P. 2019. Characterization of Key Aroma Compounds in a Commercial Rum and an Australian Red Wine by Means of a New Sensomics-Based Expert System (SEBES) - An Approach to Use Artificial Intelligence in Determining Food Odor Codes. *J. Agric. Food Chem.* 67, 4011-4022. <https://doi.org/10.1021/acs.jafc.9b00708>.

[3] Stilo F, ... Cordero C. 2019. Untargeted and Targeted Fingerprinting of Extra Virgin Olive Oil Volatiles by Comprehensive Two-Dimensional Gas Chromatography with Mass Spectrometry: Challenges in Long-Term Studies. *Agric. Food Chem.* 67(18), 5289-5302. <https://doi.org/10.1021/acs.jafc.9b01661>.

**Keywords:** quantitative metabolomics, comprehensive two-dimensional gas chromatography, volatilomics, MS-FID Fusion, artificial intelligence smelling

## LECTURES

### L79

## MACHINE-LEARNING BASED VOLATILOMICS FOR FAST SCREENING OF CULTURED-YEAST FERMENTED COFFEE

**Catherine Kiefer**<sup>(1)</sup>, **Steffen Schwarz**<sup>(2)</sup>, **Sascha Rohn**<sup>(3)</sup>, **Philipp Weller**<sup>(1)</sup>

<sup>1)</sup> *Institute for Instrumental Analysis and Bioanalytics/ Competency Center for Chemometrics CHARISMA, Mannheim University of Applied Sciences, Germany*

<sup>2)</sup> *Coffee Consulate, Germany*

<sup>3)</sup> *Department of Food Chemistry and Analysis, Institute of Food Technology and Food Chemistry, TU Berlin, Germany*

*\*Corresponding author - E-mail: c.kiefer@hs-mannheim.de*

Coffee is the second largest global commodity, and its characteristic aroma is essential for its appeal. By fermenting green coffee beans in a controlled manner, it is possible to enhance the flavour and sensory profile of the coffee beans to achieve higher specialty coffee association (SCA) gradings on specialty coffees [1]. In the literature, various bacteria and yeast strains are described for controlled coffee fermentations [2]. This could be of interest to the industry, as this would lower the production costs of such coffees. However, this could also lead to a misuse by claiming controlled fermentation on unfermented low-grade coffees.

To date, the analysis of the metabolome of fermented coffee beans is commonly based on HPLC or HPLC-MS [3] and solid phase microextraction (SPME)-GC-MS for volatilome [4]. With cup tastings, SCA trained experts compare and describe the sensory properties of coffees by the smell, aroma, sweetness, body and several more sensory attributes [5].

The aim of this study is the development of a screening technique for the confirmation of a controlled coffee fermentation. In a later step, a fingerprint-based model for the differentiation between microorganisms used for controlled fermentation will be established.

An authentic sample set was acquired on a coffee farm in brasil, by fermenting ripe and washed coffee cherries with the addition of three different known yeasts and without the addition of microorganisms, respectively. As a fast-screening method, GC-IMS was used to compare the volatile profiles of the samples. Furthermore, the sensory properties of the different fermented coffees were evaluated by experts. For complementary data, an in-depth metabolomic analysis of the coffees was carried out with HPLC-ESI-HRMS and metabolomic workflows.

The sensory profile of coffee beans is highly dependent on various factors including variety, processing, origin, profile of roasting and fermentation inoculum. To avoid adding skewness to the analysis, as many confounding variables as possible were excluded. The analysed coffee beans were from the same origin, variety, processing and profile of roasting and differ only in their fermentation inoculum. The assessed GC-IMS data is high-dimensional and partially colinear and demands for a multivariate data analysis approach. For the evaluation of HPLC-MS data, a univariate analysis was chosen to highlight possible marker substances for specific microorganisms used in fermentation.

In this talk, a multivariate evaluation of GC-IMS data for a fast classification of coffee fermented with different microorganisms will be presented. Furthermore, a univariate data analysis of HPLC-HRMS data for the tentative identification of a marker substances will be shown.

[1] Bressani et al. 2020.

[2] Aditiawati et al. 2020; da Silva et al. 2021.

[3] Elhalis et al. 2020.

[4] Lee et al. 2016.

[5] Martinez et al. 2017; Wang et al. 2020.

**Keywords:** volatilomics, machine-learning, coffee fermentation, GC-IMS, HPLC-HRMS

## LECTURES

### L80

#### **SPECTROSCOPIC APPROACHES FOR RAPID FOOD AUTHENTICITY SCREENING - RECENT APPLICATIONS AT FAO/IAEA**

**Alina Mihailova**<sup>(1)</sup>, Marivil Islam<sup>(1)</sup>, Simon Kelly<sup>(1)</sup>, Britt Maestroni<sup>(1)</sup>, Aiman Abraham<sup>(1)</sup>, Islam Hamed<sup>(1)</sup>, Christina Vlachou<sup>(1)</sup>

<sup>1)</sup> Food Safety and Control Laboratory, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Austria

\*Corresponding author - E-mail: a.mihailova@iaea.org

Traceability of food products throughout the supply chain is an integral part of ensuring the quality, safety and authenticity of the food we consume. Significant fraud cases have been reported in the European Union and many other countries over the past few years. Food fraud concerns the whole agri-food sector, from small local shops and farmers markets to supermarket chains, internet sales, and global large-scale retailers. To contribute towards assuring consumer confidence of the food supply chain, in addition to administrative controls, it is highly important to have robust analytical methods that can verify food labelling claims in an objective and independent way.

Technological advancements made over the recent years in the field of vibrational spectroscopy allow performing rapid, cost-effective non-targeted food authenticity screening. Benchtop and portable spectroscopy devices offer high sample throughput, low cost, ease-of-use in routine operations and applicability to on-site/on-line analysis. These analytical approaches can be used as Tier 1 screening techniques and have the potential to be easily integrated into quality management procedures at various stages of the food supply chain.

We present an overview of the recent applications of benchtop and portable infrared spectroscopy techniques, multispectral imaging as well as gas chromatography-ion mobility spectrometry that have been used at the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture for the verification of food authenticity and determination of geographical origin. Applications of these techniques, combined with chemometrics and data fusion, for the geographical discrimination of rice and palm oil, and the authenticity testing of Arabica coffee, will be presented. We discuss validation strategies, advantages, challenges, and future perspectives of these rapid screening approaches for food authentication.

**Keywords:** food fraud, food authenticity, geographical origin, infrared spectroscopy, gas chromatography-ion mobility spectrometry

## LECTURES

### L81

## PORTABLE NIR SPECTROSCOPY AND MACHINE LEARNING FOR A GLOBAL HONEY AUTHENTICATION AND FRAUD DETECTION

**Andrea Mara**<sup>(1)</sup>, **Marco Caredda**<sup>(2)</sup>, **Marco Ciulu**<sup>(3)</sup>, **Francesca Tilocca**<sup>(1)</sup>, **Ilaria Langasco**<sup>(1)</sup>, **Javier Saurina**<sup>(4)</sup>, **Maria I. Pilo**<sup>(1)</sup>, **Nadia Spano**<sup>(1)</sup>, **Gavino Sanna**<sup>(1)</sup>, **Oscar Nunez**<sup>(5)</sup>, **Sonia Sentellas**<sup>(5)</sup>

<sup>1)</sup> Department of Chemical, Physical, Mathematical, and Natural Sciences. University of Sassari, Italy

<sup>2)</sup> Department of Animal Science, AGRIS Sardegna, Italy

<sup>3)</sup> Department of Biotechnology, University of Verona, Italy

<sup>4)</sup> Department of Chemical Engineering and Analytical Chemistry, University of Barcelona Research Institute in Food Nutrition and Food Safety, University of Barcelona Serra, Spain

<sup>5)</sup> Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. Research Institute in Food Nutrition and Food Safety, University of Barcelona. Serra Húnter Fellow, Department de, Spain

\*Corresponding author - E-mail: amara@uniss.it

The present study aims to illustrate the potential of near-infrared spectroscopy (NIR) by portable instrumentation, combined with machine learning techniques and chemometrics, for the global authentication of honey origins and the detection of adulterants.

Beekeeping is currently facing significant challenges due to climate change, agrochemical overuse, spread of parasites, industrialization, and fraudulent practices. Recent coordinated actions led by the European Commission (From the Hives, 2021-2022) underscore the impact of honey frauds, emphasizing the necessity for fast and easy-to-use analytical techniques able to ensure the authenticity of the high numbers of products on the market. Authenticity involves adherence to declared information, regulatory standards, and safety protocols. European legislation mandates labels to specify honey origin and prohibits adulterants, reflecting consumer demand for accurate floral and territorial attribution.

Reliable analytical methods are crucial for detecting adulterants and tracing their origins, particularly in imports and internal markets susceptible to fraud. In this context, portable NIR spectroscopy offers on-site analysis capabilities, circumventing costly delays associated with laboratory testing.

This research focused on simultaneously predicting the origin of honey and detecting adulterants using a portable NIR by analyzing multifloral and unifloral honey samples from Spain and Italy. Samples were adulterated with syrups containing glucose, fructose, and maltose. Several approaches were tested for modelling, including simple chemometrics techniques and machine learning for variable selection. Linear discriminant analysis (LDA) was used to discriminate samples based on botanical and geographic origin, while partial least squares (PLS) regression was used for adulterant quantification. Different spectral pre-treatments were evaluated while genetic algorithms (GA) were used for variable selection.

The study achieved prediction accuracies of 90% and 95% for botanical and geographical origins, respectively. LDA models discriminated pure from adulterated honey samples with over 92% accuracy. PLS models demonstrated high accuracy, with performance influenced by botanical origin and the composition of adulterants.

The findings highlight the potential of NIR spectroscopy combined with chemometrics for global honey authentication, predicting origins and detecting adulteration.

**Keywords:** honey, near-infrared spectroscopy, machine learning, adulteration, traceability

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## LECTURES

### L82

## ELEMENTAL PROFILE OF HONEY: INSIGHTS RELATED TO THE ENVIRONMENTAL INFLUENCES AND THE DEVELOPMENT OF AUTHENTICATION MODELS

**Maria David**\*<sup>(1)</sup>, **Ariana Raluca Hategan**<sup>(1)</sup>, **Adriana Dehelean**<sup>(2)</sup>, **Romulus Puscas**<sup>(2)</sup>, **Gabriela Cristea**<sup>(2)</sup>, **Dana Alina Magdas**<sup>(1)</sup>

<sup>1)</sup> (1) Faculty of Physics, Babeş-Bolyai University; (2) National Institute for Research and Development of Isotopic and Molecular Technologies, Romania

<sup>2)</sup> National Institute for Research and Development of Isotopic and Molecular Technologies, Romania

\*Corresponding author - E-mail: maria.david@ubbcluj.ro

One of the oldest natural sweeteners, honey is a highly desired commodity worldwide. As a result, significant amounts of honey having various botanical and geographical origins are produced. The main chemical compounds of honey are sugars and water, having also small amounts of amino acids, proteins, vitamins, and minerals, all of which contribute to the nutritional value. In this context, the elemental content of honey is an important attribute, as it is enriched in macro-minerals (i.e. K, Mg, P) and essential trace elements (i.e. Fe, Se, Zn). Apart from these valuable elements, other potentially toxic metals need to be monitored (i.e. As, Cd, Pb), as well as emerging contaminants such as the Rare Earth Elements group (REEs). The latter unsafe elements can be present in honey and are linked to its geographical origin. It was determined that the soil elemental composition is reflected in the content of honey, as the plant uptakes these elements through the root system, which concentration ratios remain consistent in plants/flowers, and even after bees' digestion and honey production. As the REEs distribution in soil is highly influenced by hydrothermal processes, depth, or environmental pollution (i.e. mining; waste from car/device production), more research should be conducted regarding the REEs link to soil and foodstuff distribution. This work aims to further our understanding of those factors related to the REEs content in honey. In this regard, the possible connections among several essential and trace elements were screened, as well as interdependencies that influence the REEs content in honey were analyzed. For this purpose, 212 honey samples having more than 25 botanical origins and collected from six countries, were measured to determine their isotopic and elemental fingerprint. Pearson correlation was employed to assess these interdependencies and results with a high degree of generalizability were obtained because of the samples' diverse distribution. The present study also examines variations in REEs, isotopic and elemental content of honey based on geographical areas where plants grow (horizontal distribution), by comparing the profile of the samples from Romania with samples produced in other EU countries. Moreover, it was assessed whether the analyzed profile of the samples is influenced by the root depth of the plant used for honey production, by determining the discriminators among samples derived from deep-rooted tree sources (i.e. acacia, linden, chestnut, fir) and from shallow-rooted *Herbaceous* plants (i.e. colza, sunflower, raspberry, black grass). The obtained markers for the horizontal comparison were subsequently used as input for developing accurate differentiation models in terms of geographical origin by the employment of Partial Least Squares Discriminant Analysis. The same methodology was applied to the discriminators responsible for the depth influence, in order to obtain botanical authentication models.

**Keywords:** honey analysis, isotopic and elemental fingerprint, rare earth elements, elements correlation, inorganic contaminants

**Acknowledgement:** This work was supported by the MCID through the "Nucleu" Program within the National Plan for Research, Development, and Innovation 2022-2027, contract no. 27N/2023, PN 23 24 03 01.



## LECTURES

### L83

## SOYA ELEMENTOMIC FINGERPRINTING AND PREDICTIVE MODELING: PAVING THE WAY TOWARDS ADDRESSING DEFORESTATION CHALLENGES

**Maria del Mar Aparicio-Muriana**<sup>(1)</sup>, **Yunhe Hong**<sup>(1)</sup>, **Brian Quinn**<sup>(1)</sup>, **Nicholas Birse**<sup>(1)</sup>, **Chris Elliott**<sup>(1)</sup>

<sup>1)</sup> *Queen's University Belfast, United Kingdom*

*\*Corresponding author - E-mail: m.apariciomuriana@qub.ac.uk*

The escalating level of rainforest deforestation, primarily driven by the production of Forest-Risk Commodities (FRC) such as non-dairy cattle products (beef and leather), cocoa, palm, and soya, presents significant global challenges—such as loss of biodiversity, climate change, and impacts on global carbon storage. This pressing issue not only jeopardizes rainforests—often referred to as the “lungs of the earth”—but also underscores the urgent need to address deforestation driven by FRC production. The urgency of this issue is further highlighted by emerging regulations such as the EU Deforestation Regulation (EUDR) of 2023, which aims to curb deforestation associated with these commodities.

To address these challenges and enhance transparency in the soya supply chain we introduce an innovative approach to traceability and responsible sourcing within the soya industry. Our work emphasizes the development and validation of a cutting-edge analytical method capable of creating a ‘chemical map’ for the precise geographical identification of soya origin. Utilizing ICP-MS, we conducted the elemental profiling of over 300 soybean samples from the world’s top seven soya-producing countries, representing 95% of global soya production. Through the application of chemometric modelling, we developed a predictive model capable of discerning the source of soya with an accuracy rate higher than 95% and identifying the elemental markers that play a key role in the elementomic origin classification model of soya.

Our research determined that for a more accurate approach, seeds (soybeans) and soya meal should be analysed separately due to intrinsic differences in their elemental composition, as shown in models comparing both groups. Additionally, we explored the impact of deforestation on soya composition. As a preliminary study, we collected a small number of soya meal samples from impacted areas and compared their elemental analysis with samples from non-impacted areas. The results are promising; the two types of soya meal were separated according to their elemental composition, suggesting the potential for using elementomics and chemometric analysis to identify samples from deforested areas.

Our next major steps are to attain accreditation for our method, which will not only signify the robustness and reliability of our analytical approach but also establish a benchmark for quality in the industry, and to develop a cloud-based system utilizing our database of samples and chemometric models.

This initiative aligns with global efforts to counteract deforestation associated with soya production. By unravelling the complexities of soya production, comprehending its impact on deforestation, and exploring sustainable alternatives, we actively contribute to a unified effort that holds the promise of a more sustainable future for our planet.

**Keywords:** food authenticity, elemental profiling, chemometric modelling, deforestation, ICP-MS

**Acknowledgement:** *This work was funded by Agilent Thought Leader Award.*

## LECTURES

### L84

## SPECIES IDENTIFICATION OF SEAFOOD IN COMPLEX FOOD MATRICES USING NEXT GENERATION SEQUENCING TECHNOLOGY

**Julia Andronache**<sup>\*(1)</sup>, Margit Cichna-Markl<sup>(2)</sup>, Stefanie Dobrovolny<sup>(1)</sup>, Rupert Hochegger<sup>(1)</sup>

<sup>1)</sup> Austrian Agency for Health and Food Safety (AGES), Institute for Food Safety Vienna, Department for Molecular Biology and Microbiology, Austria

<sup>2)</sup> Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria

\*Corresponding author - E-mail: julia.andronache@ages.at

Food fraud is a concern for consumers in general. Seafood in particular has been identified as the food commodity with the second highest potential for fraud by the European Parliament [Pardo, 2018, Food Control]. This is promoted by significant price ranges between species of similar morphology and by complex distribution systems. As EU Regulation No. 1379/2013 requires the correct labelling of seafood, there is a need for analytical methods that can ensure food authenticity. To fulfil these legal requirements, DNA based methods such as PCR and DNA barcoding are often used for species identification in seafood. DNA barcoding combined with next generation sequencing technologies is called DNA metabarcoding. This technology allows the investigation of species mixtures and efficient sample screening for routine analysis. Therefore, we developed a DNA metabarcoding method to identify and differentiate seafood in food products.

The method targets a mitochondrial 16S rDNA fragment of 150 bp to 210 bp, depending on the primer pair. DNA was extracted from samples by a modified cetyltrimethylammonium bromide-protocol [Dobrovolny et al., 2019, Food Chemistry]. Reference sequences were downloaded from the NCBI database and aligned with the CLC software for primer design. The universal primers with the most promising *in silico* characteristics were tested with varied PCR conditions. In this study, 69 primers were tested in 101 combinations. After the final PCR conditions were established, DNA amplicons from reference material (202 samples) and processed foods (112 samples) were analyzed on Illumina iSeq 100<sup>®</sup> and MiSeq<sup>®</sup> sequencing platforms. Data evaluation was performed on the published and adapted analysis pipeline in Galaxy [Dobrovolny et al., 2022, Foods]. The creation of customized databases in combination with the Galaxy pipeline facilitated automatized data evaluation (>600 reference sequences).

Five primer systems were designed for seafood: 1) crustaceans (*Malacostraca*), 2) squids (*Coleoidea*), 3) oysters and scallops (*Ostreidae*, *Pectinidae*), 4) mussels, razor clams, sheath clams, venerids, cockles (*Mytilidae*, *Pharidae*, *Solenidae*, *Veneridae*, *Cardiidae*) and 5) sea and land snails (*Gastropoda*). We successfully identified the reference material and detected seafood species in complex and highly processed food matrices such as squid ink linguine, lobster butter and spice mixes for instant noodles. The amount of falsely declared samples ranged from 7 % to 34 %, depending on the seafood group. The systems will be validated with the aim of applying the method in official food laboratories.

Our results show that DNA metabarcoding is suited for the detection and identification of seafood in highly processed food products. Additionally, they emphasize the fact that routine monitoring of seafood is necessary to ensure food authenticity.

**Keywords:** DNA metabarcoding, food authentication, next generation sequencing, seafood

**Acknowledgement:** This research was funded by the Austrian Agency for Health and Food Safety (AGES), Institute for Food Safety Vienna, Department for Molecular Biology and Microbiology. The project is a cooperation with the University of Vienna.

### L85

## AUTHENTICATION OF RAW NUT PRODUCTS: IDENTIFICATION OF UNEXPECTED ADULTERATIONS BY TRULY UNTARGETED PEPTIDE PROFILE ANALYSIS VIA MICROLIC-IM-QTOF-MS/MS AND DE NOVO SEQUENCING

**Sammy Venegas<sup>(1)</sup>, Andreas Mauser<sup>(1)</sup>, Sabrina Gensberger-Reigl<sup>(2)</sup>, Monika Pischetsrieder<sup>\*(2)</sup>**

<sup>1)</sup> Chair of Food Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany

<sup>2)</sup> A: Chair of Food Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg B: FAU Research Center New Bioactive Compounds - FAU NeW, Germany

\*Corresponding author - E-mail: sammy.venegas@fau.de

Nuts and nut products are highly priced raw materials used in various foods produced in Germany, such as „Elisenlebkuchen“, a traditional gingerbread defined by its high oil seed content. Hence, they are an essential commodity for domestic food manufacturers. Prices vary depending on the season, climate and crop yield of a given year. Import of e.g. walnuts to Germany usually happens through a complex, international supply network, since commercial cultivation of walnut takes place mostly overseas. Therefore, nuts and especially nut products are vulnerable to adulteration with cheaper alternatives, possibly to meet global market demands or to raise profits along the supply network.

Currently, mostly targeted approaches are established to elucidate adulteration in this scenario. Targeted methods can provide highly sensitive detection of adulterations, if the origin of the blended material is known, while the presence of unexpected material remains undetected. This also applies to untargeted proteome analysis, because peptides are generally identified by database-dependent sequencing. In this study, a method is presented in which nut samples are measured by truly untargeted peptide profile analysis via microLC-ESI-IM-QTOF-MS/MS (data-dependent acquisition mode) after in-solution endoprotease Glu-C digestion. Feature detection and database-independent *de novo* peptide sequencing was performed using PEAKS® online. By omitting protein database alignment, the resulting peptide profiles are suitable for a non-target readout from the generated raw data detecting also unexpected adulterations.

Comparison of ground walnuts spiked with 1 - 50 % of various adulterants with 27 authentic reference samples from three harvest years and seven origin countries allowed for qualitative assessment of authenticity (one-class classification) with up to 96 % sensitivity with 98 % specificity. This was challenged by analyzing 10 blinded test samples of unknown composition, which were all correctly classified by this approach. The protocol was also applied to real samples supplied by a cooperation partner from a German food manufacturer.

Discriminating peptide sequences from the untargeted data set were then subjected to downstream analysis using a reviewed protein sequence database (Uniprot-swissprot). Thus, the qualitative composition of the adulterant in the test samples could be reliably identified. The identification of marker peptides could be utilized to develop targeted quantification methods in a cascade approach, which has been demonstrated by exemplary LC-QqQ-MS measurements.

In summary, the presented method enables the untargeted authentication and characterization of adulteration of walnut raw materials by unknown matrices.

**Keywords:** food fraud, walnut, untargeted shotgun proteomics, database-independent, *de novo* peptide sequencing

**Acknowledgement:** The presented work is part of the Bavarian research association SHIELD - “Safe Domestic (Organic) Food through Sensory Detection Methods”. This project is funded by the Bavarian Research Foundation and the Rudolf und Henriette Schmidt-Burkhardt Stiftung.

## LECTURES

### L86

## SPECIES AND CULTIVAR DIFFERENTIATION BY DNA BARCODING COUPLED TO HIGH RESOLUTION MELTING ANALYSIS TO DETECT FOOD ADULTERATION

Jakob Scheibelreiter<sup>(1)</sup>, Michaela Wildbacher<sup>(1)</sup>, Isabel Niederkofler<sup>(1)</sup>, Sofie Schandl<sup>(1)</sup>, Klementina Yakova<sup>(1)</sup>, Katha Pühringer<sup>(1)</sup>, Julia Andronache<sup>(2)</sup>, Stefanie Dobrovolny<sup>(2)</sup>, Rupert Hochegger<sup>(2)</sup>, Thomas Rühmer<sup>(3)</sup>, Barbara Siegmund<sup>(4)</sup>, Margit Cichna-Markl\*<sup>(1)</sup>

<sup>1)</sup> Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria

<sup>2)</sup> Austrian Agency for Health and Food Safety (AGES), Institute for Food Safety Vienna, Department for Molecular Biology and Microbiology, Austria

<sup>3)</sup> Research Centre for Fruit Growing & Viticulture Haidegg, Austria

<sup>4)</sup> Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Austria

\*Corresponding author - E-mail: [margit.cichna@univie.ac.at](mailto:margit.cichna@univie.ac.at)

Ensuring the safety and authenticity of commercial foodstuffs is essential for complying with national and international regulations, thereby protecting human health and consumer interests. However, economically motivated food adulteration is a global issue, often involving the substitution of higher-value species or cultivars with cheaper alternatives.

Differentiation between species or cultivars is frequently based on real-time polymerase chain reaction (PCR) and sequencing technologies. DNA barcoding coupled to high resolution melting (HRM) analysis plays an increasing role in routine analysis. DNA barcodes are specific regions of DNA consisting of two conserved regions, serving as primer binding sites, and a variable DNA sequence in between, allowing species/cultivar differentiation. HRM is a cost-effective post-PCR technique that exploits differences in the melting behavior of PCR products. A prerequisite for HRM is that the DNA region of interest, the DNA barcode, is amplified in the presence of a saturating intercalating dye, e.g. EvaGreen. At the end of the last PCR cycle, the PCR products are slowly melted by increasing the temperature in small increments. The intercalating dye is released and the fluorescence intensity decreases. The melting behavior of the PCR products depends on various parameters, including their length and the ratio of guanine and cytosine to adenine and thymine. Species and cultivar differentiation in food by DNA barcoding coupled to HRM analysis is frequently based on single nucleotide polymorphisms (SNPs) or microsatellites.

The high potential of DNA barcoding coupled to HRM analysis will be demonstrated on several examples, including the differentiation between edible insect species approved in the EU; the differentiation between chickpea varieties; and the differentiation between old apple and pear varieties from Styria. The lecture will address development, optimization, and validation of DNA barcoding - HRM assays. Important characteristics such as selectivity, limit of detection, limit of quantification, repeatability and reproducibility will be critically discussed. In addition, the impact of the DNA extraction protocol, PCR-HRM mix, thermocycler, and food processing will be addressed.

**Keywords:** DNA barcoding, high resolution melting analysis, food adulteration, species, cultivar

## LECTURES

### L87

#### **AN HOLISTIC SAFETY ASSESSMENT OF PLANT-BASED PROTEIN SOURCES: WHERE WE ARE AND WHAT THE MYCOBEANS ALLIANCE CAN DO**

**Chiara Dall'Asta**<sup>(1)</sup>, Luisa Calcinai<sup>(1)</sup>, Sara Cutroneo<sup>(1)</sup>, Octavian Mihalache<sup>(1)</sup>, Raquel Torrijos<sup>(2)</sup>, Tullia Tedeschi<sup>(1)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Italy

<sup>2)</sup> Department of Food and Drug, University of Parma, Italy; Laboratory of Food Chemistry and Toxicology, University of Valencia, Spain, Italy

\*Corresponding author - E-mail: chiara.dallasta@unipr.it

Human and environmental health are strictly interconnected, representing the pillars of diet sustainability. In accordance with international guidelines, substantial dietary shifts are needed to move toward sustainable diets. To lend both health and environmental benefits, the consumption of plant-based minimally processed food should be doubled in the coming years, replacing part of the animal-source food. In this framework, the ASEAN region plays a key role in providing raw materials of vegetable origin.

Besides the well-known benefits related to the nutritional properties and the high content of bioactive compounds, protein sources from vegetable origin may come with inherent risks, such as natural toxins, anti-nutritional factors and a still underexplored allergenic potential. Conversely, biotechnologies such as fermentation may offer opportunities for improving the safety profile of novel ingredients and their overall quality.

This talk will give an overview of the safety assessment workflow applied to plant ingredients intended for the preparation of plant-based food alternatives, also providing some examples of the most advanced approaches currently in use. It will also present some preliminary results on the occurrence of natural contaminants obtained within the EU-ASEAN MYCOBEANS alliance (HE MSCA Staff Exchange project).

**Keywords:** alternative proteins, plant-based food, allergens, natural toxins, risk assessment

**Acknowledgement:** This project/study/work has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101131125 - Mycobeans.

## LECTURES

### L88

#### MYCOSMART: AN ON-SITE MYCOTOXINS DETECTION SYSTEM FROM THE SYNERGY OF EU-SE ASIA COLLABORATION

**Nitsara Karoonuthaisiri**\*<sup>(1)</sup>

<sup>1)</sup> *National Science and Technology Development Agency, National Center for Genetic Engineering and Biotechnology, Thailand*

*\*Corresponding author - E-mail: nitsara.kar@biotec.or.th*

Contamination from multiple mycotoxins is a growing food safety concern worldwide due to their deleterious effects in humans and animals. The incident and impact of the issues from co-occurrence of mycotoxins are getting more serious than ever due to the climate change. It is imperative to develop a rapid, accurate and inexpensive system to detect multiple mycotoxins enabling prompt monitoring of the toxins and preventing them from entering the food chain. This issue needs a collaborative effort to address. Our consortium from the EU and SE Asia has developed and validated a multiplex microarray-based lateral flow immunoassay with a portable reader for on-site simultaneous determination of five mycotoxins – aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), T-2 toxin (T2), zearalenone (ZEA), deoxynivalenol (DON), and fumonisin B<sub>1</sub> (FB<sub>1</sub>) in rice. This technology detects multiple mycotoxins via a competitive approach. The microarray spot signals generated through a novel luminescent organic dye are captured and processed to estimate mycotoxin levels using a portable microarray reader installed with a user-friendly interface. In addition, the sample extraction solvent consists of green components (ethanol and polyethylene glycol), and the extraction procedure is very simple and ultrafast. The assay can accurately detect a wide range of multiple mycotoxins concentration in rice samples with recovery values ranging from 75 to 127%, relatively similar to LC-MS/MS method. The limits of detection obtained for AFB<sub>1</sub>, T2, ZEA, DON, and FB<sub>1</sub> were 1.80, 1.21, 1.39, 1.17, and 0.56 µg/kg, respectively. Overall, the newly developed and validated analytical system can be used as a point-of-care device for the detection and quantification of multiple mycotoxins in rice and rice-based products.

**Keywords:** multiplex microarray-based lateral flow immunoassay, on-site detection, multiple mycotoxins, rice and rice-based products, EU-Asia collaboration

## LECTURES

### L89

#### RAPID NATURAL TOXIN TESTING - EU AND SE ASIA PERSPECTIVES

**Nicholas Birse**\*<sup>(1)</sup>

<sup>1)</sup> *Queen's University Belfast, United Kingdom*

\**Corresponding author - E-mail: n.birse@qub.ac.uk*

Natural toxins are potentially hazardous naturally occurring compounds produced by plants, animals, fungi and bacteria. They have a variety of roles, some act as natural defence compounds, protecting crops from consumption, whilst others are involved in symbiotic or parasitic relationships needed to maintain soil health and fix nutrients.

These compounds, despite their natural occurrences, can be harmful to health in humans and in animals, with many now known to have carcinogenic properties, with risks to health increasing through longer term, lower-level chronic exposure. Higher levels of exposure also present significant risks, with some classes of toxins being highly potent and capable of causing incapacitation or death at low levels of exposure.

Climate is highly significant in the occurrence of many classes of natural toxins, with the result that climate change is changing the locations where different classes of natural toxins may be found, the levels at which they are found, and also impacting the efficacy of existing crop treatments.

This talk will look at ways we can learn through an extensive collaboration between European and South East Asian countries about the impending challenges likely to face both Europe and South East Asia, learning from each other's experiences of existing and ongoing climate change, and the impact it is having on food production and food safety.

The talk will then progress to looking at how we set about developing new, faster, cheaper testing technologies that can more quickly and more accurately detect when a natural toxin is present, and if the levels found present a risk for human or animal consumption. Cost and ease of use, particularly in developing nations is a key consideration, to make access to better testing technologies more equitable across the globe.

**Keywords:** toxins, mycotoxins, climate change, food safety, rapid testing

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## LECTURES

### L90

## EVALUATING THE RISKS ASSOCIATED TO CHRONIC EXPOSURE TO CHEMICAL CONTAMINANTS IN FOOD

**Rudolf Krska**<sup>(1)</sup>, **Chris Elliott**<sup>(2)</sup>

<sup>1)</sup> (1) BOKU University, Vienna, Department IFA-Tulln, Austria; (2) Queen's University Belfast, Institute for Global Food Security, Belfast, UK; (3) Austrian Competence Centre for Feed and Food, Quality, Safety & Innovation (FFoQSI), Austria, Austria

<sup>2)</sup> Queen's University Belfast, Institute for Global Food Security, United Kingdom

\*Corresponding author - E-mail: rudolf.krska@boku.ac.at

In the recently published book "Toxin-Free Food?" over 100 risk assessments were used to determine how safe food really is in Europe [1]. With special emphasis on mycotoxins, potentially harmful chemicals in food were assessed in detail to which average European consumers are chronically exposed. The identified risks were finally ranked according to their relevance. On the top of this ranking, contaminants from food processing, followed by aromatic petroleum hydrocarbons can be found [2]. This is due to their potential genotoxicity and carcinogenicity in humans and due to their wide distribution in many foods consumed daily. Interestingly, of the multitude of biotoxins being present in our food, only dietary exposure to aflatoxins produced by fungi and pyrrolizidine alkaloids produced by plants pose a potential chronic risk in Europe. Such a ranking would also be of interest to ASEAN countries. In fact, aflatoxins are in the third place. This high ranking is due to the before-mentioned high carcinogenic potency causing liver cancer in humans coupled with the high consumption of cereal-based foods in Europe. Nuts and maize, which are far more commonly contaminated with aflatoxins, are consumed to a much lesser extent in Europe. Dioxins, dioxin-like polychlorinated biphenyls, nickel and a brominated flame retardant can be ranked fourth due to their exposure through the food consumed daily. Despite their carcinogenic effects, pyrrolizidine alkaloids are "only" ranked fifth because we are exposed to these plant toxins through very specific sources, such as tea, honey or herbs. Of least concern in this risk ranking are perfluorooctane sulfonic acid, perfluorooctanoic acid and heavy metals. These substances are present in many foods, but only pose a potential risk to some European adults.

What is still unclear is the significance of chronic exposure to "cocktails" of co-occurring contaminants that we ingest daily through food. The combined risk from exposure to mixtures of toxic substances may be greater than the risks currently assessed for individual chemicals.

[1] M. Eskola, C.T. Elliott, J. Hajšlová, D. Steiner, R. Krska (2020) Towards a dietary-exposome assessment of chemicals in food: An update on the chronic health risks for the European consumer, *Critical Reviews in Food Science and Nutrition*, 60:11, 1890-1911, DOI: 10.1080/10408398.2019.1612320.

[2] R. Krska, M. Eskola, C. Elliott. *Toxin-Free Food?* (2023) Picus Verlag. ISBN 978-3-7117-3028-2.

**Keywords:** emerging contaminants, mycotoxins, food safety, climate change



## LECTURES

### L91

## **SIMULTANEOUS DETECTION OF 29 ANTIMICROBIAL RESIDUES: A SUCCESSFUL STORY OF THAI-UK COLLABORATION**

**Awanwee Petchkongkaew**\*<sup>(1)</sup>

<sup>1)</sup> *International Joint Research Center on Food Security (IJC-FOODSEC), Thailand*

*\*Corresponding author - E-mail: [awanwee@tu.ac.th](mailto:awanwee@tu.ac.th)*

Antimicrobial residues occur in many foods of animal origin due to mistakes or the deliberate misuse of antibiotics. The most serious consequence of this is the risk of spreading antimicrobial resistance through animal and human populations.

To help monitor the presence of such food contaminants in the ASEAN region a collaboration between the Institute for Global Food Security at Queen's University, Belfast and the International Joint Research Center on Food Security (IJC-FOODSEC) was established to technology transfer an LC-MSMS workflow that can simultaneously detect 29 different antimicrobial agents from four different classes of important antibiotics.

This project has proved to be highly successful and as a result research on the presence of Antimicrobials in shrimp feed has been launched. The results of this study will be presented at RAFA and future workplans will be announced.

**Keywords:** antimicrobial residues, antimicrobial resistance, LC-MSMS, Thai-UK collaboration

## LECTURES

### L92

## PROMOTING MULTI-OMICS APPROACHES FOR BLACK TEA GEOGRAPHICAL INDICATIONS TRACING WITH AI AND MACHINE LEARNING

Yicong Li<sup>(1)</sup>, Simon Haughey<sup>(1)</sup>, Nicholas Birse<sup>(1)</sup>, Natasha Logan<sup>(1)</sup>, Chris Elliott<sup>(1)</sup>, Di Wu<sup>\*(1)</sup>

<sup>1)</sup> Queen's University Belfast, United Kingdom

\*Corresponding author - E-mail: d.wu@qub.ac.uk

Tea, being the most beloved drink around the world, has grown into a global market of 122.2 billion USD and is expected to rise to 160 billion USD by 2028. Originating in Asia, tea is now being cultivated in many emerging areas, e.g. Africa and raising sustainability concerns linked to the environment and worker welfare. Black tea, especially those Geographical Indication (GI) products of higher prices, is widely traded across the continents through international supply chains. However, due to insufficient testing technologies and an established regulation system, they are often exposed to fraud challenges. Through more than 700 black tea samples collected from 9 origins, we have built a large database of none-targeted multidisciplinary fingerprinting data from our 2-tiered testing system of spectroscopy (NIR, FTIR, XRF) and spectrometry (LC-MS, ICP-MS) of IGFS. We have then developed a 'smart' innovative analytical system, by implementing state-of-the-art artificial intelligence and machine learning algorithms (LDA, RF, KNN, SVM etc.) with our powerful tools for the discrimination of geographical origin of black teas. Results have revealed the huge potential of machine learning to promote the discrimination functions and accuracy of the models, especially compared to traditional chemometrics analysis, at different levels and approaches. This has also enabled further possibilities for data fusion of deep learning and industrial applications in the future.

**Keywords:** black tea, geographical indications, AI and machine learning, spectroscopy, mass spectrometry

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## LECTURES

### L93

## NON-TARGETED DETECTION OF FOOD ADULTERATION USING AN ENSEMBLE MACHINE-LEARNING MODEL

Terence Lok Ting Lau<sup>\*(1)</sup>, Teresa Chung<sup>(1)</sup>, Yan Yan Nelly Lam<sup>(1)</sup>

<sup>1)</sup> Hong Kong Baptist University, Hong Kong

\*Corresponding author - E-mail: [terencelau@hkbu.edu.hk](mailto:terencelau@hkbu.edu.hk)

Recurrent incidents of economically motivated adulteration have long-lasting and devastating effects on public health, economy, and society. With the current food authentication methods being target-oriented, the lack of an effective methodology to detect unencountered adulterants can lead to the next melamine-like outbreak. In this study, an ensemble machine-learning model that can help detect unprecedented adulteration without looking for specific substances, that is, in a non-targeted approach, is proposed. Using raw milk as an example, the proposed model achieved an accuracy and F1 score of 0.9924 and 0.9913, respectively, when the same type of adulterants was presented in the training data. Cross-validation with spiked contaminants not routinely tested in the food industry and blinded from the training data provided an F1 score of 0.8657. This is the first study that demonstrates the feasibility of non-targeted detection with no a priori knowledge of the presence of certain adulterants using data from standard industrial testing as input. By uncovering discriminative profiling patterns, the ensemble machine-learning model can monitor and flag suspicious samples; this technique can potentially be extended to other food commodities and thus become an important contributor to public food safety.

**Keywords:** food safety, adulteration, non-target detection, machine learning, big data

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## LECTURES

### L94

## BAD APPLES OR BAD BARRELS - INTEGRITY IN EUROPEAN AND ASIAN FOOD BUSINESSES

**Saskia Van Ruth**\*<sup>(1)</sup>

<sup>1)</sup> *University College Dublin, Ireland*

\**Corresponding author - E-mail: saskia.vanruth@ucd.ie*

In view of food fraud prevention and for strategic deployment of food authentication tests, it is crucial to understand the anatomy of food fraud. The root of the problem is a human adversary, not a microbe or a pollutant as is the case with many food quality and most safety issues. This intentional aspect is a crucial difference: It does not involve accidents but a conscious decision to cross ethical boundaries. White collar criminology sees the involvement of individuals in this type of offending traditionally as a result from exposure of individuals to criminogenic conditions in an organisation. It is considered that individuals adapt to values in companies or industries that may be useful to conducting successful business transactions but which may also promote rule violation and crime. From this perspective one would expect a 'barrel of bad apples'. On the other hand, there is also a current of scientists that places emphasis on the role of the individual with some people being more attracted and selected into criminogenic organisations. Furthermore, this current considers that all persons do not react in a similar way to criminogenic pressures, temptations and opportunities. In our study we examined levels of personal integrity of employees in the European food industry and compared those with Chinese businesses. Additionally, the ethical business culture in a European food industry sector was mapped using a validated tool to measure seven dimensions of this culture (the CEV-S) and scores were compared with those of other sectors. The study showed that, independent of geographics, there are always employees with higher and lower ethical standards, Good and bad apples. It revealed also that the food industry shows similar ethical climate characteristics on average as a number of other sectors. More importantly, it showed that variation within sectors surpasses the variation between sectors. Hence, a healthy or poor ethical climate depends more on the organisation than on the industry sector. On the other hand, the additionally disclosed relationship between level of personal integrity and ethical business climate indicates that 'the bad barrel' is certainly an aspect to be considered in food fraud prevention strategies.

**Keywords:** food fraud, ethical business culture, food crime, fraud comprehension

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## LECTURES

### L95

#### DEVELOPMENT OF PORTABLE DETECTION TECHNOLOGY FOR FOOD AUTHENTICITY

**Guoliang Li**\*<sup>(1)</sup>, Jianghua Liu<sup>(1)</sup>, Zhuoqun Su<sup>(1)</sup>, Yiheng Shi<sup>(1)</sup>

<sup>1)</sup> School of Food Science and Engineering, Shaanxi University of Science and Technology, China

\*Corresponding author - E-mail: 61254368@163.com

Economically motivated meat adulteration has become the focus and difficulty of food authenticity supervision. Currently, the most effective detection technology is DNA analysis. However, the existing DNA detection methods mainly rely on sophisticated instruments and strict operating environments for nucleic acid amplification, and on-site rapid detection technologies are scarce. The bottleneck is how to identify and convert trace adulterated DNA in complex matrices into portable and readable signals. Inspired by the target specific recognition and efficient trans-cleavage activity of CRISPR/Cas12a system, we first introduced it into meat authenticity detection. Through innovative design and signal encoding of non-target nucleic acid substrates, the trace adulterated DNA signals were cleverly transformed into highly sensitive fluorescence, Raman signals, or portable colorimetric signals, achieving effective detection of low adulteration rates in complicated meat matrices. The developed technologies not only meet the demands of portable meat authenticity detection, but also provide novel approaches for on-site rapid detection of other biomarkers.

**Keywords:** meat authenticity, CRISPR/Cas12a system, DNA, on-site detection

### L96

## NATURAL DEEP EUTECTIC SOLVENTS FOR GREEN SAMPLE PREPARATION: FROM BIOACTIVES TO CONTAMINANTS EXTRACTION

Laura Carbonell-Rozas<sup>(1)</sup>, Laura Righetti<sup>(2)</sup>, Roberto Romero-Gonzalez<sup>(3)</sup>, Ivan Aloisi<sup>(4)</sup>, Hans Mol<sup>(4)</sup>, Antonia Garrido Frenich<sup>(3)</sup>

<sup>1)</sup> Department of Chemistry and Physics, University of Almeria, E-04120 Almeria, Spain, & Wageningen Food Safety Research, Wageningen University & Research, Wageningen, 6708, WB, The Netherlands., Spain

<sup>2)</sup> Wageningen Food Safety Research, Wageningen University & Research, Wageningen, 6708, WB, The Netherlands, The Netherlands

<sup>3)</sup> Department of Chemistry and Physics, University of Almeria, E-04120 Almeria, Spain., Spain

<sup>4)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: lauracr@ual.es

Natural Deep Eutectic Solvents, so-called NA(DES), are emerging green solvents increasingly used for sample preparation in recent years in order to replace the toxic organic solvents commonly used. These solvents are formed by mixing natural compounds, such as sugars, amino acids, and organic acids, in specific molar ratios, resulting in an eutectic mixture with a melting point much lower than that of the individual components. NADES present several advantages such as biocompatibility, biodegradation, low costs, tunability, stability and high extraction efficiency. Therefore, they have promising applications in food analysis and industrial processes. In the last years, the evaluation of NADES for the extraction of bioactive compounds has increased considerably; however, its application for food safety purposes is still scarce.

In this work we demonstrate the applicability of NADES as green sample preparations from two different perspectives: for the extraction of both bioactive compounds as well as contaminants prior their determination by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). First, we assessed the use of NADES in a miniaturized ultrasonic-assisted extraction (micro-UAE) of phenolic compounds from tea samples (*Camellia sinensis*). For this purpose, 11 different hydrophilic NADES were prepared and evaluated as potential extraction solvents. The experimental conditions affecting the micro-UAE were optimized to maximize extraction efficiency and to minimize number of experiments by performing a design of experiments. The NADES LGLH, composed of lactic acid:glycerol:water in a molar ratio of 1:1:3, provided the best results for the simultaneous extraction of the 17 target compounds, including catechin derivatives and other minor polyphenols. Then, several hydrophilic and hydrophobic NADES were tailored and tested for multiclass pesticides extraction. 11 model pesticides, including fungicides, herbicides and insecticides, were extracted from different food samples belonging to various commodity groups such as spinach, orange and wheat. The hydrophobic NADES composed of thymol: menthol (1:1) provided the highest recoveries for all target pesticides as well as lower matrix effect, and suitable compatibility with LC. Subsequently, this NADES was tested to extract 100 related pesticides in the target matrices.

All the evaluated NADES were characterized by Nuclear Magnetic Resonance (NMR) to confirm the formation of the supramolecular structure of the eutectic mixture. Furthermore, their physico-chemical properties such as viscosity, polarity, pH and density were measured for a better understanding of their applications. Different analytical metrics such as to AGREEprep and BAGI were used to test the greenness and the practicability of the proposed sample treatments.

Both approaches highlighted the broad spectrum of applicability of NADES in developing green sample treatments for food analysis.

**Keywords:** bioactive compounds, pesticides, NADES, green sample preparation

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### L97

## MULTIDETERMINATION OF MYCOTOXINS IN SOLID AND LIQUID FOODSTUFFS USING SUPRAMOLECULAR SOLVENTS COMBINED WITH LC-MS/MS

**Luis Muniz-Bustamante**\*<sup>(1)</sup>, **Noelia Caballero-Casero**<sup>(1)</sup>, **Soledad Rubio**<sup>(1)</sup>

<sup>1)</sup> *University of Córdoba, Spain*

\**Corresponding author - E-mail: t52mubul@uco.es*

According to the latest Rapid Alert System for Food and Feed (RASFF) report, mycotoxins represent the third most common alert within the 17 categorized risks, from both EU and non-EU countries. The International Agency for Research on Cancer (IARC) has classified mycotoxins based on their carcinogenic activity in humans (Group I, Group IIA and group IIB). This has led to the establishment of specific legislation regulating the maximum content level of each mycotoxin in different foodstuffs depending on their nutritional composition. Therefore, it is crucial the development of suitable analytical methods for the multidetermination of mycotoxins at legislated concentrations in liquid and solid foods. To date, extraction methods based on QuEChERS, solid-phase extraction, and immunoaffinity column are widely used. However, they do not cover both solid and liquid samples with the same sample treatment. Additionally, they often require a second purification stage, increasing the consumption of organic solvents and the cost of the analyses.

Supramolecular solvents (SUPRAS) are nanostructured liquids generated from colloidal solutions of amphiphilic molecules through self-assembly and coacervation processes. SUPRAS exhibit regions of different polarity within their structure, allowing for the extraction of compounds across a wide range of polarities. They possess mixed interaction mechanisms, which increase the solubilization of analytes. Additionally, they have a high number of available solubilization centres, enabling efficient extraction using low volumes of SUPRAS. Moreover, the structure of SUPRAS can be tailored to favour the extraction of compounds of interest and eliminate interferences from the matrix. This research proposes the use of SUPRAS for the microextraction of 12 mycotoxins regulated by the EU and their subsequent quantification using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The developed method was optimized and validated for five groups of solid and liquid foods, based on performance criteria for each mycotoxin according to Regulation (EU) N° 519/2014. Thanks to the properties of SUPRAS, the method extracted the analytes and eliminated matrix interferences in a single step. The developed method enables the quantification of 12 mycotoxins thanks to the high sensitivity (quantification limits 0.03-21.483 ng/mL), excellent extraction efficiency (recovery 59-120%), and high repeatability and reproducibility (relative standard deviation 3-19% and 0.3-20.1%, respectively). Besides successfully extracting 12 mycotoxins regulated by the EU from both solid and liquid samples, this method aligns with the principles of green chemistry, being simple, fast, cost-effective, and potentially serving as an alternative to current routine mycotoxin analyses in foods.

**Keywords:** mycotoxins, microextraction, supramolecular solvents, LC-MS/MS, solid and liquid foods

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## LECTURES

### L98

#### PROFILING OF PHENOLIC COMPOUNDS IN HERBS AND SPICES BY A SUSTAINABLE EXTRACTION COMBINED WITH A MINIATURIZED LC METHOD

**Francesca Rigano**<sup>\*(1)</sup>, **Katia Arena**<sup>(1)</sup>, **Roberto Lagana Vinci**<sup>(1)</sup>, **Roberta La Tella**<sup>(1)</sup>, **Paola Dugo**<sup>(2)</sup>, **Luigi Mondello**<sup>(2)</sup>

<sup>1)</sup> Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Italy

<sup>2)</sup> Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina. Chromaleont s.r.l., c/o Department o, Italy

\*Corresponding author - E-mail: frigano@unime.it

The present contribution puts together most recent trends in both food and analytical chemistry. Starting from the food pyramid, most recent graphical representations have been integrated with herbs and spices, not only to add flavour to food thus allowing a reduction of salt consumption, but also aiming to add beneficial active principles to the diet. Moreover, the valorisation of local products encourages food scientists to fully characterize typical natural products aiming at finding new source of nutraceuticals, cheaper and readily available in specific territories, respecting biodiversity and promoting food security. In this regard, this research focuses on the elucidation of the phenolic profile of different samples of Sicilian sumac and oregano, collected at different altitudes. Specifically, phenols were isolated from both matrices through a green extraction employing water and ethanol following mild heating under mechanical agitation.

From the analytical point of view, current efforts of analytical scientists are directed to greening analytical methods through the reduction of energy, solvent and sample consumption and minimization of waste. In this regards, liquid chromatography (LC) practitioners are putting their efforts toward the miniaturization of LC methods and/or reduction of total analysis time.

In the present work, a miniaturized LC method was developed on a capillary octadecylsilica (C18), packed with 2µm particles. The method was first used to build a database of retention data for more than 50 phenolic compounds. Then, Quantitative Structure-Retention Relationship (QSRR) models were generated to predict retention times of not available standard compounds, and such predictive models were used for the reliable identification of phenols in real-world samples. The use of the QSRR approach allowed to further enhance the overall greenness and feasibility of the LC method since a simple UV detector was sufficient to identify the analytes based only on retention data, avoiding the use of expensive and sophisticated detectors such as high-resolution or tandem mass spectrometers.

**Keywords:** phenolic compounds, capillary LC, sumac, oregano, green analytical chemistry

**Acknowledgement:** The authors thank Shimadzu and Merck Life Science for their continuous support.



### L99

## STRAIGHTFORWARD WHEAT QUALITY ASSESSMENT BY COMBINING NEAR-INFRARED SPECTROSCOPY AND ANALYSIS OF VARIANCE SIMULTANEOUS COMPONENT ANALYSIS

**Stephan Freitag**\*<sup>(1)</sup>, Maximilian Anlanger<sup>(1)</sup>, Maximilian Lippl<sup>(2)</sup>, Klemens Mechtler<sup>(3)</sup>, Elisabeth Reiter<sup>(2)</sup>, Heinrich Grausgruber<sup>(4)</sup>, Rudolf Krska<sup>(5)</sup>

<sup>1)</sup> BOKU University, Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Austria

<sup>2)</sup> Austrian Agency for Health and Food Safety GmbH, Institute for Animal Nutrition and Feed, Austria

<sup>3)</sup> Austrian Agency for Health and Food Safety GmbH, Institute for Sustainable Plant Production, Austria

<sup>4)</sup> BOKU University, Department of Crop Sciences, Institute of Plant Breeding, Austria

<sup>5)</sup> BOKU University, Department of Agrobiotechnology, IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics; Institute for Global Food Security, School of Biological Sciences, Queens University Belfast, Austria

\*Corresponding author - E-mail: [stephan.freitag@boku.ac.at](mailto:stephan.freitag@boku.ac.at)

Wheat is one of the most important crops for feeding humanity. For decades, near-infrared spectroscopy (NIRS) has served as a tool for assessing its quality. However, the advantages of NIRS, such as non-destructive, rapid, and cost-effective analysis, can only be exploited when a calibration for the quality parameters of interest, such as protein or moisture content, is available. In this study, we demonstrate that analysis of variance simultaneous component analysis (ASCA) on NIR spectra of wheat provides a straightforward alternative to using such calibrations. We found that the year, sample site, and their combination significantly ( $p < 0.05$ ) influenced the protein, starch, moisture, fat, fiber, and ash content of wheat samples obtained by a classic NIR-based calibration. Thus, we show that combining ASCA with NIRS simplifies NIR-based quality assessment of wheat without the need for time- and chemical-consuming calibration development, as similar conclusions can be drawn.

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**Keywords:** near infrared, chemometrics, ASCA, green analytical chemistry

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### L100

## ADVANCED MATHEMATICAL MODELLING APPLIED TO WATER-BASED EXTRACTION OF BIOACTIVE COMPOUNDS FROM APPLE POMACE USING RSM, ANNS AND RANDOM FOREST MODELS

**Francesca Carra**<sup>\*<sup>(1)</sup></sup>, **Maurizio Rinaldi**<sup>(2)</sup>, **Vincenzo Disca**<sup>(3)</sup>, **Chiara Carini**<sup>(1)</sup>, **Fabiano Travaglia**<sup>(1)</sup>, **Marco Arlorio**<sup>(1)</sup>

<sup>1)</sup> Department of Pharmaceutical Sciences, Food Chemistry, Biotechnology and Nutrition Unit, Università del Piemonte Orientale, Italy

<sup>2)</sup> Department of Pharmaceutical Sciences, Mathematics Unit, Università del Piemonte Orientale, Italy

\*Corresponding author - E-mail: francesca.carra@uniupo.it

Apple (*Malus domestica*) is one of the most widely consumed fruits globally, not only for its taste but also for the nutritional value. In fact, apples are a rich source of bioactive compounds of which vitamins, dietary fibers - mainly pectins - and a huge range of phenolic compounds such as flavonols, dihydrochalcones, anthocyanins, hydroxycinnamic acids, and hydroxybenzoic acids are the main represented. One of the most interesting compounds in apple and apple-derived product is phlorizin, showed to be a key role in the glucose reuptake mechanism [1].

From the field to the table, apples are consumed as fresh fruit or subjected to a variety of different processes to obtain juices, cider, jams, and snacks. The processing generates an enormous quantity of waste and by-products including peels, seeds, and pomace, which are often overlooked despite their significant potential [2]. These by-products are exceptional sources of bioactive compounds, particularly phenolic compounds, vitamins, and carotenoids, leading to a potential exploitation in the food, cosmetic, and nutraceutical fields [3,4]. The extraction of these bioactive compounds traditionally relied on conventional organic-solvent driven methods. However, the request for sustainable processes by the food industry catalysed the need for more eco-friendly and green extraction methodologies.

The main purpose of this research was to model and optimize a green extraction of phenolics from apple pomace, using water as solvent, up-cycling this by-product and producing high-value extracts with minimal environmental impact. Through a designed *half-factorial* experiment, 32 extractions have been conducted, varying the parameters of temperature (from 35 to 80 °C), extraction time (from 15 to 60 min.), and solid-to-liquid (S/L) ratio (from 0.1 to 0.025 g mL<sup>-1</sup>). Each resulting extract has been thoroughly evaluated with spectrophotometric assays for total phenolic content (TPC) and antioxidant capacity (AC), and with HPLC-DAD focusing on polyphenolic components.

To clarify the effects of the different extraction parameters, response surface methodology (RSM), artificial neural network (ANN) and random forest (RF) modelling techniques have been used. Although all of these mathematical models have a strong predictive capability [5,6], ANN and RF provide a superior fit. According to this research temperature and S/L ratio are the two most influential factors in the extraction process; the combination of higher temperatures and higher solid-to-liquid ratios yields better result in terms of analyses response.

Concluding, this study advanced the understanding of water-based green extraction, also highlighting the potential of advanced mathematical experimental design.

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[5] A. Weremfo et al., 2023.

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**Keywords:** apple-byproducts, green extraction, bioactive compounds, mathematical models

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## LECTURES

### L101

## GREENER FLAVOROMICS IN QUALITY CONTROL: FAST HS-GC-IMS IN COMBINATION WITH MACHINE LEARNING FOR ANALYSIS OF COMPLEX FOOD SAMPLES

Lukas Bodenbender\*<sup>(1)</sup>, Sascha Rohn<sup>(2)</sup>, Philipp Weller<sup>(1)</sup>

<sup>1)</sup> Institute for Instrumental Analysis and Bioanalytics, Mannheim University of Applied Sciences, Germany

<sup>2)</sup> Department of Food Chemistry and Analysis, Institute of Food Technology and Food Chemistry, TU Berlin, Germany

\*Corresponding author - E-mail: l.bodenbender@hs-mannheim.de

Gas chromatography - ion mobility spectrometry (GC-IMS) is a powerful technique in quality control of food, beverages, and flavor products [1]. Quality control is fundamental for food safety and compliance, as well as for the verification of authenticity and product quality. With more global food and product supply chains, higher production costs lead to an increasing trend of fraudulent products and as well, academic research on food fraud gained more attention within the last years [2]. Issues range from mislabelling and dilution up to product adulterations. However, at the same time, analytical departments are confronted with the continuous demand for cost reduction, simplification of analytical methods and to act more sustainable. Therefore, new analytical approaches are particularly important in quality control and food authenticity. In this context, HS-GC-IMS offers a robust design, high sensitivity and a working principle at ambient pressure, facilitating point of care operations [1]. Time consuming and complex sample preparation are typically not required.

Although efforts are put into the development of more sustainable methods, current approaches show two weaknesses [3]. On the one hand, samples are diluted with organic solvents and on the other hand, helium is used as a carrier gas in GC-MS. IMS in combination with chiral GC separation columns, enables solvent-free differentiation of various citrus oils based on their volatile metabolome and in particular, their enantiomeric ratios, e.g. in bitter orange (*Citrus x aurantium*) and orange (*Citrus Sinensis*). Our study reports sample preparation-free headspace sampling in combination with a fast hydrogen carrier gas-based GC. Thus, analysis time is decreased substantially in comparison to common commercially available GC-IMS systems, operated with nitrogen to a factor of more than 2.5. Additionally, this study reports on an optimized flow design of the IMS cell and higher IMS cell temperatures, by which the peak shape is improved significantly concerning FWHM and tailing factor. This is crucial for the analysis of complex flavor samples with a large number of substances and high boiling volatile organic compounds such as terpenes, terpenoids and sesquiterpenes. With the use of hydrogen benchtop generators, fast GC-IMS methods in combination with machine learning are a resource-friendly and powerful approach for the analysis of complex samples in the field of food, flavor and beverages.

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**Keywords:** GC-IMS, chemometrics, green analytical chemistry, flavoromics, chiral GC separation

## LECTURES

### L102

## MICROWAVE-ASSISTED METHODS FOR GREENER AND MORE PRACTICAL (BLUENESS) FATTY ACID PROFILING IN FOOD

**Donatella Ferrara**<sup>(1)</sup>, **Marco Beccaria**<sup>(2)</sup>, **Chiara Emilia Irma Cordero**<sup>(3)</sup>, **Giorgia Purcaro**<sup>\*(4)</sup>

<sup>1)</sup> Gembloux Agro-Bio Tech, University of Liège / University of Turin, Belgium

<sup>2)</sup> University of Ferrara, Italy

<sup>3)</sup> Department of Drug Science and Technology, University of Turin, Italy

<sup>4)</sup> Gembloux Agro-Bio Tech, University of Liège, Belgium

\*Corresponding author - E-mail: gpurcaro@uliege.be

Fatty acids (FA) are predominant components of the lipid fraction in foods, and their analysis is important for industrial applications and understanding their implications for nutrition and health. While the standard laboratory procedure for profiling fatty acid is gas chromatography coupled with flame ionization detection (GC-FID), the sample preparation can be performed following somewhat different protocols. Typically, the lipids fraction is extracted from the matrix and then the FAs are converted into their more volatile derivatives FAMEs. FAMEs are generally formed under acidic ( $\text{BF}_3$ ,  $\text{HCl}$ ,  $\text{H}_2\text{SO}_4$ ) or alkaline conditions ( $\text{KOH}$ ,  $\text{NaOH}$ ,  $\text{CH}_3\text{NaO}$ ) through esterification and/or transesterification.

This study compares different extraction and derivatization methods for FAME analysis, including the use of microwave-assisted technologies to increase the productivity and greenness of the processes. Two different microwave-assisted extractions (solvent extraction and extraction with hydrolysis) were used and the extracts were derivatized with two other different methods: the conventional derivatization with  $\text{BF}_3$  and microwave-assisted derivatization using a methanolic hydrogen chloride solution. The combinations of these extractions and derivatizations were then compared with one-step microwave-assisted extraction and derivatization and to two AOCS Official methods (Ce 2b-11 and Ce 2c-11) used as references.

The FAME were identified and semi-quantified using comprehensive two-dimensional gas chromatography coupled with a flame ionization detector and a reverse fill/flush flow modulator (GC $\times$ GC-FID).

All methods were evaluated for their greenness and practicality using two recent tools, namely AGREEprep and BAGI, respectively.

The microwave-assisted processes were demonstrated to be comparable to official methods in terms of performances. Among all the methods, the one-step microwave-assisted extraction and derivatization achieved the highest score for greenness and practicality, 0.51 and 75, respectively; while the official methods had 0.22 and 67.5, respectively. In the end, a particular focus should be given to the derivatization step. It was shown that the  $\text{BF}_3$  can be replaced by methanolic hydrogen chloride microwave-assisted derivatization. This method achieves the same performance as the other methods while enhancing greenness, operator safety, and throughput.

**Keywords:** microwave-assisted methods, fatty acids methyl esters, bidimensional chromatography, green chemistry

**Acknowledgement:** The authors thank Milestone, SepSolve, and Shimadzu for their support.

### L103

## DEVELOPMENT AND VALIDATION OF A RAPID AND GREEN ANALYTICAL TOOL FOR TOMATO PUREES AUTHENTICATION BY HIGH RESOLUTION MASS SPECTROMETRY BASED FLOW INJECTION ANALYSIS

Antonella Lamonaca<sup>(1)</sup>, Anna Luparelli<sup>(2)</sup>, Elisabetta De Angelis<sup>(2)</sup>, Linda Monaci<sup>(2)</sup>, Rosa Pilolli\*<sup>(2)</sup>

<sup>1)</sup> Institute of Sciences of Food Production, ISPA- CNR, Italy

<sup>2)</sup> National Research Council of Italy, Institute of Sciences of Food Production, Italy

\*Corresponding author - E-mail: rosa.pilolli@ispa.cnr.it

Among fruits and vegetables, tomatoes are commonly valued for their sensorial and nutritional properties due to the high content in antioxidants and bioactive compounds. Over the last decade, several metabolomic approaches were proposed to investigate tomato varieties. The aim of this study was to develop and validate a rapid analytical tool for Datterino purees authentication by flow injection analysis based on high resolution mass spectrometry detection (FIA-HRMS). In addition, a preliminary marker analysis for varietal discrimination will be presented.

The approach was developed on a set of 40 puree samples equally distributed into two groups: (A) labelled as 100% Datterino, (B) low-cost alternative purees with no varietal specifications. The sample preparation was designed to be time-effective and optimized to involve only green solvents. The metabolite extracts were analysed by FIA-HRMS in both polarity modes on a hybrid quadrupole/orbitrap MS. The total run time, including sample preparation and detection, was below 30 min. Three independent extracts were prepared for each puree sample under optimized conditions to build up the final data set (n=120). The spectra were averaged over a 30s-time range, background subtracted and grouped into accurate peaks lists. The lists were processed by MetaboAnalyst v6.0 for peak matching, imputation of missing values, data filtering and normalization. PCA by NIPALS algorithm with a V-fold cross-validation (V=7) was used as unsupervised approach to evaluate the presence of outliers and establish the number of significant PCs best describing the multivariate system. These latter provided a total explained variance of 96,2% ( $R^2X(\text{Cum})$ ) and predicted variance of 96,1% ( $Q^2$ ), with a barely valuable grouping of cases. The significant PCs were used as input for supervised pattern recognition by Linear Discriminant Analysis (LDA) with external validation. The LDA model achieved a prediction ability of 100% for the analysis set and 95% for the testing set in both groups A and B. Markers discovery by multivariate exploratory ROC (Receiver operating characteristic) analysis was carried out, with conservative filters applied for matrix preparation excluding non-informative and low intensities features. The multivariate ROC curves were automatically generated based on three multivariate algorithms: support vector machines (SVM), partial least squares-DA (PLS-DA), and random forests (RFs). All the three classification methods applied with Monte-Carlo cross-validation provided the best modelling with 100 features. With the SVM algorithm, the predictive accuracy was 96,5% with an area under the curve of 0,986 and a 95% confidence interval of 0,895-1. Comparable performances were obtained with PLS-DA (10 latent-variables) and RFs classification methods. The three lists of ranked important features were evaluated to match common candidates, and MS/MS-based identification of such candidate markers was accomplished.

**Keywords:** tomato authenticity, rapid method, green technology, high resolution mass spectrometry, flow injection analysis

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## LECTURES

### L104

## QUALITY ASPECTS OF PLANT PROTEINS FOR MEAT AND DAIRY ALTERNATIVES: MOLECULES AND METHODS

**Hans-Gerd Janssen**<sup>(1)</sup>, **Boudewijn Hollebrands**<sup>(1)</sup>

<sup>1)</sup> A - Unilever Foods Innovation Centre, Bronland 14, 6708 WH Wageningen, the Netherlands; B - Wageningen University, Laboratory of Organic Chemistry, Stippeneng 4, 6708 WE Wageningen, The Netherlands, The Netherlands

\*Corresponding author - E-mail: [hans-gerd.janssen@unilever.com](mailto:hans-gerd.janssen@unilever.com)

The growing concern about the environment and animal welfare has increased consumer demand for plant-based meat alternatives and dairy free products. Although good plant-based foods are available, their absolute market share is still low. This is at least partly because of the distinct flavour and taste of plant-based products. Their aroma is often described as beany, malty or green, and taste is reported as bitter and astringent.

The volatile molecules responsible for the off aroma and their formation routes are by now well understood. The two most important formation routes are (enzymatic or chemical) lipid oxidation and conversion of free amino acids into volatile aldehydes via the so-called Strecker degradation reaction. Understanding of the taste defects is less well developed, but lipid oxidation products, plant polyphenols, saponins and peptides are important contributors.

In our laboratory we have developed a suite of methods to support the development of improved plant-based foods. The workhorse is the 3 minutes rapid hexanal GC-MS method we previously published as a tool to monitor the efficacy of natural antioxidants. For monitoring the full aroma pattern of samples, we developed a multi-target method focussing on 226 key food odorants. Peak areas are translated to flavour intensities using literature information. Non-volatile taste species are monitored using a broad-gradient reversed-phase LC-HR MS method that covers all above mentioned classes of potential bitter species. Artificial intelligence-based tools are developed to identify short peptides based on predicted retention times and mass spectra. In contrast to existing methods for peptide identification our tools can distinguish sequence-different isobaric peptides. In the current presentation we will discuss the various tools we apply for food flavour and taste analysis. The use of these methods for quality monitoring of ingredients and finished products will be demonstrated. We will show that the bottleneck in this work no longer is the separation but is rather how to derive meaningful information from the ever-expanding data sets. Moreover, human sensory assessment is notoriously unreliable complicating the process of linking taste and aroma defects to responsible compounds.

**Keywords:** plant proteins, flavour analysis, bitter compounds, peptide identification

## LECTURES

### L105

#### ARE MEAT ANALOGUES ADEQUATE TO SUBSTITUTE MEAT? A CASE STUDY ON SOY-BASED BURGER PROTOTYPES: NUTRITIONAL ADEQUACY, PROTEIN DIGESTIBILITY, AND SAFETY ASSESSMENT

**Sara Cutroneo**<sup>\*(1)</sup>, **Barbara Prandi**<sup>(1)</sup>, **Octavian Augustin Mihalache**<sup>(1)</sup>, **Marie Cristin Baune**<sup>(2)</sup>, **Florian Singer**<sup>(2)</sup>, **Nino Terjung**<sup>(2)</sup>, **Chiara Dall'Asta**<sup>(1)</sup>, **Tullia Tedeschi**<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

<sup>2)</sup> German Institute of Food Technology (DIL), Germany

\*Corresponding author - E-mail: sara.cutroneo@unipr.it

The large switch to plant-based diets of recent years has brought to an increase in market offer for meat analogues, leading to a high variety of products. A previous explorative study highlighted high variability of products on the Italian market (Cutroneo et al., 2022, *Front Nutr*, 9:1-12). This was further confirmed in a more specific study on the comparison of protein quality and digestibility between plant- and meat-based burgers (Cutroneo et al., 2023, *Food Res Int*, 172:113183). The latter particularly pointed out that quality of raw materials affects digestibility, regardless of protein source. Furthermore, it was highlighted that there is an actual concern for co-occurrence of mycotoxins in plant-based meat analogues (Mihalache et al., 2023, *Food Res Int*, 168:112766).

This study aims to deepen these aspects, exploring if soy-based burgers can be an adequate and safe substitute to beef burgers. High quality raw materials were used for the development of new formulations of soy-based burger analogues trying to achieve a good meat-like structure, taste, nutritional composition, protein quality, and also ensure safety of products.

Soy-burger prototypes were developed at the German Institute of Food Technology (DIL). High quality soy protein isolate was used as protein-base. Protein was textured with low and high moisture extrusion. To also investigate the effect of the binder on protein digestibility, methylcellulose and transglutaminase were chosen. Through different combinations, 6 prototypes were produced and compared to the meat control (3 replicates each). Composition, protein quality and integrity, protein digestibility and peptide release after digestion were evaluated and compared. Furthermore, anti-trypsin activity and occurrence of 11 mycotoxins were investigated in both raw materials and final products.

The prototypes appeared to be adequate meat replicas (i.e., appearance, taste, texture). A comparable nutritional profile to meat was observed, achieving also a balanced amino acid profile with good content of essential amino acids. The products showed good digestibility, although not entirely comparable with the meat control, with some differences due to different ingredients (i.e., textures, binder). Anti-trypsin activity (KTI and BBI inhibitors) was confirmed in the samples, with some differences in accordance with digestibility results. Only Tentoxin (produced by *Alternaria alternata*) was observed in protein isolate, textures, and, in lower amount, in burgers.

Results highlighted the feasibility of producing high quality and safe soy-based meat analogues. Using high quality raw materials coupled with the right choice of binder, amount and type of fat, can achieve a good protein digestibility with high biological value. This is one of the first studies that investigates the safety assessment of these products. Our results highlight the importance of further investigate these aspects when considering a drastic shift in the diet.

**Keywords:** plant-based meat analogues, soy-based burgers, protein quality, protein digestibility, meat analogues safety assessment

## LECTURES

### L106

#### **SAFETY OF NEW PROTEIN SOURCES: HOW EFSA'S UPDATED NOVEL FOOD GUIDANCE SAFEGUARDS CONSUMERS' HEALTH WHILE SUPPORTING INNOVATION IN THE EU AGRIFOOD SYSTEM**

**Ermolaos Ververis**<sup>\*(1)</sup>, Reinhard Ackerl<sup>(2)</sup>, Oceane Albert<sup>(2)</sup>, Domenico Azzollini<sup>(2)</sup>, Elisa Beneventi<sup>(2)</sup>, Agnes De Sesmaisons Lecarre<sup>(2)</sup>, Antonio Fernandez Dumont<sup>(2)</sup>, Esther Garcia Ruiz<sup>(2)</sup>, Andrea Germini<sup>(2)</sup>, Maria Glymenaki<sup>(3)</sup>, Georges E.N.Kass<sup>(2)</sup>, Eirini Kouloura<sup>(2)</sup>, Marcello Laganaro<sup>(2)</sup>, Maura Magani<sup>(2)</sup>, Leonard Matijevic<sup>(2)</sup>, Vania Mendes<sup>(2)</sup>, Estefania Noriega Fernandez<sup>(4)</sup>, Irene Nuin Garciaarena<sup>(2)</sup>, Gabriela Precup<sup>(2)</sup>, Pablo Rodriguez Fernandez<sup>(2)</sup>, Ruth Roldan-Torres<sup>(2)</sup>, Annamaria Rossi<sup>(2)</sup>, Francesco Suriano<sup>(2)</sup>, Emanuela Turla<sup>(2)</sup>, Silvia Valtuena Martinez<sup>(2)</sup>, Wolfgang Gelbmann<sup>(2)</sup>

<sup>1)</sup> European Food Safety Authority (EFSA), Parma, Italy; Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece, Italy

<sup>2)</sup> European Food Safety Authority (EFSA), Italy

<sup>3)</sup> European Food Safety Authority (EFSA), Parma, Italy; Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, United Kingdom, Italy

<sup>4)</sup> European Food Safety Authority (EFSA), Parma, Italy; Nofima, Stavanger, Norway; University of Zaragoza, Zaragoza, Spain, Italy

\*Corresponding author - E-mail: ermolaos.ververis@efsa.europa.eu

In the European Union (EU), the classification of "novel food" encompasses products not significantly consumed by humans before May 15, 1997. Novel foods can be newly developed foods and food ingredients, food produced using new technologies and production processes, as well as food which has been traditionally consumed in non-EU countries and are distinct, by law, from GMO products. To access the EU market, novel foods require pre-market authorization which involves a rigorous safety assessment of novel food application dossiers submitted by food business operators. This safety assessment is performed by the European Food Safety Authority (EFSA), which also provides guidance to applicants on scientific requirements for dossier submission.

The EFSA Novel Food Scientific Guidance that explains the type and quality of scientific information EFSA needs to conclude whether the novel food is safe under the proposed conditions of use underwent its first revision of scientific content since 2016, mandated by the European Commission. This update incorporates recent EU regulatory changes in the field, leverages advancements in food research and innovation, and capitalises on EFSA's experience since the centralisation of the EU assessment process in January 2018. Key areas addressed in the update include compositional data, identity, production processes, toxicology, nutrition, exposure, and allergenicity, covering a range of products including new protein sources spanning from plants and insects to microorganisms and cell culture-derived products.

An increasing number of new protein sources have been developed in recent years, seeking approval to access the EU food market as novel foods. Consequently, the characterisation, protein quality and safety assessment of these products is becoming more and more relevant to the health and nutritional status of the EU population. This work will elucidate the EU's novel food regulatory framework, EFSA's safety assessment principles, and the main revisions implemented in the updated guidance document, with a focus on safety assessment aspects linked to novel protein sources.

Disclaimer: The views expressed in this work are those of the authors and should not be interpreted as representing the official position of EFSA. Thus, the present work is published under the sole responsibility of the authors and may not be considered as an EFSA scientific output. EFSA cannot be held accountable for any errors or inaccuracies that may appear.

**Keywords:** novel foods, food safety, alternative proteins, risk assessment, food legislation



## LECTURES

### L107

#### **METROLOGY FOR FOOD SAFETY IN THE CIRCULAR ECONOMY: TARGETED AND SCREENING METHODS FOR CONTAMINANTS IN FOOD AND RECYCLED PACKAGING**

**Chiara Portesi**<sup>(1)</sup>, **Roland Becker**<sup>(2)</sup>, **Tea Zuliani**<sup>(3)</sup>, **Burcu Binici**<sup>(4)</sup>, **Christopher Hopley**<sup>(5)</sup>, **Laura Martin**<sup>(6)</sup>, **Consolato Schiavone**<sup>(1)</sup>, **Luigi Bergamaschi**<sup>(1)</sup>, **Charlotte Hauschildt**<sup>(2)</sup>, **Michal Strzelec**<sup>(7)</sup>, **Aida Jotanovic**<sup>(8)</sup>, **Ian Allan**<sup>(9)</sup>, **Kuria Ndungu**<sup>(9)</sup>, **Bert van Bavel**<sup>(9)</sup>, **Taner Gokcen**<sup>(4)</sup>, **Erika Bester**<sup>(10)</sup>, **Huiling Liu**<sup>(11)</sup>, **Christina Riemenschneider**<sup>(12)</sup>, **Alexander Schaechtele**<sup>(12)</sup>, **Tommy Licht Cederberg**<sup>(13)</sup>, **Agnieszka Anna Niklas**<sup>(14)</sup>, **Alexander Montoya**<sup>(13)</sup>, **Elias Kakoulides**<sup>(15)</sup>, **Ludger Bruehl**<sup>(16)</sup>, **Patrycja Wojciechowska**<sup>(17)</sup>, **Karolina Wiszumirska**<sup>(17)</sup>, **Els Van Hoeck**<sup>(18)</sup>, **Karlien Cheyngs**<sup>(18)</sup>, **Ivo Leito**<sup>(19)</sup>, **Irja Helm**<sup>(19)</sup>, **Matthias Groschke**<sup>(20)</sup>, **Sasa Hren**<sup>(21)</sup>, **Thomas Brandsch**<sup>(22)</sup>, **Evangelia Kristalli**<sup>(23)</sup>, **Nizar Benismail**<sup>(24)</sup>, **Thierry Diaz**<sup>(24)</sup>, **Nadine Bohni**<sup>(25)</sup>, **Maurus Biedermann**<sup>(25)</sup>, **Sarah Hill**<sup>(5)</sup>, **Marina Zbinden**<sup>(26)</sup>, **Raymond Schelker**<sup>(27)</sup>, **Melanie Haupt**<sup>(27)</sup>, **Jan Hanusch**<sup>(28)</sup>, **Marlena Roguszewska**<sup>(29)</sup>, **Andrea Mario Rossi**<sup>(30)</sup>, **Francesco Romaniello**<sup>(1)</sup>

<sup>1)</sup> INRIM, Italy

<sup>2)</sup> BAM, Germany

<sup>3)</sup> Jožef Stefan Institute, Slovenia

<sup>4)</sup> TUBITAK, Turkey

<sup>5)</sup> LGC, United Kingdom

<sup>6)</sup> EUROLAB, Belgium

<sup>7)</sup> GUM, Poland

<sup>8)</sup> IMBiH, Bosnia and Herzegovina

<sup>9)</sup> NIVA, Norway

<sup>10)</sup> ZRS Koper, Slovenia

<sup>11)</sup> CHIRON, Norway

<sup>12)</sup> EURL for POPs, CVUA, Germany

<sup>13)</sup> Technical University of Denmark, Denmark

<sup>14)</sup> National Food Institute, Technical University of Denmark, Denmark

<sup>15)</sup> EXHM, GR, Greece

<sup>16)</sup> MRI, Germany

<sup>17)</sup> Natureef, Poland

<sup>18)</sup> Sciensano, Belgium

<sup>19)</sup> University of Tartu, Estonia

<sup>20)</sup> AxelSemrau, Germany

<sup>21)</sup> GEA, Slovenia

<sup>22)</sup> GERSTEL, Germany

<sup>23)</sup> JOTIS, Greece

<sup>24)</sup> NOAC Vittel, France

<sup>25)</sup> KL-ZH, Switzerland

<sup>26)</sup> METAS, Switzerland

<sup>27)</sup> REALCYCLE, Switzerland

<sup>28)</sup> SQTS, Switzerland

<sup>29)</sup> KB FOLIE, Poland

<sup>30)</sup> National Metrology Institute of Italy - INRiM, Italy

\*Corresponding author - E-mail: c.portesi@inrim.it

Safeguarding consumers from potential harm caused by food contamination is of paramount importance to ensure food safety. The focus on safety is a central priority for European Commission lawmakers. Furthermore, as outlined in the EU circular economy action plan, the development of a sustainable food system is a critical objective.

However, progress towards these goals can sometimes face obstacles, including the risk of eroding consumer confidence in food quality. Factors such as the increased use of recycled and sustainable packaging materials and the growing awareness of emerging contaminants necessitate immediate attention.

To ensure a high level of food safety throughout production and distribution, it is crucial to improve and harmonise analytical techniques for contaminant quantification. This constitutes the basis for reliable data and compliance with regulations and for resolving disputes and minimising financial

## LECTURES

losses within the food industry. The ScreenFood project (June 2024 -May 2027) is funded by the European Metrology Partnership and aims to develop reference methods and reference materials for quantifying contaminants in both food and food packaging, with a specific emphasis on recycled materials. These metrological tools will aid industries and official food control in guaranteeing the delivery of safe food and sustainable packaging while adhering to regulatory requirements.

Specific objectives of the project are i. To improve protocols for the quantification of the mineral oil aromatic hydrocarbons (MOAH) fraction and for the quantification of the fraction consisting of substances with three or more aromatic ring systems (3+MOAH); ii. To develop sensitive analytical procedures for detecting and quantifying per- and polyfluoroalkyl substances (PFAS) in selected matrices, in line with EU requirements; iii. To develop traceable and highly accurate reference materials for quality control and quality assurance purposes; iv. to develop screening methods addressing new/existing organic and inorganic contaminants, in virgin and recycled packaging, such as PET, and bio-based and reusable materials; v. To investigate the migration of contaminants from packaging into food simulants, as well as to foster the research in the discovery of Emerging and Novel PFAS through non-targeted screening.

The consortium brings together 28 partners from EU metrological institutes, research centres, control laboratories, EU reference laboratories and industries. A large panel of collaborators and stakeholders is supporting the consortium in keeping the project aligned with EU and industrial priorities.

This presentation will outline the project objectives and the planned advances beyond the state of the art and provide an overview of ongoing activities.

**Keywords:** food safety, sustainability, PFAS, mineral oils, food packaging

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## LECTURES

### L108

## DRIVERS FOR INCREASE OF CIRCULARITY IN THE FEED SECTOR VERSUS CONTROL MEASURE CHALLENGES

Wilma Taverne\*<sup>(1)</sup>

<sup>1)</sup> Cargill & FEFAC-European Feed Manufacturers' Federation, The Netherlands

\*Corresponding author - E-mail: Wilma\_Taverne@Cargill.com

Circularity and sustainability are important for the future of the animal sector. But it is also very important that the feed safety is guaranteed. That is resulting in other sources of ingredients which are going to be explored and used. New resources mean new contaminants (marine resources), contaminants to be measured in new matrixes, which is sometimes a challenge. Also, other processes are/will be explored, e.g., mechanical unpacking with potential risk on residues of packaging material. However, the reduction of regulatory limits for contaminants will have a negative impact on possibilities of circularity. The analytical challenges in the complex feed ingredient matrixes are reducing the availability.

**Keywords:** circularity, sustainability, animal sector, feed safety, new resources and processes

### L109

## FOOD AND FEED SAFETY VULNERABILITIES IN THE CIRCULAR ECONOMY

**Angelo Maggiore**<sup>(1)</sup>, **Bernard Bottex**<sup>(1)</sup>, **Michele Ardizzone**<sup>(2)</sup>

<sup>1)</sup> *European Food Safety Authority, KNOW Unit, Italy*

<sup>2)</sup> *European Food Safety Authority, KNOW Unit & European Food Safety Authority, GMO Unit, Italy*

*\*Corresponding author - E-mail: [angelo.maggiore@efsa.europa.eu](mailto:angelo.maggiore@efsa.europa.eu)*

The foundation of the legislation on food and feed safety in Europe has evolved over the last two decades, mostly in response to food/feed crises. Bovine Spongiform Encephalopathy and Dioxins, both linked to animal feed, highlighted the need to safeguard human and animal health through improved regulatory safety standards. As part of the Green Deal, the Commission has put sustainability at the centre of EU political and economic policies, stimulating industry, including the food and feed sector, to invest in the development of a more circular and sustainable economy. More emphasis is put on the development of a sustainable food and feed sector, addressing the need to reduce the high pressure on natural resources and the negative impact on the planet attributable to the current conventional agro-zootechnical practices. In the design of a sustainable and circular food and feed system, it is crucial to anticipate emerging risks associated with the use of new sources, practices and technologies. Potential new hazards, exposure pathways and knowledge gaps need to be explored, in order to assess the adequacy of the current food and feed safety framework for assessing future risks and ensuring protection of humans, animals and the environment under a "One Health" approach. In this respect, EFSA endeavours to ensure preparedness to future risk assessment, food/feed safety and environmental challenges. Through an extensive literature search, EFSA has mapped existing circular economy practices, gathered information, and evaluated the evidence for vulnerabilities of circular economy for food/feed safety, plant, animals, and the environment. Following consultation with stakeholders, a focused literature search was carried out to identify emerging risks to plant, animal, human health and the environment from novel foods and feeds within the framework of circular economy. The few studies that investigated risks were almost entirely focused on insects as food or feed and the substrates that they are reared on. In this area, a wide range of chemical hazards were reported including heavy metals, dioxins, PCBs, PAHs, mineral oil hydrocarbons, veterinary medicines, pesticides and the uptake of allergens by insects from the substrate e.g. gluten. Following consultation with stakeholders and based on a series of prioritisation criteria, EFSA has subsequently focused on the identification and characterization of emerging risks from "circular feed", defined as feed derived from the following three sources: 1) Food waste; 2) Former food products and 3) Agri-Food processing co/by-products. Future trends and technologies that may drive the emergence of risks for feed safety, animal health and the environment were identified in a participatory foresight exercise. It highlighted that in the transition to sustainable animal feed production, it is crucial to recognize potential new hazards, exposure pathways and knowledge gaps. Analytical challenges were identified and the need to develop and harmonise reliable analytical methodologies and metrics to identify, characterise and quantify circular products and verify compliance with the applicable legislation was recognised.

**Keywords:** emerging risks, food and feed safety, foresight, circular economy

## LECTURES

### L110

## 140 YEARS OF AOAC INTERNATIONAL: ADDRESSING FOOD ANALYSIS CHALLENGES THROUGH COLLABORATION AND CONSENSUS

**Katerina Mastovska**\*<sup>(1)</sup>

<sup>1)</sup> AOAC INTERNATIONAL, United States of America

\*Corresponding author - E-mail: KMastovska@aoac.org

For 140 years, AOAC INTERNATIONAL has been at the forefront of advancing food analysis, consistently addressing emerging method needs to ensure the safety and integrity of foods and other products. By fostering collaboration and consensus among experts, AOAC has developed standards and validated methods that are globally recognized and trusted by industry and regulatory bodies alike.

This presentation will highlight key milestones in the organization's rich history, showcasing how AOAC has adapted to scientific advancements and regulatory changes. It will delve into current initiatives and future directions, emphasizing the critical role of collaboration in tackling global food analysis challenges.

AOAC's current scientific initiatives span a wide range of analyte categories, including important nutrients, chemical residues and contaminants (such as PFAS in foods and food contact materials), microbial contaminants, gluten and food allergens, and various authenticity issues, such as botanical identity verification. These initiatives have been developed to address method needs and gaps for the analysis of novel foods (alternative protein sources), infant formula, cannabis, botanicals, and other matrices and product types.

Moreover, AOAC has been developing standards and tackling challenges related to the integration of new approaches and technologies into food testing. This includes for instance next-generation sequencing, metagenomics, non-targeted analysis, or bioassays.

Join us to celebrate AOAC's 140-year journey and discover how you can get involved in AOAC's current and future scientific initiatives, collaborating with others on the development of new voluntary consensus standards and official methods.

**Keywords:** chemical contaminants and residues, food allergens, novel foods, non-targeted analysis, bioassays

## LECTURES

### L111

#### ANALYSIS OF DIETARY CARBOHYDRATES

**Barry McCleary**\*<sup>(1)</sup>, **Yannis Vrasadis**<sup>(2)</sup>, **Sean Austin**<sup>(3)</sup>

<sup>1)</sup> *FiberCarb, Ireland*

<sup>2)</sup> *Eurofins Food, Feed & Water Testing Netherlands, The Netherlands*

<sup>3)</sup> *Nestlé Research, Vers-Chez-Les-Blanc, Switzerland*

\*Corresponding author - E-mail: [mcclary@fibercarb.com](mailto:mcclary@fibercarb.com)

Dietary carbohydrates are categorised as “Available” and “Unavailable”. Available carbohydrates are those which are digested to monosaccharides in the human small intestine and absorbed. Excessive levels of available carbohydrates in the diet, especially those based on glucose, can lead to obesity and Type II diabetes. Unavailable, dietary carbohydrates are also termed dietary fiber. These carbohydrates pass through the small intestine undigested and are partially or wholly utilised/fermented by colonic microorganisms. AOAC International has played a pivotal role in identifying analytical needs in this area and through its Expert Review Panels, provided a mechanism for evaluation of proposed analytical procedures. In this presentation, “new” methods for measurement of available carbohydrates and dietary fiber will be described and compared to methods that have been in use for several decades. The need for validated methods for *in vitro* measurement of glycaemic index will be introduced.

Specific challenges in the measurement of high viscosity and gelatinous dietary fiber components such as xanthan gum, psyllium and galactomannans will be highlighted, as well as problems in the measurement of new potential fiber sources from seaweed (algal polysaccharides), fungi (beta-glucan and manno-oligosaccharides) and insects (chitin). Non-digestible oligosaccharides are now included in the definition of dietary fibre, and health benefits are often associated with these oligosaccharides. When claims are linked to particular oligosaccharides, methods with specificity for these oligosaccharides are required to control compliance. Methods which are available for some of the commonly used oligosaccharides will be described. We will speculate on which targets may come next and the associated challenges in analysis.

**Keywords:** dietary fiber, available carbohydrates, Codex

## LECTURES

### L112

## AOAC INTERNATIONAL INITIATIVES IN CHEMICAL CONTAMINANT AND RESIDUE ANALYSIS

Lukas Vaclavik\*<sup>(1)</sup>

<sup>1)</sup> *Eurofins Food Chemistry Testing, United States of America*

\*Corresponding author - E-mail: [lukas.vaclavik@ft.eurofinsus.com](mailto:lukas.vaclavik@ft.eurofinsus.com)

AOAC INTERNATIONAL is at the forefront of advancing contaminant and residue testing, playing a crucial role in ensuring safety and quality across various industries. By actively collaborating with key stakeholders, AOAC addresses emerging challenges and gaps in standardized testing methodologies. The organization facilitates development and validation of cutting-edge analytical methods and offers resources and networking opportunities to foster consistency and reliability in testing practices. A cornerstone of AOAC's approach is the Standard Method Performance Requirements (SMPR®) documents. These comprehensive guidelines are pivotal in setting the criteria for analytical methods and steering the expert review process of workflows seeking Official Method of Analysis status. Crafted through a rigorous and collaborative process, the SMPR® documents involve contributions from industry experts, research organizations, and regulatory authorities, ensuring that the methods developed are fit for their intended purposes. This presentation will provide an overview of AOAC International's current and new initiatives in contaminant and residue testing, highlighting the development of recent SMPR® documents for determination of ethylene oxide residues and pyrrolizidine alkaloids in relevant matrices, including foods, spices, and dietary supplements.

**Keywords:** contaminants, residues, AOAC INTERNATIONAL, Standard Methods

## LECTURES

### L113

#### **PFAS: AOAC ACTIVITIES AND COMPARISON OF TWO SINGLE LAB VALIDATED METHODS IN FOOD**

**Susan Genualdi**<sup>\*(1)</sup>, Manjula Sunkara<sup>(1)</sup>, Lowri DeJager<sup>(1)</sup>, Ashley Griffin<sup>(2)</sup>, Ernest Capraro<sup>(2)</sup>, Ashten Gibson<sup>(2)</sup>, Andrew Savage<sup>(2)</sup>

<sup>1)</sup> U.S. Food & Drug Administration, United States of America

<sup>2)</sup> Nestle, United States of America

\*Corresponding author - E-mail: [susan.genualdi@fda.hhs.gov](mailto:susan.genualdi@fda.hhs.gov)

The development of analytical methods for per- and polyfluoroalkyl substances (PFAS) in food is essential for monitoring the global food supply and for assessments of dietary exposure. The AOAC PFAS working group was established in 2023 to develop a standard method performance requirement (SMPR) for analytical methods for PFAS in food and feed. The SMPR was completed in November of 2023 and a call for methods was issued in December. In 2024, AOAC issued a new initiative for PFAS in food packaging. Both Nestle and the FDA have developed analytical methods for the analysis of PFAS in food following the requirements of SMPR 2023.003. For comparison, both methods were run at a Nestle lab in Dublin, OH and an FDA lab in College Park, MD. Incurred and proficiency samples were analyzed by both methods to compare accuracy. Additionally, method performance among different types of food samples using each method was compared. Results of the method comparison will be reported along with a summary of current AOAC activities on PFAS.



## LECTURES

### L114

## AOAC GUIDANCE FOR FOOD ALLERGEN METHOD VALIDATION

**Melanie Downs**\*<sup>(1)</sup>

<sup>1)</sup> Food Allergy Research & Resource Program, University of Nebraska-Lincoln, United States of America

\*Corresponding author - E-mail: mdowns2@unl.edu

Analytical methods to detect and quantify food allergens have long been critical tools for food manufacturers and regulatory agencies. The available methods, however, have struggled to demonstrate applicability across food matrices and comparability of results. With current proposals underway at the international level to implement quantitative, risk-based approaches for precautionary allergen labelling, it is more urgent than ever that stakeholders have access to reliable and robust validated analytical methods. Starting in 2021, the AOAC Food Allergens Working Group began developing guidance on food allergen immunoassay validation. The objective of these efforts was to generate comprehensive, consensus recommendations for developers of both qualitative and quantitative food allergen methods to use in various types of method validations (e.g., single laboratory and collaborative studies). The guidance will also form the basis on which AOAC will evaluate submitted method validation data. The recommendations are built upon prior work on food allergens conducted by members of the AOAC community as well as long standing AOAC practices for collaborative validation studies. Key emerging issues addressed in the guidance include the following: reporting unit requirements, incurred test material preparation guidelines and use requirements, procedures for estimation of limits of detection and quantification, and single laboratory study designs to estimate intermediate precision and repeatability. The working group believes the guidance will be an invaluable resource for the assessment of food allergen method performance both within the AOAC approvals process and more broadly. As the current document focuses on information for method developers, subsequent work starting in 2024 will focus on guidance for end users of food allergen methods.

**Keywords:** AOAC, food allergens, ELISA, validation, immunoassay

**Acknowledgement:** *The work presented was conducted as part of the AOAC International Gluten and Food Allergens Initiative.*

## LECTURES

### L115

## CHALLENGES TO ASSESS SAFETY OF MIXTURES: "BEST PRACTICES FOR BIOASSAY TESTING OF FOOD AND OTHER COMPLEX MIXTURES"

**Maricel Marin-Kuan**\*<sup>(1)</sup>

<sup>1)</sup> *Société des Produits Nestlé, Switzerland*

\*Corresponding author - E-mail: [maricel.marin-kuan@rdls.nestle.com](mailto:maricel.marin-kuan@rdls.nestle.com)

According to the European Food Safety Agency (EFSA), mixture is any combination of 2 or more chemicals that may contribute to effects regardless of source, and spatial temporal proximity [1]. Safety assessment of mixtures is of utmost importance at scientific and regulatory level. Certainly, the progress in the *in vitro* field is a fundamental driver bringing promising solutions to address complex systems. There are, however, underlying issues in the *in vitro* field that need further attention to avoid misinterpretations: what is being measured when assessing biological activity or activities? Critical limiting factors related to the accuracy and precision for biological testing are still missing. Contributing factors to these gaps include the integration of fundamental upstream boundaries including sample preparation and lack of best laboratory practices. A framework for the assessment and harmonization of the validity of *in vitro* studies is needed to build robust ground for reliable data analyses and therefore biological interpretation towards their acceptance. In this frame, the progress of working groups involved in the initiative from AOAC Europe section on the use and application of *in vitro* assays within the end-to-end process will be presented.

Moreover, as contribution to the assessment of mixtures, a breakthrough methodology comprising effect-based analysis bioassays coupled to High Performance Thin-Layer Chromatography (HPTLC) for the detection of key toxicological events developed at Nestlé Research will be presented [2]. Moreover, the integration of Liquid Chromatography-Mass Spectrometry (LC-MS) to HPTLC allowed the structural identification of the bioactive compound(s), bringing a valuable advancement in facilitating safety assessment [3]. The above strategy has been applied to case studies illustrating the feasibility of this approach in characterizing mixtures and ultimately contributing to their safety assessment.

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## LECTURES

### L116

## HARMONIZATION ON NON-TARGETED TESTING. BABYLONIAN LINGUISTIC DIVERSITY FOR MASS SPECTROMETRY

**Marios G. Kostakis**<sup>(1)</sup>, **Thomas Gude**<sup>(2)</sup>

<sup>1)</sup> *Method Validation Working Group, Eurachem, Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Greece*

<sup>2)</sup> *AOAC-Europe, Switzerland*

*\*Corresponding author - E-mail: makostak@chem.uoa.gr*

Non-targeted methods have been gaining ground in recent decades, especially with the tremendous development of high-resolution mass spectrometry. The challenges for non-targeted methods are becoming increasingly necessary as there are limited guidelines to regulate and harmonize the methods worldwide. Eurachem and AOAC-Europe have been signed a Memorandum of understanding for this sector. Two years ago, a series of Webinars on "Trends & Challenges for Non-Targeted Methods" have been started. As a result of this webinars a joint working group was set up with the aim of providing guidelines for the harmonization of non-targeted methods, mainly based on Mass Spectrometry. So far, the starting working group was now split into five subgroups dealing with food, authenticity, environmental/water, packaging and metabolomic testing. As an initial step the five groups are summarizing definitions and workflows for non-target testing. Based on the theses results a harmonized wording and where possible a harmonized workflow incl. validation should be created. The aim of this presentation is to present the result of the comparison and to communicate the linguistic diversity between the fields of non-targeted methods.

**Keywords:** harmonisation, non-targeted methods, linguistic diversity, mass spectrometry

## LECTURES

### L117

#### PFAS EXPOSURE - SENSE AND NONSENSE

**Jacob de Boer**<sup>\*(1)</sup>

<sup>1)</sup> *Vrije Universiteit Amsterdam, The Netherlands*

*\*Corresponding author - E-mail: jacob.de.boer@vu.nl*

Because of their unique water and fat repellent properties, per- and polyfluorinated alkyl substances (PFAS) are being used in many useful applications, such as in fire extinguishers, cosmetics, non-stick cookware, fast food packaging materials, etc. However, PFAS belong to the most persistent synthetic chemicals on earth. The C-F bond is so strong that no degradation occurs. Because PFAS are also mobile, they have been distributed worldwide. In 2020, the European Food Safety Agency (EFSA) has published a new opinion on PFAS. Based on an immunotoxicological study by Grandjean et al. the maximum permissible levels of PFAS in the human body have 1,000-10,000-fold decreased. At several places in the world the production of PFAS has led to very high levels of these compounds, e.g., in the neighbourhoods of PFAS producing plants or around airports where fire brigades have used PFAS foam during their training sessions.

Safe levels of PFAS are often substantially exceeded in drinking and surface water. However, what are safe levels? Authorities are still developing and adjusting advisory values for PFAS in food, drinking water and the environment. The general trend is to lower the maximum permissible levels for PFAS. In some cases, such as in the USA and also in the Netherlands, maximum permissible levels have been lowered to <1-10 pg/L. Analytical methods are nowadays very sensitive for PFAS, but such low levels are even on the edge of what is possible. The low levels also confuse water managers. Drinking water in the Netherlands, and in Europe, may contain 100 ng/L of the sum of 20 PFAS components. That is much higher than the ecotoxicological limits for surface waters. Even the advisory levels for drinking water in the Netherlands, 4.4 ng/L for the sum of 4 PFAS components are much higher than the ecotoxicological limit for PFOS in surface water of 7 pg/L. This raises the question if we are sometimes too strict in setting limits for PFAS. It is good to follow the precautionary principle, but with all knowledge generated now on PFAS, it should be possible to provide more clarity in maximum tolerance levels for PFAS.

**Keywords:** PFAS, exposure, water

### L118

## OCCURRENCE AND EVALUATION OF ENVIRONMENTAL RISKS OF PFAS

Ondrej Parizek<sup>(1)</sup>, Veronika Svobodova<sup>(1)</sup>, Darina Dvorakova<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Jakub Tomasko<sup>(1)</sup>, Jana Pulkrabova<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: jana.pulkrabova@vscht.cz

Per- and polyfluoroalkylated substances (PFAS) are a group of organohalogenated pollutants that have received considerable attention due to their widespread presence and confirmed adverse effects on human health. PFAS are used in various applications, including ski waxes, which have been identified as a significant source of environmental contamination. Regulatory measures such as Commission Regulation 2020/784, which came into force on 4 July 2020, control the presence of PFOA in certain products sold in the European Union, including ski waxes. In addition, as of the 2023/2024 winter season, the use of fluorocarbon-based ski waxes in competitions has been banned by the relevant international sports organizations. However, these restrictions do not apply to recreational skiers, who continue to use such waxes without restriction.

The objectives of this study were (i) to conduct long-term environmental monitoring in cross-country skiing areas, (ii) to assess the impact of the fluorinated wax ban on the occurrence of PFAS in the environment, and (iii) to collect comprehensive data on the distribution of PFAS in different environmental compartments where a higher historical exposure due to ski wax use is expected. To achieve these objectives, snowmelt (n=36), soil (n=22), and surface water (n=22) samples were collected from a cross-country ski resort in Nove Mesto na Morave between 2022-2024, during major winter sports events and regional competitions. The sampling campaign also included the collection of surface water and soil samples before and after the winter season.

Target analyses focused on perfluoroalkyl carboxylic acids (C4-C18 PFCA), perfluoroalkyl sulphonic acids (C3-C13 PFSA) and fluorotelomer precursors, using ultra-performance liquid chromatography with tandem mass spectrometry and ultra-performance liquid chromatography high resolution mass spectrometry. The snowmelt samples contained predominantly PFCAs, with the highest concentrations (1113 ng/L at the start; 54-633 ng/L on the course) recorded during a major winter sports event in the 2022/2023 season. In comparison, samples from a similar event in the 2023/2024 season showed significantly lower PFCA levels at the start line (8-27 ng/L). PFCA concentrations in soil samples after the 2022/2023 winter season varied from 1.5-130 ng/g dry matter, with a notable decrease of almost 40% observed in samples collected after the 2023/2024 season compared to the previous season. The results suggest that reducing the use of fluorinated ski waxes can significantly reduce PFAS levels in the environment. Even so, the increased concentration of PFAS in soil and surface water can increase potential human exposure in adjacent urban areas. These results underline the importance of regulatory measures to reduce environmental contamination and highlight the need for continued monitoring and research into the sources and risks of PFAS exposure to human health and the overall environment.

**Keywords:** PFAS, human exposure, environmental pollution

**Acknowledgement:** This work was financially supported from the European Union program for research and innovation Horizon 2020 within the grant agreement NO. 101057014 (PARC), which we are gratefully acknowledged.

## LECTURES

### L119

#### COCKTAILS OF ENDOCRINE DISRUPTORS IN THE DIFFERENT DIETS OF FRENCH CONSUMERS

**Gaud Dervilly**<sup>(1)</sup>, **Manon Bourdeau**<sup>(1)</sup>, **Bruno Le Bizec**<sup>(1)</sup>, **Helene Moche**<sup>(2)</sup>, **Anne Platel**<sup>(2)</sup>, **Marie-Christine Chagnon**<sup>(3)</sup>, **Isabelle Severin**<sup>(3)</sup>, **Fabrice Nesslany**<sup>(2)</sup>

<sup>1)</sup> ONIRIS, INRAE, LABERCA, France

<sup>2)</sup> Institut Pasteur, France

<sup>3)</sup> UMR INSERM 1231, France

\*Corresponding author - E-mail: [gaud.dervilly@inrae.fr](mailto:gaud.dervilly@inrae.fr)

With a view to identifying main endocrine disruptors (ED) mixtures to which French consumers are exposed through food, their main diets were modelled using an adapted dimension reduction method. Seven specific diets could be modelled for adults while only one overall diet was considered for children aged 3 to 17 years. The knowledge of the contamination levels of 78 known or suspected endocrine disrupting compounds in the foods constituting these diets, collected in the frame of the second French Total Diet Study, made it possible to explore the mixtures of EDs to which consumers are exposed. Our approach made it possible to highlight, for a given diet, the substances whose exposure is statistically higher than in the diet of the general population. Thus, significantly different ED mixtures could be established for each diet. These results pave the way for studying the specific effects of these cocktails of endocrine disruptors, each of which is representative of a type of chronic exposure linked to specific diets.

**Acknowledgement:** *This work has been financially supported by the French National Research Program for Environmental and Occupational Health of Anses (2019/1/168).*

## LECTURES

### L120

## ULTRA-TRACE ANALYSIS OF EXPOSURE CHEMICALS IN PLACENTAL TISSUE, MATERNAL- AND UMBILICAL CORD BLOOD

Max Lennart Feuerstein<sup>(1)</sup>, Sebastian Granitzer<sup>(2)</sup>, Julia Füreder<sup>(3)</sup>, Kathrin Sigl<sup>(3)</sup>, Tina Bürki-Thurnherr<sup>(4)</sup>, Claudia Gundacker<sup>(2)</sup>, Benedikt Warth<sup>(1)</sup>

<sup>1)</sup> Faculty of Chemistry, Department of Food Chemistry and Toxicology, University of Vienna, 1090 Vienna, Austria & Exposome Austria, Research Infrastructure and National EIRENE Node, Austria

<sup>2)</sup> Center for Pathobiochemistry and Genetics, Institute of Medical Genetics, Medical University of Vienna, 1090 Vienna, Austria, Austria

<sup>3)</sup> Faculty of Chemistry, Department of Food Chemistry and Toxicology, University of Vienna, 1090 Vienna, Austria & University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), Austria

<sup>4)</sup> Empa, Swiss Federal Laboratories for Materials Science and Technology, 9014 St. Gallen, Switzerland, Switzerland

\*Corresponding author - E-mail: max.lennart.feuerstein@univie.ac.at

The exposome, the totality of environmental and food-related exposures, is a relevant factor for human health. Blood concentrations of chemicals are important internal markers of exposure and can have a substantial impact during embryonic development and early life. However, only limited data is available on the placental transfer of many chemicals, which is typically not systematically studied *in vivo*. Sensitive and broad multi-analyte methods are prerequisite to fully address various relevant exposure chemicals in human samples at the same time. Thus, trace analysis in low sample amounts demands for suitable extraction and acquisition methods with wide chemical coverage, high method sensitivity, and large dynamic range. Moreover, short total analysis time is required for high sample throughput. To overcome the limitations of targeted human biomonitoring (HBM), we developed a workflow aiming to identify and quantify various xenobiotics and their metabolites in low amounts of placental tissue, as well as maternal and umbilical cord blood (i.e., 50 mg or 50 µL). A 1290 Infinity II (Agilent) was used for ultra high-performance liquid chromatography (UHPLC) in combination with a 7500 Qtrap (SCIEX) for quantification using targeted tandem mass spectrometry (MS/MS). Additionally, a ZenoTOF 7600 (SCIEX) was used for non-targeted analysis and suspect screening using high-resolution mass spectrometry (HRMS). To maximize fragment spectra coverage, while ensuring high spectral quality at the same time, a combination of data dependent acquisition (DDA) and data independent acquisition (DIA) was applied. Open-source software, chemical databases, and spectral libraries were used for data processing and identity confirmation. Various endogenous compounds and xenobiotics including medical drugs, environmental-, food, and lifestyle-related chemicals were annotated using spectral libraries or identified using authentic analytical standards. In addition, >80 chemicals including plasticizers, perfluoroalkyl substances, mycotoxins, phytoestrogens and other xenobiotics were targeted using MS/MS. In a proof-of-principle study, we applied this workflow to a cohort of matched placenta, maternal blood and cord blood samples from 74 individuals and determined the placental transfer rates for various chemicals *in vivo*. Such combinations of extremely sensitive quantitative assays and suspect screening are required to further expand the coverage and depth of current HBM assays and represent essential tools for future exposome-wide association studies (ExWAS).

**Keywords:** human biomonitoring, mass spectrometry, early life exposomics

### L121

## ADVANCEMENTS IN MULTI-ELEMENT SPECIATION: A NOVEL APPROACH FOR THE IDENTIFICATION OF CHELATING COMPOUNDS USING SEC-ICP-MS/MS AND SEC-QTOF-MS WITH A FOCUS ON CADMIUM IN PLANT-BASED FOODS

Julian Cardini<sup>(1)</sup>, Jens J. Sloth<sup>(1)</sup>, Katrin Löschner<sup>\*(1)</sup>

<sup>1)</sup> Technical University of Denmark, Denmark

\*Corresponding author - E-mail: kals@food.dtu.dk

The increasing shift from meat- to plant-based diets has necessitated a deeper understanding of the presence of heavy metals and their speciation in plant-based foods. Cadmium, a toxic heavy metal, can pose significant health risks. Significant research gaps persist in the field of cadmium speciation, particularly with respect to its bioavailability and the fact that many compounds binding cadmium remain unidentified in plant-based foods. Traditional analytical methods for cadmium speciation have been predominantly reliant on Size Exclusion Chromatography (SEC) with single element detection by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

This research presents an innovative methodology that integrates SEC-ICP-MS/MS and SEC-QTOF-MS, for the identification of Cd-binding compounds in combination with simultaneous speciation analysis of multiple elements, including sulphur, phosphorus, zinc, iron, and calcium in plant-based foods. Sulphur plays a crucial role in binding cadmium via dithiol groups and can be used as a marker for proteins and peptides. Phosphorous can be equally used as marker for proteins and peptides. In addition, it is a major constituent of phytic acid, which is a well-known metal chelator. The presence of zinc, iron, and calcium might significantly influence the bioavailability of cadmium.

The extraction process for cadmium was optimized across six different matrices with varying protein content, including e.g. tiger nuts, beetroot leaves, black-eyed beans and lentils, using an ultrasonic bath and considering variables such as extraction time, solvent, and volume. This optimization yielded recoveries ranging from 2.3% to 72.3%, indicating strong binding of cadmium to some of the matrices. Depending on the molecular weight of the compounds, two size exclusion columns, with an optimal separation range of 600 kDa - 10 kDa and 7000 Da - 100 Da, were employed to separate the extracted compounds and optimized based on chromatographic peak resolution and analysis time. During the optimization procedure, four calibration compounds were utilized. A 20% enhancement in resolution was achieved during the optimisation, with an analysis time of 60 minutes. For ICP-MS/MS-detection, the signal of the six elements was optimized based on the oxygen flow in the collision/reaction cell. In-house validation was performed in terms of repeatability, intermediate precision, method repeatability, Limit of Detection, Limit of Quantification, and linearity.

Clear differences in the binding patterns of cadmium between the different plant-based foods were observed, particularly within the molecular weight range of 1000 to 7000 Da. The established methodology represents a significant advancement of cadmium speciation in plant-based foods and can be applied in future bioavailability studies.

**Keywords:** multielement speciation, SEC-ICP-MS/MS, SEC-QTOF-MS, cadmium binding compounds, plant-based foods



### L122

## SILICONE WRISTBANDS AS PASSIVE SAMPLERS TO MONITOR PESTICIDE EXPOSURE DURING FARMING PRACTICES

**Chaymae El Amraoui Aarab**<sup>(1)</sup>, Chunfang Xiao<sup>(2)</sup>, Merel Van der Meer<sup>(1)</sup>, Marie-Laure Aiello<sup>(3)</sup>, Wei-Hong Zhang<sup>(4)</sup>, Benjamin Couvreur<sup>(3)</sup>, Maxime Bonnavé<sup>(3)</sup>, Nicolas Vidick<sup>(1)</sup>, Els Van Hoeck<sup>(5)</sup>, Maryse Ledent<sup>(1)</sup>, François Serneels<sup>(3)</sup>, Joris Van Loco<sup>(5)</sup>, Eva De Clercq<sup>(1)</sup>, Laure Joly<sup>(1)</sup>

<sup>1)</sup> *Chemical and Physical Health Risks, Sciensano, Belgium*

<sup>2)</sup> *South Potato Research Center of China (SPRCC) / Enshi Academy of Agricultural Sciences (EAAS) Department of Potato Research, Hubei Province, China*

<sup>3)</sup> *Agricultural Research and extension Centre of Hainaut Province - CARAH asbl Belgium, Belgium*

<sup>4)</sup> *International Centre for Reproductive Health Belgium, Department of Public Health and Primary Care, Ghent University, Belgium*

<sup>5)</sup> *Sciensano, Belgium*

\*Corresponding author - E-mail: [Chaymae.elamraouiaarab@sciensano.be](mailto:Chaymae.elamraouiaarab@sciensano.be)

Potato is a staple crop widely grown worldwide, and the focus on sustainable and resilient cultivation techniques is increasing. This study aims to assess the exposure of Chinese and Belgian farmers and their families to plant protection products (PPPs) used in potato cultivation using silicone wristbands as passive samplers.

The research involved 42 Chinese farmers using hand sprayers and 10 Belgian farmers using tractor-mounted sprayers to distribute the PPPs. The participants were divided into three groups of 14 individuals, each based on their farming methods, which included organic farming, traditional farming, and an advanced spraying technique incorporating a warning system from Belgium. The Chinese farmers wore wristbands for two consecutive weeks during the potato crop spraying season, while the Belgian farmers wore them for one week. Control wristbands were placed in farmers' households during the same period.

A total of 63 pesticides commonly used in PPP authorized for potato cultivation in Belgium and China were selected. An analytical method was developed to quantify these pesticides in the wristbands, involving extraction with acetone, solvent exchange with acetonitrile and purification by d-SPE C18 sorbent, followed by liquid chromatography coupled to tandem mass spectrometry. The study was validated successfully at very low concentrations (i.e. 1, 10 and 100 ng/g per wristband) following SANTE/11312/2021.

Afterwards, the validated protocols were applied to the wristbands worn by the farmers. The pesticide levels on wristbands from organic farming were significantly lower by 2 orders of magnitude compared to other farming methods (100 ng/g wristband versus 10000 ng/g wristband). These consistent results were observed over the two-week period, ensuring repeatability. The number of pesticides detected per wristband ranged from 0 to 21 with hand-held sprayers in China and from 21 to 39 with tractor-mounted sprayers in Belgium. In Chinese wristbands, fluopicolide, dimethomorph, famoxadone, and pyraclostrobin were the most frequently detected pesticides, with a detection rate exceeding 75% across all farming methods. All Belgian wristbands showed the presence of eleven pesticides. Pesticide concentrations on worn wristbands were notably higher by two orders of magnitude than on household control wristbands. On average, less than five pesticides were detected in household wristbands across all farming methods. A risk and health impact assessment study is currently in progress.

**Keywords:** silicone wristbands, PPP, pesticides

**Acknowledgement:** *The Belgian Science Policy (BELSPO) international cooperation project BL/50/C-SA02 EMPOCHA provided financial support.*

## L123

**COMBINED HILIC-HRMS AND CE-HRMS APPROACHES TO CHARACTERIZE THE POLAR METABOLOME. APPLICATION TO THE BIOMARKERS OF EFFECT RELATED TO PCBs AND BPA IN HUMAN ANIMAL MODEL (PIG)**

**Maykel Hernandez-Mesa**<sup>\*(1)</sup>, **Luca Narduzzi**<sup>(2)</sup>, **Alfonso Narvaez**<sup>(2)</sup>, **Maria del Mar Delgado-Povedano**<sup>(2)</sup>, **Bruno Le Bizec**<sup>(3)</sup>, **Ana María Garcia-Campaa**<sup>(2)</sup>, **Gaud Dervilly**<sup>(3)</sup>

<sup>1)</sup> *Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain*

<sup>2)</sup> *University of Granada, Spain*

<sup>3)</sup> *ONIRIS, INRAE, LABERCA, France*

*\*Corresponding author - E-mail: maykelhm@ugr.es*

Hydrophilic interaction liquid chromatography (HILIC) and capillary electrophoresis (CE) coupled to high-resolution mass spectrometry (HRMS) are the most appropriate analytical techniques for studying the polar part of the metabolome. Therefore, their combined use is recommended to extend the coverage of the polar metabolome, particularly when multiple metabolic pathways are expected to be disrupted [e.g. in toxicological studies related to exposure to chemicals such as polychlorinated biphenyls (PCBs) or bisphenol A (BPA)].

In this study, the HILIC-HRMS and CE-HRMS methods were first compared to demonstrate the complementarity of the two analytical techniques in the study of the polar metabolome, supporting the hypothesis of the need to approach metabolomic studies from the point of view of a multi-analytical platform perspective. The performance of the two methods was evaluated by four terms of comparison such as (1) compounds detectability, (2) Pezzatti's score [1], (3) intra-day precision, and (4) ease of automatic data analysis. The HILIC-MS method proved to be a more feasible tool for polar metabolome analysis, while the CE-MS method helped identify some interesting variables that contribute to complete metabolome coverage in metabolomics studies [2].

The application of the HILIC-HRMS and CE-HRMS methods in two different studies was carried out to better illustrate their complementary in metabolomics, which has recently emerged as plausible approach in chemical risk analysis. These studies focused on revealing biomarkers of effect associated with exposure to low doses of non-dioxin-like (NDL)-PCBs and BPA, respectively, on pig metabolism as new contributions to risk assessment of these chemicals. Dietary exposure to Aroclor 1260 (i.e. a commercially available mixture consisting of 98% NDL-PCB congeners) and doses of 20 ng/kg body weight (b.w.) per day for 21 days were applied based on reported population exposure levels to NDL-PCB. Dietary exposure to BPA and doses of 4 µg/kg body weight (b.w.) per day for 21 days were selected based on the tolerable daily intake (TDI) established until 2023 (EFSA, 2015).

The combination of HILIC-HRMS and CE-HRMS methods provided a significant picture of the effects of NDL-PCBs and BPA on pig metabolism. In particular, the effect on the arginine biosynthesis pathway was demonstrated for both NDL-PCBs and BPA exposure. In addition, perturbations in glyoxylate and dicarboxylate metabolism and valine, leucine and isoleucine biosynthesis were attributed to NDL-PCB exposure, while disruptions of alanine, aspartate and glutamate metabolism were associated to BPA exposure. Notably, none of the isolated analytical methods provided a robust overview of metabolic changes in pigs, highlighting the importance of approaching the metabolome by different analytical techniques to decipher its complexity.

[1] Pezzati *et al.*, *J. Chromatogr. A*, 1592 (2019) 47-54.

[2] Narduzzi *et al.*, *J. Chromatogr. A*, 1706 (2023) 464239.

**Keywords:** non-dioxin like polychlorinated biphenyls, bisphenol A, metabolomics, hydrophilic interaction liquid chromatography-mass spectrometry, capillary electrophoresis-mass spectrometry

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## LECTURES

### L124

## LIFESTYLE PRODUCTS FOR OUR HEALTH: CAN WE ALWAYS TRUST IN PRODUCT LABELLING?

**Christian W. Klampfl**<sup>\*(1)</sup>, **Bernhard Thalhamer**<sup>(1)</sup>, **Markus Himmelsbach**<sup>(1)</sup>

<sup>1)</sup> Johannes Kepler University Linz, Austria

\*Corresponding author - E-mail: christian.klampfl@jku.at

Nutritional supplements have been facing an increasing interest over the last decade. Therefore, also the market for products belonging to this category is showing a steady growth. In most cases these nutritional supplements are marketed as having additional health benefits. Sources for their distribution range from pharmacies and drug stores to gyms and various internet stores. Often such nutritional supplements are derived from plant-based products having their origin in Indian or Chinese traditional medicine. To adopt these products to our lifestyle and facilitate consumption the original, often quite elaborate procedures for preparation, such as brewing teas or manufacturing extracts from the different plant parts, are replaced by more easily consumable dosage forms such as capsules.

Ashwagandha (*Withania somnifera* (L.) Dunal) is a recognized medicinal plant with a long history particularly in traditional Indian medicine. It has attracted substantial attention due to its advantageous pharmacological properties, such as antioxidant, adaptogenic, anti-inflammatory, and immunomodulatory effects. These therapeutic benefits are mainly attributed to bioactive ingredients present in this plant, which are called withanolides and withanosides [1]. In the present work Ashwagandha root, root powder as well as a series of Ashwaganda extracts, manufactured employing several different extraction protocols, and mainly sold in the form of capsules were investigated with respect to their content in withanolides and withanosides. Analytical methods employed ranged from simple inspection of the root powder and capsule ingredients by light microscopy to analysis of the extracts by highly sophisticated instrumentation like HPLC coupled to drift-tube ion-mobility quadrupole time-of-flight mass spectrometry. Results from these analyses were subsequently compared with the product labels and data sheets available on the internet.

[1] Paul S, Chakraborty S, Anand U, Dey S, Nandy S, Ghorai M, et al. *Withania somnifera* (L.) Dunal (Ashwagandha): A comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedical and toxicological aspects. *Biomed Pharmacother.* 2021; 143:112175.

**Keywords:** nutritional supplements, Ashwaganda, withanolides, withanosides

## LECTURES

### L125

## ANALYSING FOOD ADDITIVES: THE GOOD, THE BAD AND THE UGLY

**Joris Van Loco**\*<sup>(1)</sup>, **Séverine Gosciny**<sup>(1)</sup>

<sup>1)</sup> *Sciensano, Belgium*

\**Corresponding author - E-mail: joris.vanloco@sciensano.be*

Food additives (FAs) use in the European Union is governed by Regulation 1333/2008. A wide variety of FAs are approved for use in all types of foods across Europe. Consumers demand total transparency from food business operators, while inspection services are responsible for controlling labelling and ensuring compliance with maximum limits. Member states monitor usage and assess dietary intake, and there is constantly demands from EFSA for usage data to (re-)evaluate FAs.

In a global food market, FAs that are unauthorised or illegal in the EU might be imported from third countries through ingredients or foods or even added fraudulently. Some of these illegal FAs can harm consumers and pose a direct public health concern.

Many analytical applications have been successfully implemented but often cover very few additives and/or food matrices. Under these conditions, it is challenging and expensive to control FA levels in foods, given the large variety of products on the market. One way to make the analytical process more efficient is to develop versatile, high-throughput multi-methods that can cover the largest number of FAs and matrices while managing maximum permitted levels from ppm to ppb values. Method development is complex in this context, and validation procedures are extensive. There is also a significant lack of metrological tools such as certified reference materials, standardised methods, and proficiency tests.

The use of nanotechnologies and the presence of nanoparticles in FAs require new analytical methods for their analysis and characterisation in food. Enforcing the recent ban on titanium dioxide (E171) as an FA requires new food analysis approaches. Recently, nanoparticles in other FAs, such as E551 and E174, have been found and characterised, necessitating further developments in this area.

The challenge is further compounded by the trend of re-formulated products where (synthetic) additives are abandoned and replaced by ingredients originating from natural sources. However, molecules in these "natural-sourced" ingredients are also authorised as FAs (lemon juice for citric acid E330). With current methods, it is almost impossible to distinguish between using a natural ingredient and adding the same component as a FA.

Overall, the presentation will emphasise the imperative for advanced analytical methodologies, new metrology approaches, and sustained investigation to effectively navigate the complexities of food additives monitoring.

**Keywords:** food additives, nanoparticles, metrology, multi-methods

## LECTURES

### L126

#### VALIDATION AND APPLICATION OF AN LC-ESI-MS/MS MULTI-COMPOUND METHOD SIMULTANEOUSLY DETERMINING SIX B-VITAMINS IN A BROAD RANGE OF FOODS

Zylla Marie Oane<sup>(1)</sup>, Annbjørg Bøkevoll<sup>(1)</sup>, Susanna Marie Petrova<sup>(1)</sup>, Tone Myhra<sup>(1)</sup>, Eystein Oveland\*<sup>(1)</sup>

<sup>1)</sup> *Institute of Marine Research, Norway*

\**Corresponding author - E-mail: eystein.oveland@hi.no*

B-vitamins are important micronutrients for humans, as well as for the animals, plants, and microorganisms integral to food production. Our objective is to develop a standardized analytical method capable of accurately determining multiple B-vitamins across diverse food matrices using mass spectrometry. We have developed a versatile LC-ESI-MS/MS triple quadrupole method that simultaneously analyzes six B-vitamins in a broad range of foods: B1 (thiamine), B2 (riboflavin), B3 (nicotinamide, nicotinic acid), B5 (pantothenic acid), B6 (pyridoxine, pyridoxal, pyridoxamine) and B7 (biotin). The method is also applicable to animal feed, as monitoring supplements is important to ensure animal welfare, growth, and ultimately high-quality meat. LC-ESI-MS/MS is a powerful analytical tool enabling discrimination and quantification of several vitamins and their different vitamin forms in the same analytical run. The method introduced is optimized for a variety of food matrices covering high levels of fat, protein, and carbohydrates. The analytical performance characteristics (LOQ, trueness, reproducibility) from our single lab validation was mostly within acceptable ranges according to NMKL procedures. The method is currently being investigated in AOAC International and ISO as a new standard for simultaneously analyzing several B-vitamins in a broad range of foods. With the advent of newer methodology, a revised consensus on reporting B-vitamin content will be essential, particularly due to the varying activity of different vitamin forms.

**Keywords:** B-vitamins, mass spectrometry, LC-ESI-MS/MS, standardization

## L127

**DETERMINATION OF BIOACTIVE COMPOUNDS IN 19 ITALIAN VARIETIES OF CHILI PEPPERS BY MEANS OF LIQUID CHROMATOGRAPHY HIGH RESOLUTION MASS SPECTROMETRY AND IN VITRO ENZYME INHIBITORY EFFECT**

**Cinzia Cafarella**<sup>(1)</sup>, **Francesca Rigano**<sup>(1)</sup>, **Aristeidis S. Tsagkaris**<sup>(2)</sup>, **Anna Louckova**<sup>(2)</sup>, **Katia Arena**<sup>(1)</sup>, **Luigi Mondello**<sup>(3)</sup>, **Paola Dugo**<sup>(3)</sup>, **Jana Hajslova**<sup>(2)</sup>

<sup>1)</sup> *Messina Institute of Technology c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Italy*

<sup>2)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

<sup>3)</sup> *Messina Institute of Technology and Chromaleont s.r.l. c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, former Veterinary School, University of Messina, Italy*

\*Corresponding author - E-mail: [cicafarella@unime.it](mailto:cicafarella@unime.it)

Chili peppers, the fruits of plants from the genus *Capsicum* within the Solanaceae family, originate from the Americas and have become essential to the cuisine and culture of numerous regions worldwide. They have attracted considerable attention for their potential health benefits, primarily due to a range of phytochemicals, particularly phenolic compounds, which are believed to exhibit various biological activities. This study aims to comprehensively elucidate the phenol profiles of 19 distinct chili pepper varieties cultivated in Italy and to evaluate their inhibitory effects on enzymes with significant biochemical functions. The samples were macerated in deionized H<sub>2</sub>O or EtOH at 4°C for 24, filtered and the extracts were analyzed by Ultra-High-Performance Liquid Chromatography High Resolution Mass Spectrometry (UHPLC-HRMS) to monitor their phytochemical composition by applying a targeted metabolomic screening. Moreover, "in-house" developed assays were performed to evaluate any bioactivity potential based on the activity of acetylcholinesterase (AChE, involved in the cholinergic system), butyrylcholinesterase (BChE, involved in the cholinergic system), pancreatic lipase (PNLIP, involved in dietary triglyceride metabolism),  $\alpha$ -glucosidase (GLU, involved in monosaccharide hydrolysis) and tyrosinase (TYR, involved in skin pigmentation). Additionally, the total antioxidant capacity and the total phenolic content (TPC) were measured using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay and a Folin-Ciocalteu assay, respectively. The most abundant compounds identified included capsaicin, dihydrocapsaicin, luteolin, apigenin, and quercetin derivatives. Furthermore, the results demonstrated that the ethanol extracts contained a considerably greater number of polyphenol compounds compared to the aqueous extracts. In terms of enzyme inhibition, the ethanol extracts exhibited stronger biological activities than the aqueous extracts. In detail, the tested extracts were classified to three categories demonstrating i) mild (70%) inhibition against each enzyme. Dose-response curves (6.25-100 mg/mL) were prepared for the extracts inducing a strong effect (>70%) and half maximal concentrations (IC<sub>50</sub>) were in the range 15-69 mg/mL. Importantly, the Pearson correlation coefficients (r) were calculated, and strong correlations were noticed (r>0.5), e.g., between GLU and BChE inhibition induced by ethanolic extracts. By elucidating the connections between the phytochemical composition and functional attributes of these widely consumed products, this study will contribute to a better understanding of the nutritional and health significance of incorporating diverse chili pepper cultivars into the human diet.

**Keywords:** chili pepper, HPLC-HRMS, chemical composition, biological activity, enzyme inhibition

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### L128

#### ADVANCED CHARACTERIZATION OF NOVEL CANNABIS CULTIVARS

**Matej Maly**\*<sup>(1)</sup>, **Frantisek Benes**<sup>(1)</sup>, **Zuzana Binova**<sup>(1)</sup>, **Federica Pollastro**<sup>(2)</sup>, **Stefano Salomone**<sup>(2)</sup>, **Gianpaolo Grassi**<sup>(3)</sup>, **Vincenzo Disca**<sup>(2)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> University of Piemonte Orientale, Italy

<sup>3)</sup> Canvasalus Srl, Italy

\*Corresponding author - E-mail: matej.maly@vscht.cz

*Cannabis sativa* L., one of the oldest medicinal plants, has been intensively studied over the last 70 years. Initially, research focused on delta-9-tetrahydrocannabinol ( $\Delta^9$ -THC) and cannabidiol (CBD), two major secondary metabolites with profound antioxidant, anti-inflammatory, and neuroprotective effects. Recently, however, the scientific interest has shifted towards other secondary metabolites, hundreds of which occur in *Cannabis* plants and many of them possess interesting biological activities. The characterization and quantification of these 'emerging' compounds, in some cases present at low concentrations, is a significant analytical challenge.

In collaboration with the University of Eastern Piedmont and Canvasalus Breeding Research Company, we have characterized ethanolic extracts from the dried inflorescences of five new *Cannabis* cultivars which were classified by targeted UHPLC-HRMS/MS analysis (39 phytocannabinoids quantified) as CBD, CBG and CBC dominating. The fifth cultivar (chemotype V) contained only negligible amounts of phytocannabinoids (> 0.1% w/w), on the other hand, the target screening against extensive library indicated high content of non-phytocannabinoid compounds. For more in-depth investigation, an ethanolic extract prepared from 100 grams of this chemotype was subjected to preparative chromatography, the isolated compounds were then further purified and characterized by NMR. The most interesting compounds represented by bioactive flavonoids such as cannflavin A, canniprene, and a newly identified 5-methoxy-dihydrodenbinobin, could be quantified in all extracts. Surprisingly, the concentration of the latter compound (not detected in the CBD and CBG cultivars) was even an order of magnitude higher in the CBC dominating cultivar compared to 'cannabinoids free' cultivar. This discovery led to further investigations using preparative chromatography on the CBC cultivar to isolate other derivatives of 5-methoxy-dihydrodenbinobin, which are expected to have potent anti-tumor properties. Overall, the extreme variability among *Cannabis* cultivars results in diverse pharmacological effects, and the ongoing evolution of breeding techniques, along with advanced analysis, promises new treatments and a deeper understanding of unique *Cannabis* plants.

**Keywords:** *Cannabis sativa* L., phytocannabinoids, UHPLC-HRMS/MS, preparative chromatography, flavonoids

## L129

**SUPPORTING THE OFFICIAL SENSORY EVALUATION OF VIRGIN OLIVE OILS WITH INNOVATIVE STRATEGIES BASED ON VOLATILE COMPOUNDS ANALYSIS: THE ROLE OF HS-GC-IMS AND MACHINE LEARNING**

**Enrico Valli**<sup>(1)</sup>, Rosalba Tucci<sup>(2)</sup>, Ilaria Grigoletto<sup>(2)</sup>, Enrico Casadei<sup>(1)</sup>, Chiara Cevoli<sup>(1)</sup>, Silvia Mingione<sup>(3)</sup>, Nicola Balzani<sup>(3)</sup>, Sara Barbieri<sup>(2)</sup>, Cesare Rossini<sup>(4)</sup>, Alessandro Battaglia<sup>(4)</sup>, Fabio Battaglia<sup>(4)</sup>, Andrea Giomo<sup>(5)</sup>, Alessandra Bendini<sup>(1)</sup>, Tullia Gallina Toschi<sup>(1)</sup>

<sup>1)</sup> Department of Agricultural and Food Sciences, Interdepartmental Centre for Industrial Agrofood Research; Alma Mater Studiorum - Università di Bologna, Italy

<sup>2)</sup> Department of Agricultural and Food Sciences; Alma Mater Studiorum - Università di Bologna, Italy

<sup>3)</sup> Olitalia S.r.l., Italy

<sup>4)</sup> LabService Analytica, Italy

<sup>5)</sup> Andrea Giomo, MLI-Cloud, Italy

\*Corresponding author - E-mail: enrico.valli4@unibo.it

Olive oil companies, as well as public and private laboratories, need to rapidly assess the quality and purity of olive oil samples. Such high-throughput screening methods can successfully contribute to improve the overall efficiency of official controls. Sensory analysis of virgin olive oils (performed through a well-established and effective method, known as Panel test) plays a pivotal role in quality control but suffers from a few drawbacks: it requires a panel of trained experts who need to meet in the same equipped room, a limited number of samples can be evaluated per day, and assessors may be affected by sensory fatigue. In this framework, a non-destructive screening method based on gas chromatography coupled with ion mobility spectrometry (HS-GC-IMS) for the analysis of volatile compounds, with the application of machine learning and chemometric techniques, can represent a useful tool to support the Panel test. It may be exploited as a routine method both through targeted and untargeted approaches. A set of around 100 commercial virgin olive oils was sensory assessed by five different panels and robustly classified, applying a decision tree based on the panels' agreement, previously set-up and published. Moreover, the volatile fraction of these oils was analysed in five laboratories by HS-GC-IMS with a targeted approach in which fifteen relevant molecules, as monomers and dimers, were considered to build separate predictive models. The commercial category of each oil was predicted based on volatile compounds profiles using PLS-DA models, with a focus on the borderline samples between the extra virgin and virgin, as they are particularly relevant in the real quality control scenario. The herein-described inter-laboratory study showed satisfactory results: most of the samples were correctly classified by the models in the same quality grade with respect to robust sensory classification. For the untargeted approach, 198 commercial virgin olive oils were sensory assessed by a professional panel. Then, the same oils were analysed by HS-GC-IMS and the data were extracted using the CSV mode from the .MEA file. Pre-processing is based on the transformation of the CSV file (1818 rows x 4500 columns wide) to an image. The images, each for every sample, were processed using the machine learning approach consisting of training, testing (to determine the performance index), and validation of the model. Different boosting and bagging techniques were compared with traditional statistics, both on classification and regression modes. All computations were executed in Python 3 environment using Scikit-learn 1.5.0 and OpenCV 4.10.0 libraries. The herein-discussed findings, together with previous investigations that will be briefly presented, support the use of HS-GC-IMS with machine learning and chemometrics as a useful tool to support the Panel test in the olive oil quality control.

**Keywords:** extra virgin olive oil, volatile compounds, sensory analysis, HS-GC-IMS, machine learning

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## LECTURES

### L130

## ANALYSIS OF VOLATILES AND $\alpha$ -DICARBONYL COMPOUNDS IN MAILLARD REACTION PRODUCTS DERIVED FROM 2'-FUCOSYLLACTOSE AND AMINO ACIDS

Kwang-Geun Lee\*<sup>(1)</sup>

<sup>1)</sup> *Department of Food Science and Biotechnology, Dongguk University, South Korea*

\**Corresponding author - E-mail: kwglee@dongguk.edu*

This study investigated the volatile and alpha-dicarbonyl compounds ( $\alpha$ -DCs) produced during Maillard reactions between 2'-fucosyllactose (2'-FL) and various amino acids. The effects of pH, temperature, reaction time, and amino acid concentration on  $\alpha$ -DC production were evaluated. Fucose generated the most  $\alpha$ -DCs, whereas 2'-FL produced the least.  $\alpha$ -DC formation increased with increasing pH, reaction time, temperature, and amino acid concentration. Among the amino acids evaluated, threonine elicited the highest  $\alpha$ -DC production. In total, 50 volatile compounds were identified, with 2'-FL and lactose primarily forming furan and furan derivatives. In particular, 2'-FL yielded greater amounts of 2-furfural, 2-acetylfuran, 5-methylfurfural, furfuryl alcohol, and 2-furanmethanol than other monosaccharides. These findings highlight the potential of 2'-FL as a flavouring agent and enhance our understanding of  $\alpha$ -DC formation during food processing and storage.

**Keywords:** human milk oligosaccharide, 2'-fucosyllactose, Maillard reaction,  $\alpha$ -dicarbonyl compounds, volatile

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## LECTURES

### L131

## UNTARGETED BENCHTOP VOLATILOMICS AT TRACE LEVELS IN FOODS AND FERMENTATIONS - WHY THE GAS PHASE IS SO MUCH MORE THAN HOT AIR

Philipp Weller\*<sup>(1)</sup>

<sup>1)</sup> *Institute for Instrumental Analytics, Faculty of Biotechnology, Mannheim University of Applied Sciences, Germany*

*\*Corresponding author - E-mail: p.weller@hs-mannheim.de*

The term "volatilomics" addresses the comprehension of the volatilome, which depicts an important, but yet scarcely understood part of the metabolome - in systems biology, but also in other fields, such as food analysis. Here, omics approaches are often also called "foodomic" strategies and meanwhile are an integral part in food authentication and quality control. Volatilomics are an elegant way of correlating volatile organic compounds (VOCs) via the gas phase from the matrix with specific properties of that product: authenticity, quality or provenance are just a few examples. This is in particular relevant, as a major part of the aroma-relevant compounds in foods, e.g. in roasted coffee, citrus oils or saffron belong to the VOC fraction, which can be analyzed without sample contact by using the headspace over the sample.

A major challenge is the complexity of the enormous number of different substances found, which often are not relevant as individual species, but rather their total "fingerprint", resulting of all amenable substances. This high-dimensional spectral information cannot be interpreted without applying powerful machine learning algorithms or chemometrics. These data are combined with modern machine learning techniques to extract the maximum possible information from products to improve quality and confirm authenticity.

VOCs are also an important source of information in fermentation processes. As integral part of modern biotechnology, they feature an enormously complex gas phase, which is so far not part of existing, molecule-specific monitoring strategies.

Typically, the required techniques are laboratory-based and not useable at the so-called "point-of-need", which limits their use. In this context, gas chromatography - ion mobility spectrometry (GC-IMS) is one of the most promising emerging benchtop techniques of the last years, in particular, when paired with modern machine learning and deep learning algorithms.

This presentation will demonstrate principles and examples of benchtop "volatilomics" approaches in food and fermentation processes, that in the future could be used directly at the location where they are needed.

**Keywords:** ion mobility spectrometry, foodomics, machine learning, gas chromatography, process analytics

## LECTURES

### L132

## ENTOMETABOLOMICS OF EDIBLE INSECT SPECIES REARED UNDER DIFFERENT CONDITIONS

**Katerina Sebelova**<sup>\*(1)</sup>, **Katerina Sonntag**<sup>(1)</sup>, **Ondrej Pospisil**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: sebelovk@vscht.cz*

Edible insects represent an appealing source of future food with the potential to cover the increasing global need for nutrients. In the EU, 4 species have been authorized as a novel food (Regulation (EU) 2015/2283). However, there are still many gaps in the availability of comprehensive laboratory strategies enabling further improvement of insect-based product quality, safety and/or authenticity assessment and bridging these attributes with rearing conditions.

In the presented study, a total of 10 insect species (including *Acheta domesticus*, *Tenebrio molitor*, *Locusta migratoria*, and *Alphitobius diaperinus*) obtained from different local farmers were collected over the course of two years. These samples differed not only in rearing conditions but also in the way of killing. Non-target screening, entometabolomics, based on U-HPLC-HRMS/MS followed by multivariate analysis of generated data was employed to characterize both polar and non-polar extracts. Safe distinguishing of individual species was achieved, with polar extracts resulting in tighter clustering. Moreover, it was possible to identify the rearing sites of a given species, due to the association between metabolome and particular feeding conditions. Potential markers of species and killing methods were selected. For example, dopamine-3-O-sulfate, a compound associated with cuticle sclerotization, which was detected as a marker of insects in the adult development stage. The selected markers were used to establish the HRMS/MS spectral library which is essential for testing marker selectivity. In case of nonpolar extracts, lipidomic screening against MS-Dial lipids library illustrated the differences in lipid composition on feed (for example, lipids with bound fatty acid 20:5 were detected in cricket samples fed fish or insect meal). The possibility of enhancing the nutritional value of insects by feeding them bioactive compound-rich feed has been documented. Not only the increase /profile change of carotenoids, tocopherols, and essential fatty acids in the mealworm tissues was observed when their sources such as carrot, spinach, and sunflower seeds were added to the feed, but also the change in the entire metabolome and lipidome was recorded. The results of this study demonstrate the potential of entometabolomic analysis as a challenging tool complementing conventional other omics currently used in laboratories concerned with this 'new' food commodity.

**Keywords:** edible insects, entometabolomics, high resolution mass spectrometry

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## LECTURES

### L133

#### TRANSLATION OF DART-HRMS TOOLS FROM THE BENCH TO THE INDUSTRIAL POULTRY PRODUCTION: A QUICK GUIDANCE FOR THE STANDARDIZATION OF POULTRY MEAT PIGMENTATION

**Alessandra Tata**<sup>\*(1)</sup>, Carmela Zacometti<sup>(1)</sup>, Andrea Massaro<sup>(1)</sup>, Sonia Falappa<sup>(2)</sup>, Simona Ceroni<sup>(2)</sup>, Salvatore Catania<sup>(1)</sup>

<sup>1)</sup> *Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

<sup>2)</sup> *Fileni Alimentare SPA, Italy*

*\*Corresponding author - E-mail: atata@izsvenezie.it*

Broiler feed ingredients undergo changes according to fluctuations of market prices, therefore, costs of feed ingredients, leading to high feed costs, can determine the replacement of some components of the ration. Unfortunately, in some cases, a rough check of these nutrient macro-categories is insufficient to avoid intestinal performance issues, which can lead to dysregulated energy metabolism or variations of the intestinal absorption capabilities of the broilers. As a proof of principle, in this diagnostic/prognostic study, direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) was used in an untargeted manner to analyze fresh poultry tissues of pigmented and non-pigmented broilers to investigate the metabolic causes of lack of pigmentation in an industrial poultry farm. Statistical analysis was applied to the DART-HRMS data to retrieve the molecular features that characterize the poultry meat of pigmented and non-pigmented broilers. Higher abundance of oxidized lipids, high abundance of oxidized bile derivatives, and lower levels of lipophilic vitamins, such as tocopherol isomers (Vitamin E) and retinol (Vitamin A), were captured in non-pigmented than in the meat of pigmented broilers. In this study, the DART-HRMS system performed well in retrieving valuable chemical information from broilers that explained the differences between the two groups of broilers in absorption of the lipophilic xanthophylls and the subsequent lack of proper broiler pigmentation in affected broilers. This study provided data that allowed the farm management to: i) increase the amount of administered Vitamin E to compensate for the oxidation of lipids observed in final products, ii) evaluate the possibility of restoring the use of sunflower oil in the feed, iii) improve control of the feed granulation to prevent oxidation (small fine grains ( $\leq 2$  mm) oxidize faster than bigger grains ( $> 2$  mm)), and iv) evaluate the use of anti-oxidant emulsifiers in the feed. In addition, conventional rapid analyses were used: i) color parameters of pigmented and non-pigmented broilers were measured to rationalize the color differences in abdominal fat, leg skin and leg muscle, and ii) macronutrients were determined in broiler leg muscle, to capture a detailed picture of the pathology and exclude other possible causes. The results suggest this technology could be useful in providing near real-time feedback to aid in veterinary decision-making in poultry farming and improve quality and standardization of the final product for the consumers.

**Keywords:** translational science, DART-HRMS, poultry meat, from lab to real life, industrial poultry production

### L134

## QUECHUP A COMPREHENSIVE SAMPLE PREPARATION PARTICULARLY RELEVANT FOR THE FOOD EXPOSOME CHARACTERIZATION

**Julien Parinet**\*<sup>(1)</sup>, Thierno Diallo<sup>(1)</sup>, Yassine Makni<sup>(1)</sup>, Thierry Guerin<sup>(2)</sup>

<sup>1)</sup> ANSES, Laboratory of Food Safety, France

<sup>2)</sup> ANSES, Strategy and Programmes Department, France

\*Corresponding author - E-mail: julien.parinet@anses.fr

Since 2005 and Christopher Wild's famous article [1], a great deal of work has been carried out to better understand the impact of the mixtures of contaminants to which we are exposed throughout our lives. The contaminants present in our bodies come from a variety of sources, of which food is one of the main ones. However, characterizing the diversity of contaminants present in food matrices is a major public health challenge, because we now know, through the study of cocktail effects, that the effects of even low-level substances can be significant and difficult to anticipate. It is therefore essential to develop analytical methods that are as exhaustive as possible, i.e. capable of extracting and detecting the widest possible range of contaminants to which we are exposed. In the face of these many challenges, we can rely on high-resolution mass spectrometry and its three main approaches: "targeted", "suspected" and "non-targeted". However, although these instruments enable us to search for traces of unexpected contaminants without *a priori*, we still need to ensure that we have extracted the contaminant(s), their metabolites and any by-products that could potentially affect our health. For this purpose and in order to characterize as broadly as possible the diversity of contaminants likely to be present in a sample, while reconciling practicality, low economic cost, preparation time and efficiency, we have developed, in the frame of AlimOmic project ([www.alimomic.fr](http://www.alimomic.fr)), the QueChUP method.

The QueChUP method is a sample preparation based on the logic of the well-known QuEChERS extraction procedure [2] brilliantly developed by Anastassiades and colleagues in 2002. However, unlike the QuEChERS method, QuEChUP includes the principle of sequential extraction with a minimum of two sequences employing two different solvents (or solvent mixtures). This adaptation enables us to extract a greater diversity of contaminant typologies, as it is based on the difference in affinity between an analyte of interest and a solvent. We therefore evaluated and applied QuEChUP to different types of matrices (fish products and baby food purees) and to a wide range of contaminants (pesticides, veterinary products, alkaloids, mycotoxins, PFAS) in order to demonstrate the suitability of our extraction method. As a result, we achieved much higher identification rates with QuEChUP than with QuEChERS.

[1] Wild C, P. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology 2005. *Cancer Epidemiol Biomarkers Prev*.

[2] Anastassiades M, Lehotay SJ, et al. Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach for the determination of pesticide residues 2002. *European Pesticide Residues Workshop, EPRW, Rome, Book of Abstracts*.

**Keywords:** sample preparation, contaminants, food, exposome

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## L135

**ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-ION MOBILITY-HIGH-RESOLUTION MASS SPECTROMETRY METABOLOMICS ON PIG ORGAN EXPOSED TO ANTIBIOTICS FOR FOOD SAFETY ASSESSMENT**

**Nicolo Riboni\***<sup>(1)</sup>, **Maurizio Piergiovanni**<sup>(1)</sup>, **Maria Pia Fabbri**<sup>(2)</sup>, **Monica Mattarozzi**<sup>(1)</sup>, **Antonella Cavazza**<sup>(1)</sup>, **Maria Olga Varra**<sup>(2)</sup>, **Giovanni Loris Alborali**<sup>(3)</sup>, **Federico Scali**<sup>(3)</sup>, **Adriana Ianieri**<sup>(2)</sup>, **Sergio Ghidini**<sup>(4)</sup>, **Emanuela Zanardi**<sup>(2)</sup>, **Federica Bianchi**<sup>(5)</sup>, **Maria Careri**<sup>(5)</sup>

<sup>1)</sup> *Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy, Italy*

<sup>2)</sup> *Department of Food and Drug, University of Parma, Italy*

<sup>3)</sup> *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy*

<sup>4)</sup> *Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy*

<sup>5)</sup> *Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy*

\*Corresponding author - E-mail: nicolo.riboni@unipr.it

The growing demand for antibiotic-free food requires both the use of effective analytical strategies in monitoring raw materials and the improvement of production processes. Although most of analytical methods are based on targeted approaches [1], comprehensive evaluation of the metabolomic changes related to the use of antibiotics requires the application of untargeted strategies. In the context of a growing interest in metabolomics, ultra-high-performance liquid chromatography-ion mobility-high-resolution mass spectrometry (UHPLC-IMS-HRMS) represents the best tool to study the complexity of the metabolome. In addition to the enhanced specificity, sensitivity and availability of large spectral databases of UHPLC-HRMS, IMS can improve the depth of coverage by separating ions according to their collisional cross-section (CCS).

In this study, UHPLC-IMS-HRMS was applied for the metabolite profiling of pig organ meat (liver, kidney and diaphragm), either treated or untreated with antibiotics. Spectra were acquired in the positive and negative electrospray ionization modes, operating in data independent High-Definition MSE acquisition mode, allowing for the simultaneous acquisition of CCS values, precursor and fragment ions [2]. To control the quality of metabolomics data of meat samples, a strategy for data filtering was based on RSD within QCs samples 0.8. The obtained spectra were matched with those stored in online libraries, including Human Metabolome Database, Food Metabolome Database, LIPID MAPS, KEGG, Drugbank Food and Drug Administration database. A mass tolerance of 5 and 10 ppm for precursor and fragment ions respectively, the fragmentation pattern, and an isotope similarity >80% were used for annotation. In addition, the experimental CCS of the selected features was compared with the calculated value obtained using AllCCS2 algorithm [3] with a tolerance of 5%. The features responsible for the discrimination between antibiotic-free and positive samples were annotated, identifying 48 metabolites. A similar approach is in progress for the analysis of kidney and diaphragm. Finally, selected biomarkers will be evaluated to develop targeted quantitative methods for safety assessment of antibiotic-free meat.

[1] T. Ramatla et al., *Antibiotics* 2017, 6, 34, doi:10.3390/antibiotics6040034.

[2] N. Riboni et al., *J. Agric. Food Chem.*, 2023, 71, 15407-15416, doi: 10.1021/acs.jafc.3c04532.

[3] H. Zhang et al., *J. Anal. Chem.*, 95 (2023) 13913-13921, doi: 10.1021/acs.analchem.3c02267.

**Keywords:** metabolomics, high-resolution mass spectrometry, ion mobility spectrometry, ultra-high performance liquid chromatography, antibiotic-free meat

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### L136

#### **FIRST PROOF OF CONCEPT: APPLYING UNTARGETED METABOLOMICS AND MOLECULAR NETWORKING VIA LC-DDA-HRMS/MS TO HIGHLIGHT QUALITY AND REACTION MARKERS IN PROCESSED FOODS**

**Soha Farah<sup>(1)</sup>, Mathieu Cladiere<sup>\*(1)</sup>, Melina Ramos<sup>(1)</sup>, Even Le Roux<sup>(1)</sup>, Barbara Rega<sup>(1)</sup>, Valerie Camel<sup>(1)</sup>**

<sup>1)</sup> *Université Paris Saclay, INRAE, AgroParisTech, UMR SayFood, France*

*\*Corresponding author - E-mail: mathieu.cladiere@agroaparistech.fr*

Characterizing the chemical fingerprint of a processed food is a major challenge to advance food quality. New untargeted approaches based on LC-HRMS analysis can unlock the potential to increase our understanding on how chemical components linked to different food quality dimensions react and evolve during the manufacturing processes, thus contribute to food quality design.

This project addresses that actual challenge in food analytical chemistry and aims to develop such new approach on a range of baked products (sponge cakes) generated under controlled processing parameters (time and temperature of cooking) to generate a diversity of chemical profiles. Our approach is based on LC-data-dependent-acquisition-HRMS/MS (LC-ddA-HRMS/MS) analysis to evaluate whether the use of molecular networks can assist us in better understanding the reactional links between compounds.

Before proceeding with this approach, we wanted to ensure that sponge cake preparation is repeatable and that we can distinguish between our sponge cakes based on their processing conditions. This was confirmed by PCA test.

We then developed the LC-ddA-HRMS/MS acquisition method by optimizing various analytical parameters such as Top N and exclusion time with a data treatment workflow on MZmine. Finally, we optimized the parameters for computing a molecular network on GNPS2 to see if we could highlight reactional links between compounds.

The resulting molecular network revealed three main groups within a cluster, which included precursors of the Maillard reaction, such as sugars and amino acids. These groups are linked to clusters of reaction products, as they are more present in the most intensely processed products, but they are not yet identified.

To the best of our knowledge, this proof of concept is the very first one demonstrating that untargeted metabolomics approach can be applied to processed foods, showing that molecular networks could help us to better understand reactional links with processing and formulation. This is also the first time this method is applied to such a complex matrix, which marks the originality of our work. Continuing our analysis could provide valuable insights into the chemical transformation occurring during the processing of food products.

**Keywords:** untargeted, molecular network, processed food, chemical fingerprint, method development

## LECTURES

### L137

#### **NMR METABOLOMICS TO STUDY THE EFFECT OF DIFFERENT DRYING TECHNIQUES ON EDIBLE INSECTS: THE CASE STUDY OF ACHETA DOMESTICUS (HOUSE CRICKET)**

**Mattia Spano**<sup>\*(1)</sup>, **Giuseppina Adiletta**<sup>(2)</sup>, **Irene Ferri**<sup>(3)</sup>, **Stefania Garzoli**<sup>(1)</sup>, **Paola Russo**<sup>(2)</sup>, **Luisa Mannina**<sup>(1)</sup>

<sup>1)</sup> *Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Italy*

<sup>2)</sup> *Department of Chemical Engineering Materials Environment, Sapienza University of Rome, Italy*

<sup>3)</sup> *Department of Veterinary Medicine and Animal Sciences (DIVAS), University of Milan, Italy*

*\*Corresponding author - E-mail: mattia.spano@uniroma1.it*

In recent years, an even growing need of alternative and sustainable food sources is occurring. In this context, edible insects, traditionally used in different areas of the world, have been introduced in Europe as Novel Foods in 2019.

However, several aspects regarding these matrices have to be explored in-depth, namely nutritional and safety properties, as well as the effects of technological treatments on these profiles.

In this study, *Acheta domesticus* samples at two different stages of the vital cycle (nymph and adult) were subjected to different drying methodologies namely freeze-drying, oven, and microwave (using several parameters) to analyze the potential changes that occur in the metabolite profile, thus affecting the nutritional properties.

Proximal analysis and NMR metabolomics were applied to define both macronutrients profile and metabolites one.

NMR analysis allowed to detect several classes of metabolites namely sugars, amino acids, organic acids, fatty acids, sterols, and other compounds whose qualitative and quantitative features were strongly affected by both vital cycle stage and drying processes.

The obtained results underlined how also in the case of edible insects, production and processing practices are important to be studied since, as for "typical" foodstuffs, they strongly define chemical and nutritional properties of these innovative matrices. In the aim of improving the knowledge regarding edible insect nutritional properties and safety, this information represents an important starting point.

**Keywords:** edible insects, NMR metabolomics, drying processes

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## LECTURES

### L138

## RECENT DEVELOPMENTS AND OUTLOOK OF EU POLICY ON CONTAMINANTS WITH A FOCUS ON RELATED ANALYTICAL CHALLENGES

Frans Verstraete\*<sup>(1)</sup>

<sup>1)</sup> *European Commission, Belgium*

\*Corresponding author - E-mail: [Frans.Verstraete@ec.europa.eu](mailto:Frans.Verstraete@ec.europa.eu)

The EU legislation on contaminants Council Regulation (EEC) No 315/93 of 8 February 1993 provides that food containing a contaminant in an amount which is unacceptable from the public health viewpoint shall not be placed on the market (food can only be placed on the market when it is safe). Furthermore, it is foreseen that contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain and in order to protect public health, maximum levels for specific contaminants shall be established where necessary.

Following requests of the European Commission, the Panel on Contaminants in the Food Chain (CONTAM) from the European Food Safety Authority (EFSA) has completed in recent years several scientific opinions on contaminants in feed and food, reviewing the possible risks for animal and human health due to the presence of these substances in feed and food.

In the presentation, recent and future developments on EU legislation on contaminants in food shall be presented. Climate change, changes in dietary patterns, novel/new foods, Green deal policies entail new challenges for the safety of the food chain. In addition, in order to ensure a high level of food safety it is necessary not to address single contaminants individually but also address more attention to the combined exposure to multiple contaminants.

These new developments entail analytical challenges for an effective EU policy on contaminants in food. Indeed, for an effective risk management and enforcement, it is not only sufficient that a method of analysis is available, but the method of analysis must also be reliable, sensitive, quick and preferably cheap.

**Keywords:** contaminant, EU policy, legislation, analytical challenges

### L139

## ADDRESSING EXPOSOME CHALLENGES WITH STREAMLINED FOOD SAMPLE PREPARATION BASED ON SUPRAMOLECULAR SOLVENTS

Noelia Caballero-Casero\*<sup>(1)</sup>, Ana Ballesteros-Gomez<sup>(1)</sup>, Soledad Rubio<sup>(1)</sup>

<sup>1)</sup> University of Córdoba, Spain

\*Corresponding author - E-mail: a42caasn@uco.es

Currently, the approach to assessing the risk of chemical exposure in populations has evolved. Instead of considering the effects of individual chemical compounds on health, studies now focus on the cumulative exposures a person experiences throughout their life. Among the multiple sources of exposure, diet is undoubtedly one of the primary pathways for potential chemical risks. Analyzing the dietary exposome is crucial for understanding how these exposures affect human health. However, the complexity and variability of food matrices, along with the high number of structurally different contaminants, pose a significant analytical challenge where sample treatment is a key element. This treatment must ensure efficient multi-extraction of compounds across a wide polarity range and the elimination of interferences while being sustainable, simple, fast, and cost-effective. Supramolecular solvents (SUPRAS) offer multiple opportunities for developing innovative sample treatment platforms for the targeted and non-targeted analysis of contaminants in food samples by liquid chromatography-mass spectrometry (LC-MS). SUPRAS are nanostructured liquids generated from colloidal solutions of amphiphilic molecules through spontaneous self-assembly and coacervation processes. The synthesis of SUPRAS is spontaneous, atomically efficient, requires minimal or no energy, and can be carried out in situ in liquid samples with which they are immiscible. These solvents provide microenvironments of different polarities, multiple binding sites, high surface area, and the ability to tailor their properties by selecting the amphiphilic molecule or the coacervating agent.

This communication aims to present the main advances in using SUPRAS to develop analytical platforms for food analysis, enabling the determination of multi-analytes in multi-matrices by LC-MS. The intrinsic properties of SUPRAS that allow the efficient extraction of multi-analytes and the progress made in designing SUPRAS with restricted access properties enabling the removal of major sample macrocomponents, will be discussed. Additionally, recent applications developed by our research group in food analysis will be presented. These include the extraction of bisphenols and oxy-PAHs from solid and liquid food matrices, as well as the extraction of multiple structurally unrelated contaminants, both regulated (e.g., mycotoxins) and unregulated (e.g., emerging contaminants) in very different matrices, combining the use of SUPRAS with targeted and non-targeted analysis. Thus, SUPRAS-LC-MS analytical platforms have enabled the development of innovative strategies that simplify the assessment of potential chemical exposure through diet for the population.

**Keywords:** supramolecular solvents, sample treatment, multi-analyte extraction, food analysis, dietary exposome

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## LECTURES

### L140

#### CHALLENGES IN QUANTITATIVE VOLATILOMICS OPEN NEW OPPORTUNITIES IN FOOD QUALITY ASSESSMENT: THE ROLE OF MULTIDIMENSIONAL ANALYTICAL PLATFORMS

Andrea Caratti<sup>(1)</sup>, Angelica Fina<sup>(2)</sup>, Fulvia Trapani<sup>(2)</sup>, Simone Squara<sup>(2)</sup>, Erica Liberto<sup>(1)</sup>, Chiara Cordero\*<sup>(1)</sup>, Stephen E Reichenbach<sup>(3)</sup>, Qingping Tao<sup>(4)</sup>, Daniel Geschwender<sup>(4)</sup>

<sup>1)</sup> Department of Drug Science and Technology, University of Turin, Italy

<sup>2)</sup> University of Turin, Italy

<sup>3)</sup> University of Nebraska - Lincoln, United States of America

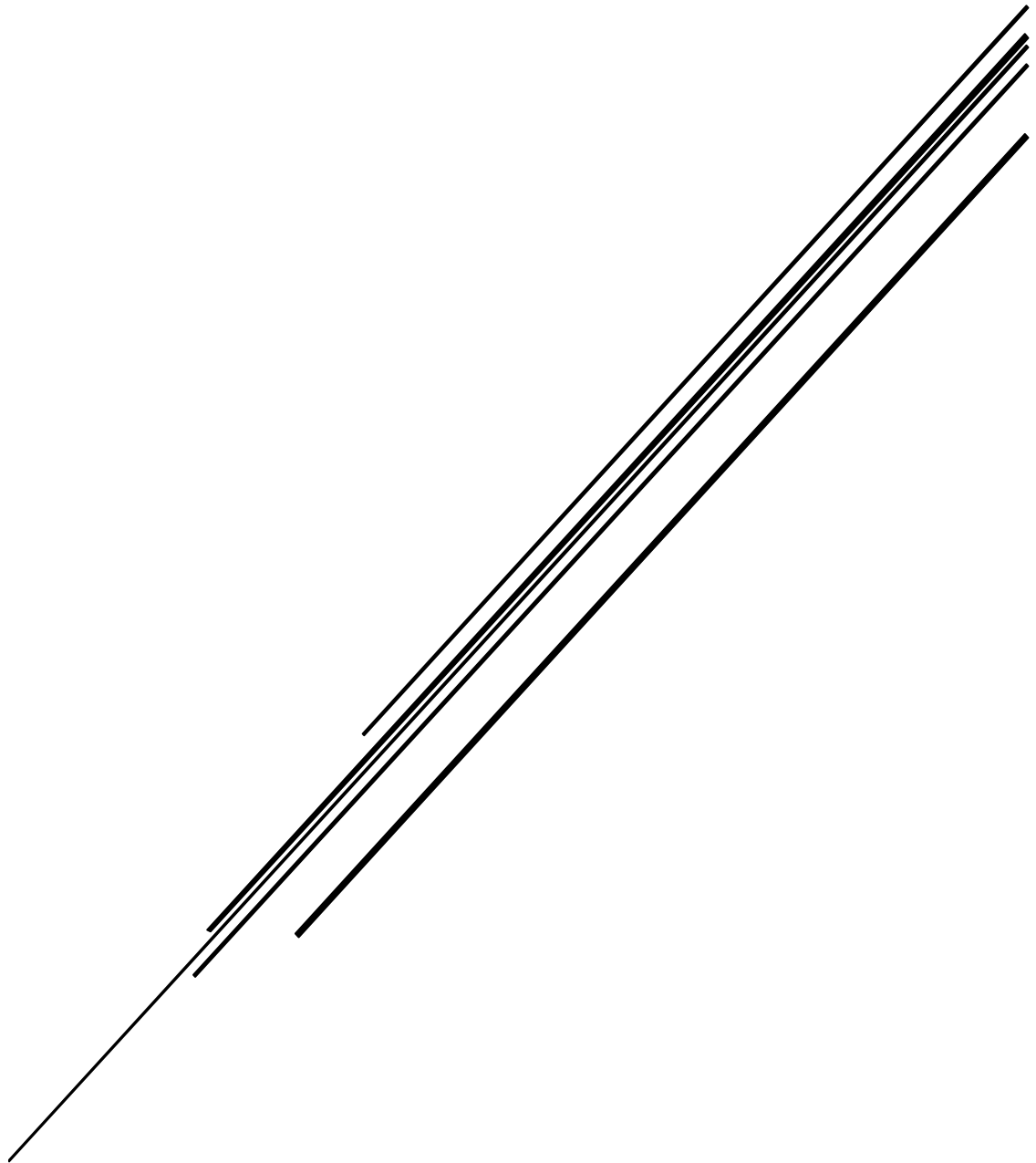
<sup>4)</sup> GC Image, United States of America

\*Corresponding author - E-mail: chiara.cordero@unito.it

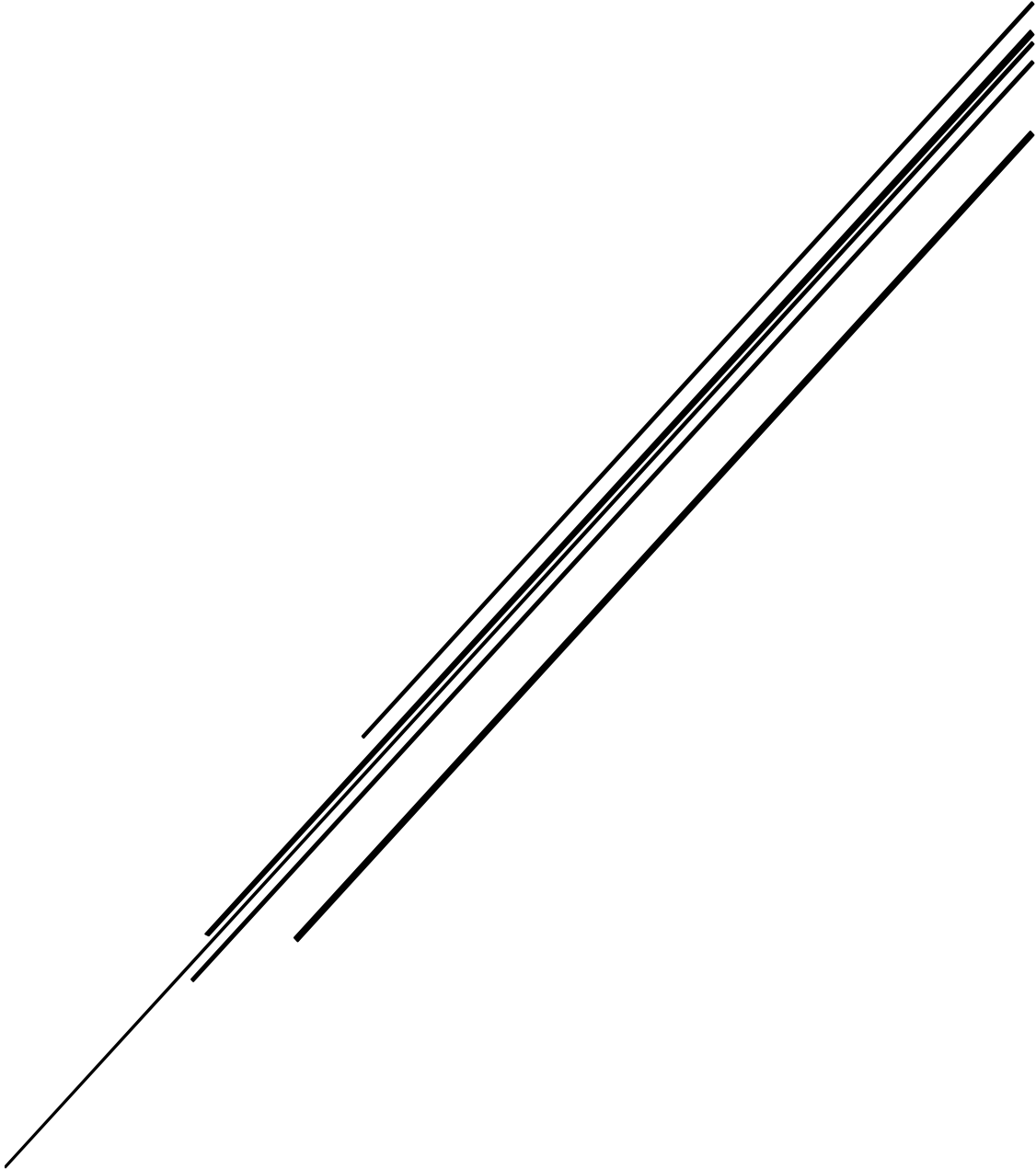
Effective investigation of food volatilome using comprehensive two-dimensional gas chromatography with parallel detection by mass spectrometry and flame ionization detector (GC×GC-MS/FID) provides valuable insights related to industrial quality. However, the lack of accurate quantitative data hinders the transferability of results over time and across laboratories. This study employs quantitative volatilomics using multiple headspace solid phase microextraction (MHS-SPME) on a large selection of hazelnut samples (*Corylus avellana* L.) representative of the top-quality selection desired by the confectionery industry. Through untargeted and targeted fingerprinting based on image pattern recognition robust classification models validate the significance of chemical patterns strongly correlated with quality parameters such as botanical and geographical origin, post-harvest practices, and storage time and conditions. By quantifying marker analytes, Artificial Intelligence (AI) tools are developed, including augmented smelling based on sensomics with blueprints related to key-aroma compounds and spoilage odorants; decision-makers for rancidity levels and storage quality; and origin tracers. Reliable quantification allows AI to be applied confidently, potentially driving industrial strategies.

**Keywords:** comprehensive two-dimensional gas chromatography, accurate odorants quantitation, Artificial Intelligence decision-makers, quantitative fingerprinting, aroma blueprint

# POSTER SESSIONS



# ALLERGENS



# ALLERGENS

## A1

### **UNINTENDED ALLERGEN PRESENCE (UAP): CROSS-CONTACT, CONTAMINATION OR FRAUD. REAL-LIFE CASES FROM THE FOOD INDUSTRY**

**Marleen Voorhuijzen<sup>\*(1)</sup>, Nathalie Smits<sup>(1)</sup>, Andries Koops<sup>(1)</sup>, Yvonne Westphal<sup>(1)</sup>**

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: marleen.voorhuijzen@wur.nl

The European Union (EU) states that consumers must be informed about the intentional use of any of the 14 priority allergens. In case of prepacked foods this entails clear indication on the list of ingredients and for unpacked foods consumers must be informed otherwise. Despite this regulation, Wageningen Food Safety Research (WFSR) receives regularly inquiries for laboratory analysis from the Dutch Food and Consumer Product Safety Authority when a consumer experienced an allergic reaction to an unlabelled food product. Such reaction can be caused by undeclared or unintended allergen presence (UAP) and can be the result of cross contact, contamination or even fraud. UAP is a major cause of food allergy or intolerance incidents in sensitised consumers. Although PAL (Precautionary Allergen Labelling, e.g. 'this product may contain') was introduced to protect consumers from UAP, currently no harmonised regulation regarding PAL (when to use, how to phrase it) is in place within the EU. This lack of regulation results in many unsubstantiated disclaimers by producers, which in turn leads to a limitation in food choice for affected consumers and an increased risk of incidents. To show the importance of harmonised regulation and monitoring of PAL to minimize the risk of UAP and thereby the risk of incidents, here recent examples of real-life cases of consumed foods that resulted in hospitalization of allergic consumers are given. In addition to regulation and harmonisation, these examples underline the importance of taking a multidisciplinary approach consisting of protein-based methods ELISA and LC-MS, and DNA-based methods qPCR and sequencing for detection, identification, and possible source-tracing of UAP presence in commercially available food products.

# ALLERGENS

## A2

### **MULTI-ALLERGEN QUANTIFICATION IN FOOD USING CONCATEMER-BASED ISOTOPE DILUTION MASS SPECTROMETRY: A COLLABORATIVE STUDY**

**Maxime Gavage**<sup>(1)</sup>, **Kaatje Van Vlierberghe**<sup>(2)</sup>, **Marc De Loose**<sup>(2)</sup>, **Christof Van Poucke**<sup>(2)</sup>, **Anne-Catherine Huet**<sup>(3)</sup>, **Nathalie Gillard**<sup>(3)</sup>

<sup>1)</sup> CER Groupe, Belgium

<sup>2)</sup> ILVO Flanders Research Institute for Agriculture, Fisheries and Food, Belgium

<sup>3)</sup> CER Groupe, Analytical Laboratory, Belgium

\*Corresponding author - E-mail: m.gavage@cergroupe.be

Food allergen analysis is crucial for effective allergen management and ensuring accurate food labeling, thereby protecting consumers with allergies. The need for robust, specific, and sensitive detection methods has led to the increasing adoption of mass spectrometry (MS) over the past decade. MS primarily analyzes specific peptides from enzymatically digested proteins extracted from food samples. Recently, several MS-based methods for detecting single or multiple food allergens have been developed, with some demonstrating validated sensitivity and accuracy in single-laboratory settings.

Despite the advancements, inter-laboratory validation remains a critical step toward standardizing food allergen analysis methods. While inter-laboratory studies using immunoassays or DNA-based techniques exist, there has been a notable absence of such studies for MS-based methods.

The "Allersens" project was a 4-year research program funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment in 2016. During the course of this program, we developed and validated an MS-based method for quantifying four major food allergens—egg, milk, peanuts, and hazelnuts—in processed food products. The quantification strategy was based on standard addition method, and stable isotope-labelled concatemer was used as the internal standard.

To assess the feasibility of harmonization across analytical laboratories, we engaged nine European laboratories in a collaborative study using our method. Participants received the analytical procedure, blank and incurred cookie matrices, standards, and the stable isotope-labelled concatemer internal standard. The study evaluated the method's selectivity, sensitivity, trueness, and precision based on the collected quantification results.

Our findings demonstrate the potential of this MS-based method and quantification strategy for food allergen analysis. Practical challenges associated with food allergen analysis in a routine analytical lab were highlighted. Notwithstanding these challenges, multiple participants were able to detect and quantify the different allergens with sensitivity and accuracy in agreement with AOAC INTERNATIONAL *Standard Method Performance Requirements* (SMPR® 2016.002). The encouraging results of this pioneering interlaboratory study represent an additional step towards harmonization among laboratories testing for allergens.

**Keywords:** food allergen, mass spectrometry, concatemer internal standard, collaborative study, harmonization

**Acknowledgement:** *The research that yielded these results was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 15/10 ALLERSENS.*

# ALLERGENS

## A3

### DETECTION OF FOOD ALLERGENS IN MICROBIAL FERMENTATION FOOD PRODUCTS USING UNTARGETED LC-HRMS PROTEOMICS

**Christof Van Poucke**<sup>\*(1)</sup>, **Marie-Alice Fraiture**<sup>(2)</sup>, **Nancy H.C. Roosens**<sup>(2)</sup>, **Rik Orval**<sup>(2)</sup>, **Frederic Debode**<sup>(3)</sup>, **Mathieu Dubois**<sup>(4)</sup>, **Nathalie Gillard**<sup>(4)</sup>, **Marc De Loose**<sup>(1)</sup>

<sup>1)</sup> ILVO Flanders Research Institute for Agriculture, Fisheries and Food, Technology and Food Science Unit, Belgium

<sup>2)</sup> Sciensano, Transversal Activities in Applied Genomics (TAG), Belgium

<sup>3)</sup> CRA-W Walloon Agricultural Research Centre, Life Sciences, Belgium

<sup>4)</sup> CER Groupe, Analytical Laboratory, Belgium

\*Corresponding author - E-mail: [Christof.vanpoucke@ilvo.vlaanderen.be](mailto:Christof.vanpoucke@ilvo.vlaanderen.be)

Within an aging and an on health focused society the consumption of food supplements and vitamins is constantly rising. Many of the ingredients used in these products are produced through microbial fermentation. Because of the direct intake and the fact that these products are often consumed by elderly people or persons with a weakened health, their purity is of primordial importance. In previous research however different unexpected impurities (genetical modified organisms, allergens, ...) were detected.

Currently to check for each of these biological possible impurities a plethora of methods is needed as often specific targeted methods are used. Within the ENSURED project (RF 22/6359) an innovative and universal open strategy to detect different impurities of biological origin are being developed. Developed methods will focus on either the genomic or the proteomic level to look at the possibility to monitor microbial fermentation products for these different biological impurities.

At the proteomic level, in a first step the possibility of untargeted bottom-up proteomics using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS) to detect food allergens (focusing on soy during method development) was investigated. Using different food supplements and vitamins in different galenic forms (tablets, capsules, liquid, powders) the sample preparation was optimised in function of the recovery.

Next this optimised extraction protocol was evaluated for its ability to allow the untargeted detection of food allergens in food supplements at different concentrations. Different acquisition modes (data dependent (DDA) and data independent acquisition (DIA)) were compared and different bio-informatic tools and software criteria were evaluated to minimize false positive results.

**Keywords:** food allergens, food supplements, untargeted analysis

**Acknowledgement:** *The research that yielded these results is funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RF 22/6359 ENSURED.*



# ALLERGENS

## A4

### IDENTIFICATION OF GLUTEN-SPECIFIC DNA MARKERS

**Tamara Kutateladze**<sup>\*(1)</sup>, Tamar Koberidze<sup>(2)</sup>, Kakha Bitskinashvili<sup>(1)</sup>, Nelly Datukishvili<sup>(2)</sup>, Boris Vishnepolsky<sup>(1)</sup>

<sup>1)</sup> Ivane Beritashvili Center of Experimental Biomedicine, Georgia

<sup>2)</sup> Ilia State University, Georgia

\*Corresponding author - E-mail: kutateladzet@yahoo.com

Foods containing gluten are among the most important allergens and can cause significant health disorders. Gluten proteins determine the allergenicity of crops such as wheat, barley, rye, and couscous. To prevent the disease, sensitive individuals should not use the allergenic product. In addition, gluten proteins are also closely related to the baking quality of wheat flour and the quality of pasta and other processed products made from wheat. Therefore, reliable identification of gluten in foods is essential for food safety assessment, health protection, and efficient food production technology. Gluten proteins consist of two main groups, glutenins and gliadins. This study focused on wheat (*Triticum aestivum*) high molecular weight (HMW) glutenin subunit gene and gamma- and omega-5 gliadin genes. Several sets of oligonucleotide primers targeting these genes were designed using bioinformatics tools. DNeasy plant mini kit (Qiagen) was applied for genomic DNA extraction. A conventional polymerase chain reaction (PCR) method was used for DNA amplification. PCR products were evaluated by agarose gel electrophoresis. After optimization of PCR conditions, the best markers for the HMW glutenin subunit, gamma- and omega-5 gliadins genes were identified. Examination of other plant species has confirmed the high specificity of wheat gluten DNA markers. The results indicate that the new efficient PCR-based DNA markers allow accurate identification of wheat glutenin and gliadin and can be successfully used for gluten control in food.

**Keywords:** high molecular weight (HMW) glutenin subunit gene, gamma- and omega-5 gliadin genes, polymerase chain reaction (PCR), DNA markers

**Acknowledgement:** This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [Grant STEM-22- 637].

## ALLERGENS

### A5

#### EFFECT OF FOOD PROCESSING ON PCR-BASED DETECTION OF MAIZE AND WHEAT ALLERGENS

**Tata Ninidze**<sup>(1)</sup>, **Tamar Koberidze**<sup>(1)</sup>, **Kakha Bitskinashvili**<sup>(2)</sup>, **Tamara Kutateladze**<sup>(2)</sup>, **Boris Vishnepolsky**<sup>(2)</sup>, **Nelly Datukishvili**<sup>(1)</sup>

<sup>1)</sup> *Ilia State University, Georgia*

<sup>2)</sup> *Ivane Beritashvili Center of Experimental Biomedicine, Georgia*

\*Corresponding author - E-mail: [tata.ninidze.1@iliauni.edu.ge](mailto:tata.ninidze.1@iliauni.edu.ge)

Maize (*Zea mays*) and wheat (*Triticum aestivum*) are widespread and important cereal crops worldwide. In the food industry, they are often used as grains and flours and are also present as a main source or ingredient in various processed products. However, wheat and corn are recognized as strong food allergens. Avoiding allergenic foods is the only way to prevent allergic diseases. Therefore, allergen detection is crucial for food safety and health protection, accurate labelling, and consumer information. This study aimed to evaluate the influence of food processing on maize and wheat allergen genes for the development of effective allergen detection methods. For this purpose, uniplex and multiplex polymerase chain reaction (PCR) methods were applied to amplify fragments of different sizes of allergen genes. Wheat and maize grains, flour, and various processed products were investigated. Food products were bought from supermarkets and also prepared in the laboratory under different conditions. Wheat low molecular weight (LMW) glutenin as well as three important maize allergens namely chitinase (Zea m 8), phospholipid transfer protein (Zea m 14), and zein were investigated. A comparison of DNA extraction methods revealed the CTAB method as a highly efficient method for isolating amplifiable genomic DNA from processed food samples. Agarose gel electrophoresis of PCR products revealed allergen genes in most of the products tested. However, the amplified fragments were degraded according to the severity and duration of food processing. The outcomes demonstrated that the detection of allergens depends on food matrices, forms of processing, efficiency of primers, and amplicon length. Testing of various processed products revealed the best PCR methods for detecting maize and wheat allergens in highly processed foods. The results indicate that the PCR methods described in this study can be used to assess allergens in a food safety monitoring system.

**Keywords:** maize allergens, PCR, allergen detection, food processing

**Acknowledgement:** *This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [Grant STEM-22- 637].*

## ALLERGENS

### A6

#### COMPARISON OF FIVE ELISA KITS FOR THE DETERMINATION OF SOY IN PEA-BASED PRODUCTS

Anne-Catherine Huet<sup>(1)</sup>, Amandine Lamote<sup>(2)</sup>, Rogier De Back<sup>(3)</sup>, Frederic Mansy<sup>(3)</sup>, Nathalie Gillard<sup>(1)</sup>

<sup>1)</sup> CER Groupe, Analytical Laboratory, Belgium

<sup>2)</sup> CER Groupe, Belgium

<sup>3)</sup> Cosucra, Belgium

\*Corresponding author - E-mail: [ac.huet@cergroupe.be](mailto:ac.huet@cergroupe.be)

Under the Regulation EU 1169/2011 on the provision of food information to consumers, the mandatory labelling of substances or products causing allergies or intolerances have been established. Amongst the 14 listed, we find soybeans and soy-derived products; whilst not using any soy or soy-derived products on their production sites, the problem of soy-detection still directly concerns food industries like Cosucra, producing pea-based products.

As analytical laboratory and National Reference Laboratory, CER Groupe evaluated the commonly used ELISA method for the detection of soy in a series of products such as several varieties of pea, faba bean and Pisane® (commercial pea-protein). The aim of this work was to compare the performances and fitness for purpose of five commercial ELISA kits, able to detect and quantify soy allergens. A quality control has been added to each assay to complete the comparison. The evaluated kits with corresponding providers are as follows: AgraQuant Soy 10002015 (Romer Labs), RidaScreen FAST Soya R7102 (R-Biopharm), Soya ELISA kit II M2117 (Morinaga), Soy protein residue Enhanced assay Essoyprd 48 (ELISA Systems) and Veratox for soy allergen 8410 (Neogen).

The results will be revealed in detail in the present communication, some ELISA kits are definitively not adapted for detecting soy in pea-based products, due to known cross-reactivity (R-Biopharm) or targeting only native proteins (ELISA Systems). The kit provided by Morinaga showed interferences for the matrix Pisane® but not for the pea as ingredient while no interferences for this specific matrix were observed with the kit developed by Neogen.

This comparative study showed that food allergen analysis remains a real challenge for the agrofood industry and the related fields of food analysis; this also has an impact on consumers and food agencies.

# ALLERGENS

## A7

### PROFICIENCY-TESTING SCHEME FOR HISTAMINE DETECTION IN FISHERY PRODUCTS

Sabrina Bouakache<sup>\* (1)</sup>, Abdelkader Boubetra<sup>(1)</sup>, Antoine Gorse<sup>(1)</sup>, Anne Tirard<sup>(1)</sup>

<sup>1)</sup> BIPEA, France

<sup>\*</sup>Corresponding author - E-mail: sbouakache@bipea.org

Histamine, a biogenic amine, is a toxin generated by bacteria in the fish's tissues. Histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. Thus, this metabolite is an indicator of fish quality and a biomarker for quality control during the food production and transportation.

The number of laboratories performing the analyses of histamine has gradually increased in recent years. There are various analytical methods available for quantifying histamine in food samples, with most relying on chromatographic analysis. These latter are considered more conventional compared to enzymatic method which is a valuable alternative for laboratories [1].

Bipea set up a regular proficiency-testing scheme intended to the detection and quantification of histamine in fishery products. Homogeneous and stabilized samples of naturally or artificially contaminated fishes were prepared and shipped to the laboratories that were required to return their results indicating the applied methods. The statistical treatment of the data was performed by BIPEA according to ISO 13528 standard [2]. Assigned (consensus) values were calculated from the participants' results according to comparable methods and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 standard [3].

The collected results enable a comparison of histamine quantification based on the analytical method performed. The assigned values obtained from the enzymatic method are likely aligned with those calculated from the conventional methods, although these methods are very different in principle [4].

Participation in proficiency testing schemes is a requirement of ISO 17025 standard to ensure that laboratory results are validated by external controls.

[1] Karen Rodríguez-Núñez and al. Enzymatic detection of histamine: Applications, challenges, and improvement potential through biocatalyst engineering, *Food Control*, Volume 162, 2024, 110436, ISSN 0956-7135. <https://doi.org/10.1016/j.foodcont.2024.110436>.

[2] ISO 13528:2022, Statistical methods for use in proficiency testing by interlaboratory comparisons, 2022.

[3] ISO 17043:2023, Conformity assessment -- General requirements for proficiency testing, 2023.

[4] Jie Yu and al. Advances in technologies to detect histamine in food: Principles, applications, and prospects - Trends in Food Science & Technology, Volume 146, April 2024, 104385-<https://doi.org/10.1016/j.tifs.2024.104385>.

**Acknowledgement:** *We extend our sincere thanks to all the laboratories that participated in the interlaboratory tests, which allowed us to conduct this study.*

## ALLERGENS

### A8

#### VALIDATION STUDY OF LATERAL FLOW DEVICE FOR DETECTING SHELLFISH PROTEINS

Takao Kurata<sup>(1)</sup>, Kenichi Aburatani<sup>(1)</sup>, Daichi Tanaka<sup>(1)</sup>, Masatoshi Sakai<sup>(1)</sup>, Masayoshi Tomiki<sup>(1)</sup>, Takako Nishi<sup>\*(1)</sup>

<sup>1)</sup> Morinaga BioScience Inc., Japan

\*Corresponding author - E-mail: t-nishi-ja@morinaga.co.jp

Various seafood is consumed worldwide, and seafood-related food allergies are also widely known. Regarding seafood-related allergies, crustaceans are the most well-known allergen, and the symptoms can develop later in life. Crustaceans, such as shrimp, crab, and lobster, are considered priority food allergens worldwide, including in the United States, the EU, and many other countries. Therefore, it is mandatory in many countries to state on the food package label when any crustaceans are used as an ingredient. Mollusks, such as squid, octopus, and oyster, are also known as seafood-related food allergens. Declaring them on food package labels is also mandatory in many countries, like Australia, Canada, and the EU. Crustaceans and mollusks are collectively referred to as shellfish, and they contain similar proteins (tropomyosin), the major crustacean proteins causing food allergies.

We developed a lateral flow device testing kit named the Rapid Test Pro II for Shellfish that can detect crustaceans and mollusks protein in one device. The composition of the extraction solution used in this kit is the same as our Food Allergen ELISA Kit II series, which has excellent extraction efficiency, ensuring reliable test results. Moreover, since the extraction solution composition is the same across all our allergen test kits, the same extracted solution can be exploited to test multiple allergens.

In this study, we evaluated the detection range on many kinds of seafood, the reactivity to incurred foods, and the cross-reactivity to various foods. When we tested various seafood samples, our kit could detect all shellfish samples in this experiment; however, the non-shellfish samples, like some fish, were negative results. In the incurred food validation, all samples had positive results. These results showed that our Rapid Test Pro II for Shellfish is suitable for detecting shellfish proteins in various foods.

**Keywords:** lateral flow, crustacean, seafood, test kit, allergen

## ALLERGENS

### A9

#### ALLERLIST: A PROOF-OF-PRINCIPLE DATA SPACE TO IMPROVE POST-MARKETING MONITORING OF POTENTIAL NEW FOOD ALLERGENS

**Christof Van Poucke**<sup>(1)</sup>, **Maxime Gavage**<sup>(2)</sup>, **Femke Van Zwynsvoorde**<sup>(1)</sup>, **Samuel Bosch**<sup>(1)</sup>, **Anne-Catherine Huet**<sup>(2)</sup>, **Romy Gaddiseur**<sup>(3)</sup>, **Isabel Taverniers**<sup>(1)</sup>, **Marc De Loose**<sup>(1)</sup>, **Nathalie Gillard**<sup>(2)</sup>

<sup>1)</sup> ILVO Flanders Research Institute for Agriculture, Belgium

<sup>2)</sup> CER Groupe, Analytical Laboratory, Belgium

<sup>3)</sup> CHU Liège, Clinical Chemistry Department, Belgium

\*Corresponding author - E-mail: [christof.vanpoucke@ilvo.vlaanderen.be](mailto:christof.vanpoucke@ilvo.vlaanderen.be)

With a growing world population, where forecasts predict a total number of about 10 billion people by 2050, the need to provide enough healthy and sustainable food products to feed the world is becoming more and more clear. This implies that the classical protein consumption pattern, mainly consisting of meat and fish, will need to be diversified with new alternative protein sources.

These new alternative protein sources entail amongst others different nutritional, functional, food safety and sensory challenges which need to be addressed prior to introduction onto the market. An important part of the food safety aspect lies in food allergenicity, as with these alternative proteins it might be expected that new allergens might be introduced. This could be caused by both cross-reactivity with existing allergens as well as *de novo* sensitisation.

Current tools for evaluating new potential food allergens are flawed and lack the ability to predict *de novo* sensitization. The Allerlist project aims to develop an innovative workflow that integrates two interconnected databases—one for protein data and the other for clinical data—alongside immunological and *in vitro* digestibility assays. This system will link *in silico* properties of new protein sources with laboratory data and clinical outcomes to facilitate the identification of emerging allergens. As a proof of concept, the workflow will be applied to three legumes: lentil, pea, and chickpea, based on a preliminary literature review. The ultimate goal is to evaluate whether this approach can be used as a post-marketing tool to detect new potential food allergens and assist regulatory bodies in assessing the need for mandatory labelling of new or emerging food ingredients.

**Keywords:** food allergens, protein diversification

**Acknowledgement:** The research that yielded these results is funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RF 23/01 ALLERLIST.

# ALLERGENS

## A10

### 2023-2024 ALLERGENS IN FOOD MONITORING IN LOMBARDIA AND EMILIA ROMAGNA REGIONS (ITALY), THE EXPERIENCE OF FOOD CONTROL DEPARTMENT, IZSLER, BRESCIA

**Barbara Bertasi**<sup>(1)</sup>, **Laura Ragni**<sup>(1)</sup>, **Renato Bertolassi**<sup>(1)</sup>, **Sara Arnaboldi**<sup>(1)</sup>, **Marina Nadia Losio**<sup>(1)</sup>, **Enrico Pavoni**<sup>(1)</sup>

<sup>1)</sup> *Food Safety Department, IZSLER, Italy*

*\*Corresponding author - E-mail: barbara.bertasi@izsler.it*

The label compliance respect to the ingredients' list in food products is a great concern for safety of allergic subjects. The onset of an allergic event and the severity of the symptoms are highly variable and can be caused by the presence of small quantities of allergenic molecules. Environmental contamination can occur in plants with multiple production lines and sometimes voluntary addition of undeclared materials is done for economic reasons. To date, in the considered regions, there is no a specific governmental sampling plan (still under improvement); however, many producers check voluntarily their products; in addition other samples are analysed for local authorities on demand. During the passive monitoring period, 2021 different food types, labelled as allergens-free, were analysed for one or more targets; in details 1450 samples were checked for gluten (gluten-free samples contain 20 mg/Kg); in this case 89% of positive samples were Italian products. Also, 4% of samples were positive for other allergens. Among these, 38% were positive for  $\beta$ -lactoglobulin (sweets, pasta, meat, gastronomy goods, 38 % were positive for egg proteins (swabs, sweets, sauces), and 17% for nuts (sweets, biscuits and gastronomy goods). For  $\beta$ -lactoglobulin, positives were distributed between Italian and imported products (50%); for egg proteins 56% of positives were Asiatic foods. The presence of undeclared allergens can be explained by different reasons, associated also with the different origins of the products. The trace contamination in Italian products can be supposed mostly due to incorrect management/separation of different production lines in the same plant. In products of Asian origin, the problem can also be increased by translation issues and the lack of international rules for product labelling. In any case the data obtained underline the importance of allergen controls on domestic and imported foods.

**Keywords:** monitoring, gluten, egg, lactoglobulin

# ALLERGENS

## A11

### LC-HRMS/MS FOR DETECTION OF MUSTARD AND GLUTEN IN BAKERY PRODUCTS

Sabina Noviello<sup>\*1)</sup>, Manila Bianchi<sup>2)</sup>, Sara Morello<sup>2)</sup>, Chiara Dalvit<sup>1)</sup>, Giancarlo Biancotto<sup>1)</sup>, Roberto Stella<sup>1)</sup>

<sup>1)</sup> Istituto Zooprofilattico Sperimentale delle Venezie, Italy

<sup>2)</sup> Istituto Zooprofilattico Sperimentale Delpiemonte Liguria E Valle D'Aosta, Italy

\*Corresponding author - E-mail: snoviello@izsvenezie.it

Food allergy is an important global public health issue with growing prevalence in the developed world and significant impact on patients' lives. In order to safeguard the health of consumers, according to Reg. 1169/2011, 14 allergens must be declared on the labels of food products in the European Union. The methods mainly used for detecting food allergens are ELISA and PCR methods, but both may suffer from limitations such as matrix effect, cross-reactivity and limited reproducibility. Such technical limitations may determine the impossibility of demonstrating the reliable absence of allergens. This is the case, for example, of mustard, the presence of which in several food commodities is hard to be proved, due to important cross-reactivity against *Brassicaceae*. So far, no reference methods for the detection of mustard are available in Italy, and this implies the need for producers to declare the potential presence of mustard in flour and bakery products. Hence, robust and reproducible instrumental methods for the specific identification of allergens in different food types are highly needed.

In this context, the present research is aimed at developing a targeted semi-quantitative bottom-up proteomics method based on LC-HRMS/MS operating in parallel reaction monitoring mode to detect gluten and mustard in food matrices, such as flour, cereals, and bakery product.

The protein sequence of the target allergens in the reference database (gliadin and glutenin for gluten, sin a1 for mustard), was subjected to an *in-silico* digestion to select characteristic marker peptides that uniquely identify the proteins of interest. We developed and validated an analytical protocol involving an extraction phase followed by filtration with amicon filters, reduction of disulfide bonds, alkylation of reduced cysteine residues, enzymatic digestion with trypsin and purification prior to LC-HRMS/MS analysis.

The validation study was conducted in three independent working sessions involving two operators and different types of food commodities, which were fortified at 10 µg/g with gluten and mustard for a total of 20 spiked samples. The specificity of the method was verified by analyzing 20 different blank samples attesting for the absence of interfering signals. The calibration points (0 and 10 µg/g) were prepared freshly in each working session by spiking gluten or mustard extract in negative sample extract. The identification of peptides was achieved by recording 4 precursor-to-product ion transitions for two specific peptides for gluten and one for mustard. The use of isotopically labelled internal standard peptides having the same sequences as the selected peptides, but containing <sup>13</sup>C and <sup>15</sup>N, allowed to reduce the analytical variability.

**Keywords:** gluten, mustard, allergens, LC-HRMS/MS

**Acknowledgement:** We thank the Italian Ministry of Health for financial support (project RC IZS VE 12/22 - CUP B23C22000960001)



## ALLERGENS

### A12

#### VALIDATION OF A HIGHLY SENSITIVE PEANUT ELISA KIT FOR DETECTING PEANUT IN PROCESSED FOODS

**Masayoshi Tomiki<sup>(1)</sup>, Mitsuko Takahashi<sup>(1)</sup>, Masatoshi Sakai<sup>(1)</sup>, Takao Kurata<sup>(1)</sup>, Takako Nishi<sup>\*(1)</sup>**

<sup>1)</sup> *Morinaga BioScience, Inc., Japan*

*\*Corresponding author - E-mail: t-nishi-ja@morinaga.co.jp*

Peanut allergy is a serious health concern, and its prevalence necessitates reliable methods for peanut detection in processed foods. Existing challenges include hidden allergens due to cross-contamination during manufacturing, where different products with varying ingredients share production lines. Additionally, processing can denature peanut protein, making it less detectable by conventional antibodies.

To address these challenges, we developed a highly sensitive ELISA kit specifically designed to detect peanut protein in both raw materials and processed foods. This kit utilizes a special extraction buffer and antibody combination to overcome the limitations of denatured proteins. We validated the performance of this kit by measuring its limit of detection, limit of quantification, and recovery rates in various processed food matrices. This validated kit provides a valuable tool for food manufacturers, regulatory agencies, and others involved in ensuring the safety of the food supply for peanut-allergic consumers.

**Keywords:** food allergen, peanut, ELISA, immuno assay

# ALLERGENS

## A13

### VALIDATION OF A GREEN EXTRACTION PROTOCOL FOR THE DETECTION AND QUANTIFICATION OF GLUTEN PROTEINS USING A LATERAL FLOW TEST

**Evanthia Angeli<sup>(1)</sup>, Dimitrios Foulos<sup>(1)</sup>, Theodora Kapsali<sup>(1)</sup>, Georgios Papageorgiou<sup>(1)</sup>, Antonios Ntantasios<sup>(1)</sup>**

<sup>1)</sup> ProGnosis Biotech, Greece

\*Corresponding author - E-mail: e.angeli@prognosis-biotech.com

Gluten consists of two primary groups of proteins: glutenins and prolamins. Prolamins, including gliadin in wheat, hordein in barley, and secalin in rye, are the alcohol-soluble fraction of gluten. Consumption of gluten can be harmful to individuals with gluten intolerance, such as those with celiac disease or wheat allergies. To protect these individuals, standards for food labelling have been established, classifying products as "gluten-free" when gluten content is below 20 mg/kg and "very low gluten" when it is below 100 mg/kg.

Lateral flow tests provide a reliable and efficient method for detecting gluten contamination in foodstuffs, helping to ensure that products meet these regulatory standards and safeguard consumer health.

Prognosis Biotech has developed a new lateral flow immunochromatographic assay, the Gluten Free Test (E19XX), which allows for the quantitative detection of gluten protein residues in food products, including those labelled as gluten-free, as well as in CIP (Cleaning-in-Place) solutions and on working surfaces.

The aim of this study was to evaluate the recovery levels of gliadin in spiked samples—comprising raw materials, commercially available products, and reference materials—using an organic solvent-free extraction buffer compared to a conventional organic solvent-based extraction protocol.

This innovative system uses a three-line lateral flow test combined with a reader device and standard curves to produce reliable quantitative results. Reference materials were used to establish these curves, while a simple extraction protocol, utilizing prefilled sample tubes, makes the method user-friendly.

For validation, gluten-free labelled samples were spiked at two different levels, including the limit of quantification (LOQ), with a gliadin solution. The samples were then extracted using both an ethanol-free extraction buffer and an ethanol-containing extraction buffer.

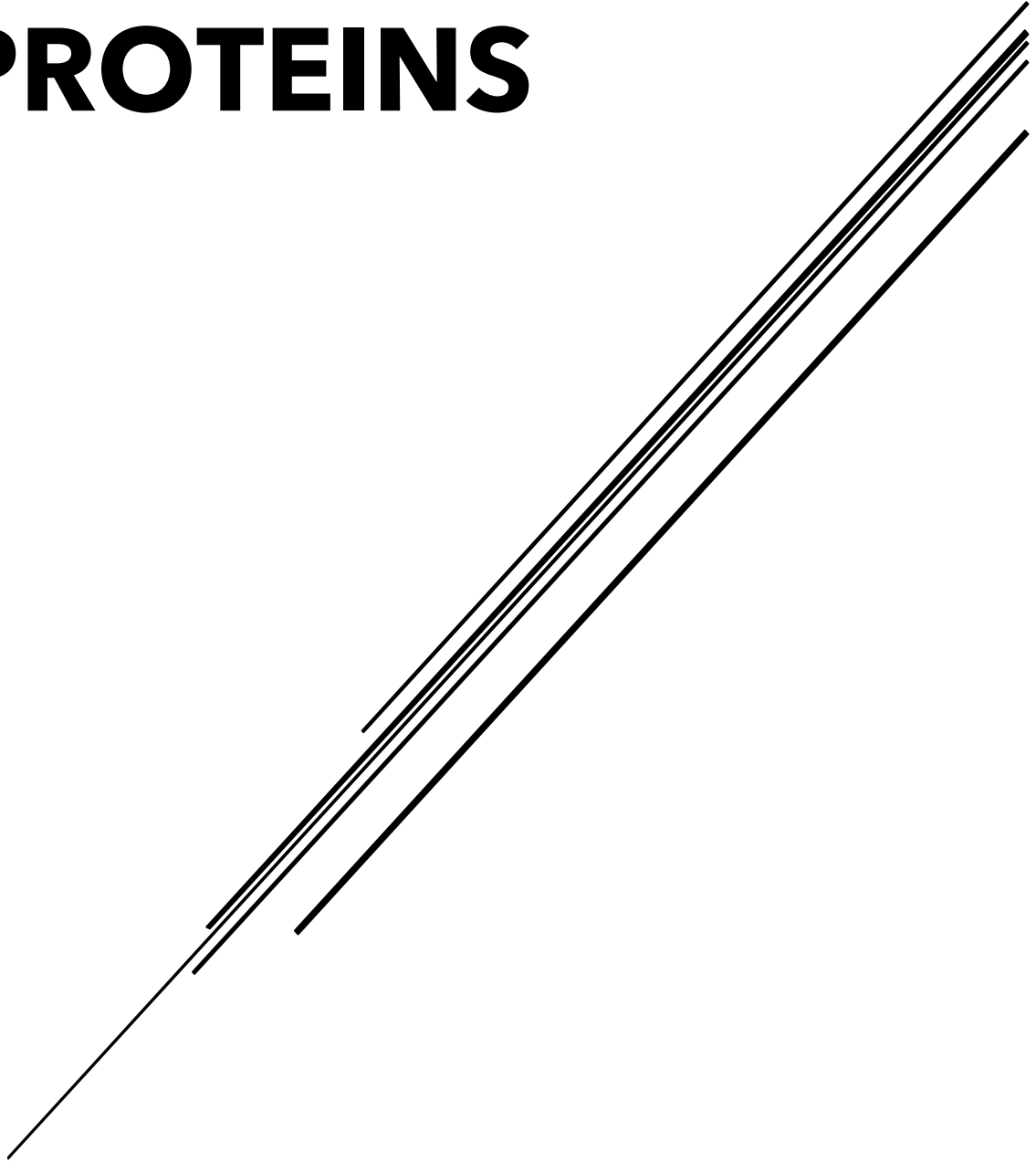
The test demonstrated robust recovery and coefficient of variation (CV) levels within the acceptable range in both cases.

The test has a limit of quantification (LOQ) of 5 ppm gluten/2.5 ppm gliadin for food samples and CIP solutions, while the limit of detection (LOD) for visual interpretation of qualitative results is 3 ppm and 0.5 µg/100 cm<sup>2</sup> for surface testing. The presence of a third (hook) line helps to prevent false-negative results due to extreme amounts of the allergen.

This test is fast, practical, and accurate, providing reliable quantification while saving time, making it an invaluable tool for ensuring the safety of gluten-free products.

**Keywords:** lateral flow test, allergen testing, green extraction, ProGnosis Biotech, gluten free test

# **ALTERNATIVE PROTEINS**



# ALTERNATIVE PROTEINS

## B1

### EVALUATION OF LOCUSTA MIGRATORIA PROTEIN DIGESTIBILITY

Marta Barberis<sup>(1)</sup>, Raffaella Colombo<sup>(2)</sup>, Adele Papetti<sup>\*(1)</sup>

<sup>1)</sup> University of Pavia & OnFoods Foundation Parma, Italy

<sup>2)</sup> University of Pavia, Italy

\*Corresponding author - E-mail: [adele.papetti@unipv.it](mailto:adele.papetti@unipv.it)

The interest about the use of edible insects is increasing day by day because they could be promising, healthy and sustainable source of high-quality protein to feed a large number of consumers (1). In this context, the evaluation of the insects' nutritional properties has become a priority since the European Food Safety Authority (EFSA) included them in the Novel Foods group. The definition of protein digestibility and amino acid bioaccessibility of insects is mandatory to define the nutritional quality of such nutrients in terms of amino acids composition, relative amino acids requirement, and amino acids digestibility in the upper gastrointestinal tract (2). Therefore, the aim of the present research project was to evaluate *Locusta migratoria* protein digestibility by a simulated *in vitro* digestion approach (3). An *in vitro* workflow based on the INFOGEST static digestion protocol was set up to investigate the digestibility of individual amino acids in oven-dried *Locusta migratoria* proteins (4-5) obtained by applying a microwave-assisted acid hydrolysis (MAAH). The simulated digestion included oral, gastric, and intestinal phases performed using simulated fluids consisting of proper electrolytes and enzymes mixtures (5). At the end of the digestion and after the enzymes inactivation, proteins and large peptides precipitation was performed and digestible (supernatant) and indigestible (pellet) fractions were obtained by subsequent centrifugation (6). A full factorial design for two level and three factors was used to identify and optimize MAAH conditions to be applied to the digestible fraction. The considered parameters of the hydrolysis process were time (20 - 120 min range), temperature (125 - 180°C range), and power (500 - 800 W range). The response expressed as hydrolysis yield was considered and monitored by HPLC-DAD analysis.

The full factorial design experiments identified the effects and interactions of the limiting parameters on the acid hydrolysis process. In particular time and temperature have a significant positive effect on the hydrolysis process, while power has an opposite effect on the hydrolysis yield. In addition, operating at the highest temperature for the longest time (180°C and 120 min) the maximum interaction was registered, and this results in the highest hydrolysis yield. Therefore, the optimized conditions for MAAH to be applied to the insect digestible fraction were 120 min, 180 °C, and 500 W.

The selection of the best condition to hydrolyze the indigestible fraction are ongoing to have a complete understanding of the total insect protein content.

**Keywords:** alternative proteins, *Locusta migratoria*, protein digestibility, *in vitro* digestion protocol

### B2

#### NOVEL PROTEINS IN THE EU: EXPLORING THE INTERPLAY BETWEEN RISK ASSESSMENT AND SOCIETAL INSIGHTS FOR COMMUNICATION

**Marcello Laganaro**<sup>(1)</sup>, **Giorgia Zamariola**<sup>(1)</sup>, **Domagoj Vrbos**<sup>(1)</sup>, **Esther Garcia Ruiz**<sup>(1)</sup>, **Maria Glymenaki**<sup>(2)</sup>, **Vania Mendes**<sup>(1)</sup>, **Alejandra Munoz Gonzalez**<sup>(1)</sup>, **Irene Nuin Garciaarena**<sup>(1)</sup>, **Gabriela Precup**<sup>(1)</sup>, **Ruth Roldan-Torres**<sup>(1)</sup>, **Ermolaos Ververis**<sup>(3)</sup>, **Andrea Germini**<sup>(1)</sup>, **Anthony Smith**<sup>(1)</sup>

<sup>1)</sup> European Food Safety Authority (EFSA), Italy

<sup>2)</sup> European Food Safety Authority (EFSA); Department of Metabolism, Digestion and Reproduction, Faculty of Medicine, Imperial College London, United Kingdom, Italy

<sup>3)</sup> European Food Safety Authority (EFSA); Dept. of Hygiene, Epidemiology and Medical Statistics, School of Medicine, National and Kapodistrian University of Athens, Greece, Italy

\*Corresponding author - E-mail: marcello.laganaro@efsa.europa.eu

In the European Union, foods and food ingredients not significantly consumed before May 1997 are considered novel. Current market developments and EU and national food policy strategies generate increasing interest in alternative protein sources. However, the acceptance of novel proteins is subject to consumers' knowledge and perception. The European Food Safety Authority (EFSA) is responsible for assessing novel foods' safety and communicating the outcomes to risk managers and consumers, empowering informed decisions.

This work outlines the scientific requirements for safety assessments of novel protein sources, including microorganisms, fungi, algae, insects, plants, and cell cultures. Tailored considerations are required based on the complexity, production process, and nutritional dietary contribution. Additionally, this work also explores public perception of these protein sources using social research data from literature and social media. Varying levels of knowledge and risk perception exist among consumers for different types of novel proteins.

To accommodate the different scientific and social aspects, targeted risk communication objectives are considered appropriate. Increasing public understanding and knowledge of risks through awareness-raising and presenting risk assessment findings according to the "enlightenment" communication objective is most appropriate for novel protein sources with low knowledge and risk perception from the public, such as insects, microorganisms, fungi, algae, and plants. Conversely, the "confidence-building" and "cooperative decision-making" objectives aim to establish or enhance trustful relationships between the industry and institutions and resolve existing or potential differences in views. These approaches are most appropriate for novel proteins derived from cell or tissue culture, which showed high knowledge and low risk perception from the public. Different risk communication approaches through past examples are illustrated.

Disclaimer: The views expressed in this work are those of the authors and should not be interpreted as representing the official position of EFSA. Thus, the present work is published under the sole responsibility of the authors and may not be considered as an EFSA scientific output. EFSA cannot be held accountable for any errors or inaccuracies that may appear.

**Keywords:** novel proteins, novel foods, risk communication, risk assessment, EFSA

## ALTERNATIVE PROTEINS

### B3

#### **BLACK SOLDIER FLY (HERMETIA ILLUCENS L.) WHOLE AND FRACTIONATED LARVAE: IN VITRO PROTEIN DIGESTIBILITY AND EFFECT OF LIPID AND CHITIN REMOVAL**

**Maria Giulia Bonomini**\*<sup>(1)</sup>, **Barbara Prandi**<sup>(1)</sup>, **Augusta Caligiani**<sup>(1)</sup>

<sup>1)</sup> *University of Parma, Italy*

\**Corresponding author - E-mail: mariagiulia.bonomini@unipr.it*

When evaluating novel protein sources, protein digestibility is of fundamental importance, being it one of the parameters used to assess protein quality. Given that chitin might interfere with insect protein digestion and that the influence of insect fractions on overall protein digestibility is currently not well understood, this study aimed at investigating the impact of lipids and chitin on the protein digestibility of black soldier fly larvae and of insect-based ingredients derived therefrom by defatting or protein isolation processes. An *in vitro* simulated gastro-intestinal digestion was carried out on all samples following the INFOGEST method, generally used for humans. Both undigested matrices and digesta were thoroughly characterized in terms of amino acid profile, SDS-PAGE gel electrophoresis, and proteomic/peptidomic analyses. Additionally, solubilized proteins and degree of hydrolysis (DH%) after digestion were also assessed. The results showed that chitin impaired protein digestion as the protein isolate showed the highest protein solubilization, DH%, and number of peptides and proteins identified by HR/MS, while the chitin-rich fraction had the lowest. Remarkably, the preferred C-terminal cleavage sites across all samples matched the specificity of the enzymes used, thus indicating that insect proteins and the presence of chitin did not alter the enzymatic specificity compared to other matrices.

**Keywords:** insect proteins, *in vitro* digestion, *Hermetia illucens*, proteomics, peptidomics

**Acknowledgement:** *The authors would like to acknowledge Dr. Andrea Faccini for his contribution.*

## ALTERNATIVE PROTEINS

### B4

#### CHARACTERIZATION OF EGGS FROM CHICKENS FED WITH INSECT-ENRICHED DIETS

Maria Giulia Bonomini\*<sup>(1)</sup>, Katia D'ambra<sup>(2)</sup>, Giovanna Minelli<sup>(2)</sup>, Domenico Pietro Lo Fiego<sup>(2)</sup>, Augusta Caligiani<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

<sup>2)</sup> University of Modena and Reggio Emilia, Italy

\*Corresponding author - E-mail: mariagiulia.bonomini@unipr.it

The demand for functional foods offering additional beneficial effects beyond their nutritional components is on the rise. Consequently, exploring alternative protein sources such as eggs produced by hens fed with insect-enriched diets holds a significant value in addressing this dietary challenge. Insects represent a highly nutritious feed source with low environmental impact for monogastric species, and their ability to convert agri-food by-products into valuable biomass is already known. For these reasons, the aim of this study was to assess the effects of implementing black soldier fly larvae in the diet of hens on eggs. The larvae were reared on plant-based substrates rich in carotenoids, which naturally enriched them in these compounds, and incorporated in the experimental diet of the hens for a three-months period. The effect of the larvae-enriched diet was studied for different parameters such as egg deposition and qualitative criteria (weight, defects, component incidence). Finally, analysis of the protein and lipid fractions were carried out on the egg yolk. The results showed that the eggs from the experimental group showed overall more defects but, at the same time, the eggs from the control group showed more defects in the same egg. In general, the addition of the carotenoid-enriched larvae in the feed resulted in a natural improvement of egg yolk color without significantly reducing the quality of the egg. The main carotenoids present in the yolk (lutein and xanthin) were higher in the experimental eggs, while their true protein content remained mostly unchanged. However, the analysis of amino acids showed that the lysine content increased in the experimental eggs, which is of great interest as lysine is one of the most commonly limiting amino acids in vegetarian diets.

**Keywords:** alternative proteins, carotenoids, egg quality, insect-enriched diet

## ALTERNATIVE PROTEINS

### B5

#### **RAPID AND EFFICIENT MICROWAVE DIGESTION FOR TRACE METALS ANALYSIS OF ALTERNATIVE PROTEINS**

Samuel Heckle<sup>(1)</sup>, Macy Harris<sup>(1)</sup>, Layla Abu-Al-Halaweh<sup>(1)</sup>, Axel Schoener<sup>(1)</sup>, Bobbie McManus<sup>\*(1)</sup>

<sup>1)</sup> CEM Corporation, United States of America

<sup>\*</sup>Corresponding author - E-mail: [bobbie.mcmanus@cem.com](mailto:bobbie.mcmanus@cem.com)

With rising populations and environmental concerns, the need for sustainable and nutritious alternative protein sources continues to increase. Plant-based proteins have been the leading choice to fulfil this need. As they grow, plants are known to take up metals from the ground. As a result, plant-based products may have an inherently higher baseline level of certain metals than animal-based products. Even further, manufacturers may introduce ingredients and additives during the processing and manufacturing process for additional nutritional and/or flavor that could contribute to trace metal content.

Toxic heavy metals such as arsenic, cadmium, lead, and mercury are known to cause adverse effects on human health; therefore, it is critical to regulate certain elements within food products. Trace metals analysis relies heavily on a robust, reliable, and reproducible sample preparation technique. In this study, trace metal concentrations, including toxic metals as well as nutritive metals and salts, are measured and compared for a variety of plant-based proteins. The trace metals are measured by ICP-MS analysis, following microwave digestion of the proteins. This approach provides a rapid, efficient and simple process for trace metal analysis of plant-based proteins.

**Keywords:** digestion, trace metals analysis, ICP-MS, alternative proteins



### B6

#### EMULSIFYING AND PHYSICO-CHEMICAL PROPERTIES OF MAILLARD REACTION PRODUCTS FORMED BY SPIRULINA PROTEIN EXTRACT WITH SACCHARIDES

Dohyoung Lee<sup>(1)</sup>, Kwang-Geun Lee<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Science and Biotechnology, Dongguk University, South Korea

<sup>\*</sup>Corresponding author - E-mail: kwglee@dongguk.edu

Proteins are natural emulsifiers that can function as an emulsifier for food because it is amphiphilic molecules. Through the Maillard reaction with saccharides, the emulsifying properties of protein can be improved. In this study, the emulsifying and physicochemical properties of Maillard reaction products (MRPs) formed by Spirulina (*Arthrospira maxima*) protein extract (SPE) with gum Arabic (GA), dextrin (DE) and glucose (GL) were investigated. Maillard reaction was performed in conventional and ultrasound-assisted wet-heating methods at each time conditions. The degree of glycation (DG) was increased all sample as the heating time increased, and the highest DG was indicated in MRPs with DE. The EAI was significantly increased by 28.87-44.22% in conventional wet heating at 5 h and 43.01-45.57% in ultrasound-assisted at 90 min (US-90min) compared to the control as heating time increased. However, the ESI was significantly decreased by 83.36-88.50% in conventional wet heating at 5h and 72.16-89.52% in US-90min. The levels of browning index, Amadori compounds and melanoidins increased by 191.6%, 15.4% and 7. % at most with increasing heating time, while the levels of phycocyanin decreased by 33.5% at most. The protein solubility decreased with heating time increased, and all samples indicated isoelectric points in the pH range of 3-4. The absolute value of the zeta potential decreased by up to 8.03% as heating time increased, but it can be stable because the absolute value is above 30 mV in all samples. The average droplet size was smallest at  $707.97 \pm 33.12$  nm in GA-3h. Most samples had a droplet size below 1,000nm, indicating that the emulsions were stable enough for commercial applications. In brief, gum Arabic is more suitable than other saccharides to improve the emulsifying properties of spirulina protein extract. The ultrasound-assisted can improve Maillard reaction for using emulsifiers because it can reduce the Maillard reaction time and indicated in better emulsifying properties.

**Keywords:** Spirulina protein extract, saccharide, gum arabic, Maillard reaction, emulsifying property

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## ALTERNATIVE PROTEINS

### B7

#### PLANT-BASED PROTEINS: EVALUATING THE POTENTIAL APPLICATIONS AS ALTERNATIVE PROTEINS IN TOMATO POWDER

**Neriman Ezgi Cifte<sup>(1)</sup>, Mecit Halil Oztop<sup>\*(1)</sup>**

<sup>1)</sup> Middle East Technical University, Turkey

\*Corresponding author - E-mail: mecit@metu.edu.tr

With rising environmental concerns, the food industry is under increasing pressure to find new ways to utilize sustainable protein sources. Plant-based proteins are emerging as key components in this shift, redefining food production. In this study, pea protein and RuBisCO were explored for their potential in the production of functionalized tomato powders. Tomato juice, including 3% (w/w) maltodextrin to increase production yield by reducing stickiness, was mixed with 1% (w/w) plant protein and subjected to high shear homogenizing as the pre-homogenization step. High pressure homogenization, the main homogenization conducted at 100 MPa, was particularly emphasized due to the low solubility of plant proteins at the pH level of tomato juice. This process increased the stability of the solution by preventing phase separation during the spray drying period, where the inlet and outlet air temperatures were 150 and 85 °C, respectively. The research findings showed that for tomato powders, with the inclusion of olive powder to enhance flavor, RuBisCO-enriched samples exhibited significantly higher total phenolic content and antioxidant capacity compared to those containing pea protein ( $p < 0.05$ ). However, flavor profile analysis, schematized on a spider diagram, highlighted a consumer preference for the pea protein-enriched samples, which were rated highly for especially their fresh tomato taste and balance of sweetness and acidity. The RuBisCO formulations, on the other hand, were less favored, receiving lower scores due to their weaker tomato flavor, strong astringency and undesirable aftertaste. Although the addition of olive powder resulted in a slight improvement, they were still not found to be successful in flavor perception. While RuBisCO has the potential to enhance the nutritional profile of foods, further research is needed to optimize its sensory properties to better meet consumer expectations. This study demonstrated the challenges of incorporating plant proteins in foods from both a production and sensory perspective.

**Keywords:** plant protein, pea protein, RuBisCO, tomato powder, sensory analysis

**Acknowledgement:** This research was funded by the Partnership for Research and Innovation in the Mediterranean Area (PRIMA) H2020 GA2032: "FunTomP-Functionalized Tomato Products".

# ALTERNATIVE PROTEINS

## B8

### CHARACTERIZATION OF BIOACTIVE SENSORY SIGNIFICANT SUBSTANCES IN PLANT-BASED PROTEINS

**Anna Louckova<sup>(1)</sup>, Jan Kyselka<sup>(2)</sup>, Frantisek Benes<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>**

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> Department of Dairy, Fat and Cosmetics, University of Chemistry and Technology in Prague, Czech Republic

\*Corresponding author - E-mail: jana.hajslova@vscht.cz

Plant-based diets involving alternative proteins are today at the center of interest of consumers and presents challenging topic for researchers. The availability of plant-based alternatives to animal products is therefore growing rapidly. Key materials for their production are protein concentrates. They can however contain various co-isolated sensory-active secondary metabolites such as polyphenols, transferred to these products from the source plants, causing unpleasant, especially bitter and astringent, aftertastes (off-flavors). The aim of this study was to characterize profiles of these substances in a large set of samples (n=106), consisting of oil seeds protein concentrates (soy, rapeseed, sunflower) from facility, where the raw materials, process intermediates and by-products (molasses) were available, and from additional set of oil-seed and plant proteins purchased from Czech market. The samples were extracted with aqueous methanol and analyzed using ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). For the purpose of target screening of polyphenols, an in-house library of more than 600 polyphenolic and other compounds occurring in plants was created providing information about availability of MS/MS spectra. The presence of many of them was detected in all the tested plant proteins. Variability in the phenolic profiles were observed in proteins from the same plant source but from different producers, documenting the impact of plant variability and/or manufacturing process. Across the chain raw material - intermediates - final product, significant decrease but no complete elimination of polyphenols was observed with accumulation of these compounds in the by-products. This was especially evident with several typical metabolites, for example daidzein and genistein in soy proteins. In hemp seed proteins in addition to phenolics also traces of phytocannabinoids were detected.

**Keywords:** plant-based protein, secondary metabolites, oil-seed proteins, polyphenols

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### B9

#### **ANALYSIS OF INSECT-BASED FEED: INTEGRATION OF CHITIN DETERMINATION INTO THE WEENDE METHOD**

**Michael Hellwig**<sup>(1)</sup>, **Patrick Sudwischer**<sup>(2)</sup>, **Werner Sitzmann**<sup>(2)</sup>, **Björn Krüger**<sup>(3)</sup>

<sup>1)</sup> TU Dresden, Chair of Special Food Chemistry, Germany

<sup>2)</sup> International Research Association Feed Technology e.V. (IFF), Germany

<sup>3)</sup> C. Gerhardt GmbH & Co. KG, Germany

\*Corresponding author - E-mail: michael.hellwig@tu-dresden.de

Insects are increasingly used as a sustainable and protein-rich ingredient in feedstuff for fish, pets, pigs and poultry. Insect meals offer a favorable nutrient composition containing essential amino acids, minerals and fatty acids that are beneficial for animal nutrition. However, a significant difference between insect meals and conventional protein sources is the presence of chitin. From an analytical viewpoint, chitin has an impact on the determination of crude protein content in these products. The nitrogen content in chitin, which represents about 6.89% of its mass, is determined as crude protein during analysis, leading to an overestimation of the actual crude protein content. In the classical nutrient analysis method (Weende analysis), chitin is also assigned to the crude fiber fraction together with other polymeric compounds. The aim of this work was to analyze chitin content using a low-cost method that does not require specialized equipment such as LC- or GC-MS and is therefore suitable for feed monitoring, insect production and processing.

The proposed analytical technique is based on classical chemical methods such as crude fibre content and nitrogen content, which allows easy implementation into existing feed analysis practices. The process involves alkaline hydrolysis of the sample with 0.25 M sodium hydroxide, followed by drying of the residue at 103°C for 4 hours. Chitin remains as the only compound containing nitrogen in the sample. The nitrogen content of the residue is then determined by the Kjeldahl method.

From these data, the chitin content can be calculated stoichiometrically based on the nitrogen measurement. During method validation, a recovery rate of over 95% and a precision below 5% was achieved, indicating the reliability and precision of the method. The method shows the ability to detect chitin content in relevant amounts (up to 2%) in feed samples with a measurement uncertainty of less than 10%. The method was successfully applied to determine the chitin content in various products from insect farming and processing, including three insect species and various developmental stages of the mealworm (*Tenebrio molitor*), such as larvae, pupae and beetles.

**Keywords:** insect protein, chitin, Weende analysis, feed

# **AUTHENTICITY, TRACEABILITY, FRAUD**



## C1

### DETECTION OF 5-HMF CONTENT AS AN INDICATOR OF HONEY ADULTERATION WITH INVERTED SUGAR SYRUP

**Somaiah Almubayedh<sup>(1)</sup>, Amani Albalawi<sup>(1)</sup>, Abdullah Akbar<sup>(1)</sup>, Tagreed Alsalem<sup>\*(2)</sup>**

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

<sup>2)</sup> King Saud University, Saudi Arabia

\*Corresponding author - E-mail: 444203081@student.ksu.edu.sa

Honey is the only sweetener that can be preserved and consumed in its natural state. This unique substance does not need to be purified or treated before it is consumed, in accordance with the Saudi Food and Drug Authority's regulation (SFDA.FD/GSO 147: 2021). A requirement of the SFDA stipulates that honey must not be heated to the point where its basic composition is affected and its quality has been compromised, and it should not contain any additives (e.g., water, sugar, colors). The proposed work aims at the development of a rapid, accurate, and non-destructive method for detecting levels of 5-HMF formed in Acacia honey as a result of the adulteration process via IS inverted sugar syrup by using Fourier transform infrared and attenuated total reflectance (FTIR-ATR) spectroscopy coupled with chemometric tools like Principal Component Analysis (PCA) for classification and qualitative estimation, and the Partial Least Square Regression (PLS-R) for quantitative estimation and as a calibration model. Identify the 5-HMF content of honey (natural, heated, and stored) and that produced by adulteration with IS. In addition, assess the change in primary sugar levels in honey caused by 5-HMF formation during heating, storage, and adulteration.

**Acknowledgement:** Saudi Food and Drug Authority

## C2

### EVALUATION OF INNOVATIVE TOOLS FOR RAPID AND ACCURATE AUTHENTICATION OF GROUND BLACK PEPPER

Ilona Nordhorn<sup>(1)</sup>, Andrea Massaro<sup>(2)</sup>, Michele Suman<sup>(3)</sup>, Roberto Piro<sup>(2)</sup>, Alessandra Tata<sup>(2)</sup>, Carmela Zacometti\*<sup>(2)</sup>, Carsten Baessmann<sup>(1)</sup>

<sup>1)</sup> Bruker Daltonics GmbH & Co KG, Germany

<sup>2)</sup> Istituto Zooprofilattico Sperimentale delle Venezie, Italy

<sup>3)</sup> Advanced Laboratory Research, Barilla G. e R. Fratelli S.p.A. Catholic University Sacred Heart, Department for Sustainable Food Process, Italy

\*Corresponding author - E-mail: czacometti@izsvenezie.it

The European Commission published in 2021 the results of the first coordinated control plan on the authenticity of herbs and spices on the European market carried out by 21 EU Member States, Switzerland and Norway. As resulted from this large investigation 17% of black pepper is suspicious of adulteration. This contribution evaluates three non-targeted method for authentication of ground black pepper by Near Infrared Spectroscopy (NIR), Gas Chromatography Ion Mobility Spectrometry (GC-IMS), Direct Analysis in Real-Time High Resolution Mass Spectrometry with Trapped Ion Mobility Quadrupole Time of Flight Mass Spectrometry (DART-TIMS-QTOF-MS) coupled to multivariate statistical analysis on the same sample set. Our evaluation of multiple tools included authentic black pepper samples (from 8 different countries and four harvesting seasons) and samples spiked with non-functional material (pinhead and spent) and exogenous materials (green lentil, olive kernel, black mustard, sesame, garlic, corn flour, rice flour, chili, papaya). The percentage of adulteration ranged between 5% and 30%.

First, our laboratory developed and validated a method by NIR that achieved high overall accuracy, sensitivity and specificity rates on test set, validation set with multiple operators and proficiency test. One of the most underrated issues of non-targeted methods is the time spent to manually average, normalize and send the data to the statistician for statistical assessment of the acquired data. For this reason, we developed a local web application that allowed the direct interrogation of the statistical model.

Afterwards, we developed and validated a GC-IMS classifier that showed high overall accuracy  $\geq 90\%$  both on withheld test set 1 and 2. HS-GC-IMS is characterized by the destruction of the sample as compared to spectroscopy methods. Moreover, the analysis of a single sample by HS-GC-IMS takes about 17 min, which is a longer time as compared with those of DART-MS and NIR spectroscopy.

The capability of DART-QTOF-MS with and without TIMS was also evaluated for black pepper authentication. Analysis times were 5 s per sample and therefore significantly shorter than for NIR and GC-IMS analyses. Unsupervised statistical analysis in form of Principal Component Analysis (PCA) revealed a clear discrimination of atypical samples from those authentic. Machine learning classifiers, based on DART-QTOF-MS data, are being built-up and validated. Although the addition of a separation step after ionization using TIMS did not further improve the discrimination, it showed great potential in terms of identifying specific marker compounds for adulterants through cleaner MS/MS spectra and collision cross-section values as an additional identification criterion.

Moreover, further challenges of the tools with independent sample set need to be systematically performed in consecutive studies to control the performances of the methods for black pepper authenticity over a longer time frame.

## C3

### COMBATING HAZELNUT FRAUD: A SYSTEMATIC COMPARISON BETWEEN MULTI-ISOTOPE, SPECTROSCOPIC AND LIPIDOMIC FINGERPRINTING METHODS

**Berta Torres-Cobos**<sup>\*(1)</sup>, **Soriana Beatrice Nicotra**<sup>(1)</sup>, **Monica Rosell**<sup>(2)</sup>, **Albert Soler**<sup>(2)</sup>, **Merce Rovira**<sup>(3)</sup>, **Agusti Romero**<sup>(3)</sup>, **Vincent Baeten**<sup>(4)</sup>, **Juan Antonio Fernandez Pierna**<sup>(4)</sup>, **Francesc Guardiola**<sup>(1)</sup>, **Alba Tres**<sup>(1)</sup>, **Stefania Vichi**<sup>(1)</sup>

<sup>1)</sup> *Department of Nutrition, Food Sciences and Gastronomy, Faculty of Pharmacy and Food Sciences, University of Barcelona, Spain*

<sup>2)</sup> *MAiMA Group, Applied Mineralogy, Geochemistry and Hydrogeology, Department of Mineralogy, Petrology and Applied Geology, Water Research Institute (IdRA), University of Barcelona, Spain*

<sup>3)</sup> *Institute of Agrifood Research and Technology (IRTA), Spain*

<sup>4)</sup> *Quality and Authentication of Products Unit, Knowledge and Valorization of Agricultural Products Department, Walloon Agricultural Research Centre, Belgium*

*\*Corresponding author - E-mail: bertatorres@ub.edu*

Hazelnuts are one of the most consumed nuts worldwide due to their versatility and outstanding sensorial and nutritional qualities, which are greatly influenced by the hazelnut's cultivar and provenance. Differences on geographical and botanical origin are also reflected in hazelnuts market price, which makes them a target of economically motivated fraudulent practices. Hence, efficient and robust methods to authenticate hazelnuts and safeguard consumers are needed.

To verify hazelnut cultivars and provenance, we tested the efficiency of the unsaponifiable fraction (UF), and triacylglycerol (TAG) fingerprinting obtained through gas chromatography-mass spectrometry, along with near (NIR) and medium infrared spectroscopy (MIR) fingerprinting. Additionally, we compared the efficiency of these lipidomic approaches with multi-isotope analysis (bulk  $\delta^{18}\text{O}$ , and  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$  of the main fatty acids) for authenticating hazelnut geographical origin. Individual partial least square discriminant analysis classification models were developed on a large sample set to discriminate the cultivar - "Tonda di Giffoni" samples from other cultivars produced in Spain (n=193) - and origin of "Tonda di Giffoni" hazelnuts produced in four distinct regions (n=207). Models were externally validated, and the performance of each method was assessed through model fitting, rates of correct classification, efficiency, applicability and transferability. The methods were tested on the same sample sets from up to four harvest years.

Results of the study showed that, although the isotopic method is robust and easily transferable across laboratories, it provides lower classification rates than lipidomic approaches. Among the latter methods, UF and NIR fingerprinting emerged as the best-performing approaches, presenting the highest correct classification rates for both cultivar and origin models (>94%). These methods proved to be fit-for-purpose tools for hazelnut geographical and varietal authentication. Additionally, despite the laborious procedure required for the UF method, its workload can be reduced by up to 70% if combined with TAG fingerprinting, achieving an accuracy >91%.

**Keywords:** geographical and varietal authentication, unsaponifiable fraction, triacylglycerol, spectroscopy multi-isotope



### C4

#### **DETECTION OF GELATIN SPECIATION, HEAVY METAL CONTAMINATION AND FRAUDULENT LABELING OF UNREGISTERED DIETARY SUPPLEMENTS AVAILABLE IN ONLINE STORES**

**Alanoud Alsaqabi**<sup>(1)</sup>, **Khalid Alshehail**<sup>(1)</sup>, **Faisal Alrowais**<sup>(1)</sup>, **Hassan Almalki**<sup>(1)</sup>, **Bandar Bin Helel**<sup>(1)</sup>, **Mohammed Alkhidhr**<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: aasaqabi@sFDA.gov.sa

Dietary supplements have become an indispensable aspect in modern life, offering a convenient and accessible way to fill nutritional gaps and promote overall well-being. The growing popularity of dietary supplements necessitates transparency and consumer awareness regarding their ingredients. Gelatin, a key capsule ingredient, is derived from animal sources, Pig skins and bovine hide and bones constitute the primary commercial sources of gelatin. In light of the religious dietary guidelines adhered to by Muslims, Jews, Hindus, and other communities worldwide, it is essential to ensure that gelatin-derived food items are devoid of pork or beef components for some communities. The aim of this study is to detect gelatin speciation, heavy metal contamination and labelling adulteration in unregistered vitamins and minerals sold directly to consumers online in Saudi Arabia.

A descriptive cross-sectional study was conducted involving purchasing 84 products from a well-known website for healthy and natural products, these samples consisted of various vitamins and minerals in different forms. Liquid chromatography mass spectrometry (LC-MS/MS) was employed with Multiple Reaction Monitoring (MRM) for gelatin speciation. The method achieves detection of gelatin in mixtures with a validated Limit of Quantitation (LOQ) of 1% w/w. All samples underwent a heavy metals analysis for three toxic elements, Arsenic (As), Cadmium (Cd) and Lead (Pb) using ICP-MS.

The results indicate that among 66 gelatin-containing samples tested for porcine gelatin, 19 (28.8%) tested positive. Similarly, among the 66 samples tested for bovine gelatin, 24 (36.3%) were positive. Additionally, 6 (9%) of the samples were found to contain a mixture of both porcine and bovine gelatin. Regarding heavy metal analysis results indicated that all samples tested for Cd and Pb were within accepted limits, whereas seven products exceeded the maximum accepted levels for As (ranging from 1.6 to 23.24 mg/kg), surpassing safety limits set by USP 2016.

For labelling accuracy, the analysis revealed concerning inconsistencies. None of the 19 products containing porcine gelatin specified the source on the label, simply mentioning gelatin presence. Furthermore, two products labeled as "veggie capsules" were found to contain porcine gelatin. These findings highlight the need for ongoing monitoring of online supplement sales to ensure consumer safety and product quality. Moreover, it is important to ensure companies to clearly disclose gelatin sources on product labels, particularly for consumers adhering to religious or dietary restrictions, thereby fostering transparency, and facilitating informed purchasing decisions.

**Keywords:** dietary supplement, gelatin, fraudulent labelling, heavy metal, porcine gelatin

### C5

#### NON-TARGETED LC-HRMS FINGERPRINT AND MULTIVARIATE CHEMOMETRIC METHODS FOR CHARACTERIZATION AND CLASSIFICATION OF HONEY FROM DIFFERENT GEOGRAPHICAL ORIGINS

**Benedetta Fanesi<sup>(1)</sup>, Danica Mostoles<sup>(2)</sup>, Andrea Mara<sup>(3)</sup>, Gavino Sanna<sup>(3)</sup>, Sonia Sentellas<sup>(4)</sup>, Javier Saurina<sup>(2)</sup>, Oscar Nunez<sup>(4)</sup>, Deborah Pacetti<sup>(1)</sup>, Paolo Lucci<sup>(1)</sup>**

<sup>1)</sup> *Università Politecnica delle Marche, Italy*

<sup>2)</sup> *University of Barcelona, Spain*

<sup>3)</sup> *University of Sassari, Italy*

<sup>4)</sup> *University of Barcelona, Generalitat de Catalunya, Spain*

\*Corresponding author - E-mail: [b.fanesi@univpm.it](mailto:b.fanesi@univpm.it)

Nowadays, the quality of natural products is an issue of great interest in our society due to the increase in adulteration cases in recent decades. In this context, honey is one of the most appreciated and requested food because of its numerous beneficial properties, however its production is scarce, and frauds are common. To prevent fraudulent practices, it is therefore necessary to develop feasible methodologies to authenticate and guarantee honey's origin. Non-targeted fingerprinting metabolomic strategies combined with multivariate chemometric methods have been recently proposed as potential methodology to address food authentication issues. The aim of the study was to test the reliability of this approach to distinguish honey production among the different continents, countries, and countries within the same continent. A C18 reversed-phase chromatography under universal gradient elution, consisting of 0.1% formic acid in water and acetonitrile, coupled to high-resolution mass spectrometry (HRMS) using a Q-Orbitrap mass analyzer was applied to address the characterization and classification of honey samples from different production regions using chemometrics. Analytes were recovered by simply dissolving honey samples in cold water without any sample treatment procedure, followed by a 1:1 dilution with methanol. The proposed non-targeted LC-HRMS method using electrospray ionization in negative mode was applied to the analysis of 184 samples coming from four continents (i.e., Europe, Asia, America, Oceania), and involving eleven countries. The chemical fingerprints of honey were subsequently subjected to chemometric analysis performing principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The proposed methodology was able to classify and authenticate the production continent of the analyzed honey samples by PLS-DA, with overall cross-validation sensitivity and specificity values higher than 95.2% and classification errors in the range of 0.8-20%. When assessing honey geographical production country, overall sensitivity and specificity values higher than 75%, and classification errors below 35%, for most of the classes, were accomplished, results that can be considered acceptable taking into consideration the high botanical variability involved and the number of analyzed samples. Classification performance improved when a PLS-DA classification decision tree was employed. As a conclusion, non-targeted UHPLC-HRMS fingerprinting is a suitable methodology for the characterization, classification, and authentication of honey samples from different geographical origins, without the requirement of using commercially available standards for quantification nor the necessity of metabolite identification.

**Keywords:** honey, authenticity, mass spectrometry, chemometrics, non-targeted analysis

### C6

#### CHARACTERIZATION AND DETECTION OF FRASS FROM TENEBRIO MOLITOR LARVAE BY NEAR INFRARED SPECTROSCOPY TECHNIQUES

**Abigaël Anselmo**<sup>\*(1)</sup>, Pascal Veys<sup>(1)</sup>, Audrey Pissard<sup>(1)</sup>, Vincent Baeten<sup>(1)</sup>

<sup>1)</sup> Walloon Agricultural Research Centre (CRA-W), Belgium

\*Corresponding author - E-mail: a.anselmo@cra.wallonie.be

Insects naturally produce frass during their rearing. This residue is defined by the European Commission (2021) as a mixture of insect excrement, food substrate, insect parts, and dead eggs. The inclusion of frass in insect meal produced by companies is prohibited. Nevertheless, with the development of insect meal production, frass could potentially be used to decrease the quality of the final product while maintaining the same information on composition and price. Consequently, the development of methods to detect the presence of frass in insect meal is needed to comply with legislation. To address this issue, this study investigates the application of Near Infrared (NIR) Spectroscopy techniques to characterize and detect frass, as they are fast and non-destructive methods that allow the use of untreated samples. Samples of frass from *Tenebrio molitor* larvae were produced internally during a 15-day *T. molitor* rearing period. These samples were used to adulterate *T. molitor* larvae meal at 20%. In the first step, the adulterated sample, the frass sample, and the *T. molitor* larvae meal sample were analysed by NIR Spectroscopy (NIRS) to develop models and predict their composition. Results indicated a protein content of 63.29% for the *T. molitor* larvae meal, 15.91% for the frass sample, and 55.35% for the adulterated sample. The chitin content never seemed to exceed 10%, nor did the lipid content. In the second step, these samples were analysed by NIR Microscopy (NIRM) combined with chemometrics to detect the presence of frass in the adulterated sample. Based on a screening method and PLS-DA analysis, 44 spectra out of 1224 were identified as frass in the adulterated sample, with a sensitivity of 0.036 and a specificity of 1. These results are promising and highlight both the fact that frass has a much less interesting chemical composition than insect meal and the possibility of detecting it in a sample. To improve predictive models for chemical composition, it is essential to conduct chemical analyses on samples and perform other analyses by reducing the quantity of frass used to adulterate the samples.

**Keywords:** frass, vibrational spectroscopy, chemometrics, insect

### C7

#### ADVANCE OF PCR APPROACHES FOR EFFICIENT TRACKING OF OIL CROPS IN PROCESSED FOODS

**Nelly Datukishvili**<sup>(1)</sup>, **Tamara Kutateladze**<sup>(2)</sup>, **Kakha Bitskinashvili**<sup>(2)</sup>, **Kakha Karchkhadze**<sup>(1)</sup>, **Boris Vishnepolsky**<sup>(2)</sup>, **Tata Ninidze**<sup>(1)</sup>

<sup>1)</sup> Iliia State University, Tbilisi, Georgia

<sup>2)</sup> Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia

\*Corresponding author - E-mail: neli\_datukishvili@iliauni.edu.ge

Oil crops are widely used to produce processed food products, especially edible oils. However, many of them are recognized as important food allergens. The modern food industry faces a major challenge regarding contamination and fraudulent alteration of food ingredients as well as adulteration of oils. Therefore, reliable identification of oilseed crops is needed for food authentication and traceability, quality and safety assessment, proper labelling, consumer information, and health protection. The aim of this study was to develop and optimize new polymerase chain reaction (PCR) methods for the detection of important oleaginous crops such as soybean (*Glycine max*), maize (*Zea mays*), sunflower (*Helianthus annuus*) and canola (*Brassica napus*). The research involved several steps, such as the identification of species-specific genes and DNA sequences by *in silico* genome data analysis; design of oligonucleotide primers using bioinformatics tools; genomic DNA extraction; DNA quantification by spectrophotometer; development and optimization of uniplex, multiplex, and nested PCR systems; evaluation of genomic DNAs and PCR products by agarose gel electrophoresis. *Seeds, flours, and various processed food products, including cold-pressed and refined cooking oils were examined.* Different DNA isolation and PCR approaches were applied. A comparison of the obtained results revealed the best PCR primers, DNA extraction, and PCR amplification methods for each oil crop and foodstuff. New DNA markers targeting species-specific genes of soybean lectin, corn zein, sunflower helianthinin, and rapeseed acetyl-CoA carboxylase (BnAcc) were identified by uniplex PCR methods. Triplex PCR was developed for the simultaneous identification of soybean, maize, and sunflower. Notably, the nested PCR methods allowed efficient detection of sunflower and rapeseed in 700 µl of oil including refined and used cooking oil. Testing of various food products *demonstrated* that the new PCR methods are very sensitive and specific tools to detect traces of soybean, maize, sunflower, and rapeseed even in highly processed food products. Most importantly, this study can significantly facilitate the economical and accurate detection of oil crops to advance food authenticity and traceability.

**Keywords:** oil crops, processed food, multiplex and nested PCR, oil authenticity, food traceability

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### C8

#### EXPLORING THE CHEMICAL PROFILE OF GREEK HONEY VARIETIES VIA UHPLC-TIMS-QTOF-MS: CHEMICAL CHARACTERIZATION AND STATISTICAL ANALYSIS

Panagiotis Loukas Gialouris<sup>(1)</sup>, Eleni Nastou<sup>(1)</sup>, Dafni Preza-Mayo-Kataki<sup>(1)</sup>, Reza Aalizadeh<sup>(1)</sup>, Marilena Dasenaki<sup>(1)</sup>, Nikolaos Thomaidis<sup>\*(1)</sup>

<sup>1)</sup> *Laboratory of Analytical Chemistry, Chemistry Department, National and Kapodistrian University of Athens, Greece*

*\*Corresponding author - E-mail: ntho@chem.uoa.gr*

Honey is widely recognized as a natural sweetener and holds a significant position among highly esteemed foods. Its complex composition predominantly consists of sugars, water, minerals, volatiles, and polyphenols [1]. Notably, the phenolic compounds present in honey are closely associated with its antioxidant properties, which substantially influence the overall quality of the product. Previous studies have established a link between bioactive compounds found in honey and various health benefits, including anti-inflammatory and anti-cancer effects. Thus, the bioactive compounds support honey's economic and nutritional value, making their determination of utmost importance. Cutting-edge analytical techniques play a key role in this approach. Honey's bioactive compounds are mainly studied using Liquid Chromatography (LC), coupled with either Low-Resolution or High-Resolution Mass Spectrometry (MS) techniques [2]. In this study, a novel approach utilizing Ultra-High-Performance Liquid Chromatography-Trapped Ion Mobility Spectrometry-Quadrupole Time-of-Flight-Mass Spectrometry (UHPLC-TIMS-QTOF-MS) was implemented for the precise chemical characterization of various Greek honey samples. For this approach, more than 200 honey samples from different geographical regions of Greece were analyzed from common and rare varieties, such as pine, thyme, oak, fir, orange, chestnut, arbutus, and heather. Following a target screening workflow 35 compounds were determined, while additional 65 compounds were identified through suspect screening. As a result, 100 compounds were reported in total, enabling a wide chemical characterization of Greek honey. Several analytes were reported for the first time in specific investigated varieties, and the acquired Collision Cross-Section (CCS) experimental values enhanced the confidence of their identification. In addition, statistical analysis revealed quantitative and qualitative differences among the investigated honey varieties. For instance, the average concentration of 2-cis,4-trans-abscisic acid was notably higher in arbutus honey compared to any other investigated variety.

[1] Gialouris et al, Development and validation of a high-throughput Headspace Solid-Phase Microextraction Gas Chromatography-Mass Spectrometry methodology for target and suspect determination of honey volatiles, *Heliyon*, (2023).

[2] Koulis et al, Thorough investigation of the Phenolic Profile of Reputable Greek Honey Varieties: Varietal Discrimination and Floral Markers Identification using Liquid Chromatography-High-Resolution Mass Spectrometry, *Molecules*, (2022).

**Keywords:** honey, chemical characterization, HRMS, origin, authenticity

### C9

#### **DIRECT ANALYSIS IN REAL TIME COMBINED WITH HIGH RESOLUTION MASS SPECTROMETRY FOR RAPID FOOD AUTHENTICITY TESTING: EXAMPLE OF BLACK TRUFFLES**

**Ilona Nordhorn**\*<sup>(1)</sup>, **Klemens Losso**<sup>(2)</sup>, **Matthias Rainer**<sup>(3)</sup>, **Carsten Baessmann**<sup>(1)</sup>

<sup>1)</sup> Bruker Daltonics GmbH & Co KG, Germany

<sup>2)</sup> Department Food Technology & Nutrition, MCI | The Entrepreneurial School, Austria

<sup>3)</sup> Institute of Analytical Chemistry and Radiochemistry, Leopold-Franzens University of Innsbruck, Austria

\*Corresponding author - E-mail: [ilona.nordhorn@bruker.com](mailto:ilona.nordhorn@bruker.com)

Food fraud is a major issue in the food industry leading to financial losses for food processors as well as inflicting lasting damage in the trust of consumers. Due to the motivation of financial profit, particularly expensive products are prone to adulteration. Truffles are considered a luxury product with prices ranging up to 1000 - 2000 €/kg for the Périgord truffle (*Tuber melanosporum* Vittad.). Other black truffle species, for example the Asian truffle (*T. indicum* Cooke et Masee) are morphologically highly similar to the Périgord truffle but much cheaper in price. Therefore, detection of adulterated truffle batches is of utmost importance, leading to a demand for analytical solutions that deliver highly reliable results, while simultaneously being easy-to-operate and fast for high-throughput analyses.

In this study, we developed a comprehensive workflow for the differentiation of different black truffle species utilizing Direct Analysis in Real Time (DART) ionization (DART JumpShot source, Bruker Daltonics) in combination with high resolution Quadrupole Time-of-Flight Mass Spectrometry (Impact II VIP QTOF-MS, Bruker Daltonics). Unsupervised and supervised multivariate statistical models built using the software MetaboScape (Bruker Daltonics) revealed a successful discrimination of the truffle samples according to their species. Subsequently, to evaluate the prediction capabilities, a support vector machine-based classification was performed. Therefore, the sample set was split into training and test groups. Classification was successful and all test samples were predicted correctly. In the next step, to explore candidate marker compounds, features with the largest contribution to the species discrimination were annotated using tools for untargeted unknown identification included in MetaboScape. In detail, annotation was performed based on the information of accurate mass, isotope pattern, and fragmentation pattern, either by a fully automated spectral library search or by a semi-automated annotation workflow comprising elemental composition prediction, structure assignment and *in silico* fragmentation.

Compared to chromatographic methods, DART-QTOF-MS offers significantly shorter analysis times of 15 s per sample and reduced solvent consumption. All in all, DART-QTOF-MS paired with chemometrics presents a fast, robust, and resource-saving method for counteracting black truffle fraud.

**Keywords:** food authenticity, chromatography-free, DART, QTOF-MS

## C10

### SAMPLE PREPARATION- AND CHROMATOGRAPHY-FREE ANALYSIS OF CINNAMON PRODUCTS ENABLED BY DIRECT ANALYSIS IN REAL TIME

**Ilona Nordhorn**\*<sup>(1)</sup>, **Carsten Baessmann**<sup>(1)</sup>

<sup>1)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: [ilona.nordhorn@bruker.com](mailto:ilona.nordhorn@bruker.com)

Cinnamon is a spice with widespread use as an aromatic additive in many cuisines, fragrances and cosmetics across the world. There are different species of cinnamon, of which Ceylon cinnamon (*Cinnamomum verum*) and cassia cinnamon (*Cinnamomum cassia*) are the two most prominent ones. Besides the differences in taste, the coumarin content and price are two important aspects for consumers. Coumarin, which is present in cassia cinnamon in significant amounts, is of concern due to its hepatotoxic properties. As coumarin is only found in trace amounts in Ceylon cinnamon, frequent cinnamon consumers are advised to use it in preference. Ceylon cinnamon, however, is more expensive than cassia cinnamon putting it at a high risk for food fraud in form of substitution or dilution especially in its powdered form.

For food quality, authenticity and safety analysis, robust and informative analytical techniques are needed. Chromatographic techniques in combination with mass spectrometry are often proposed but as chromatographic methods are unsuited to solid form testing, food products must first be extracted or dissolved into solution, adding a time-consuming sample preparation step that can introduce errors and potential contaminants.

This challenge can be overcome by using Direct Analysis in Real Time (DART) ionization which allows for the direct analysis of solids, liquids, and gases. In addition, by being a chromatography-free technique results are received within seconds rather than minutes.

We present here the application of DART ionization in combination with Quadrupole Time-of-Flight Mass Spectrometry (QTOF-MS) for the rapid analysis of solid and liquid cinnamon samples including ground cinnamon, cinnamon sticks, herbal tea and cereals. No sample preparation was carried out, and samples were analyzed directly in their solid or liquid state, respectively. Due to the use of a QTOF mass spectrometer, full MS scans and MS/MS spectra were acquired alternately in a single run so that both the spectral fingerprint of the sample and structural information for compound annotation were obtained. With this method, coumarin and further typical compounds like cinnamaldehyde and methyl cinnamate were detected, enabling a confident differentiation of the two cinnamon species. The results prove the capability of chromatography-free DART-QTOF-MS for rapid screening analysis. It not only enables the identification of unlabeled cinnamon samples, but also allows to address potential adulteration or contamination through a non-targeted screening approach. On top, this chromatography-free workflow reduces the amount of solvent and consumables used, thereby improving the total cost of ownership and environmental footprint.

**Keywords:** cinnamon, DART, QTOF-MS, chromatography-free

## C11

### USE OF CHEMICAL AND ISOTOPIC FINGERPRINTS TO ESTABLISH THE GEOGRAPHICAL ORIGIN OF "NERO DEI NEBRODI" PIG MEAT

**Federica Litrenta**<sup>(1)</sup>, **Matteo Perini**<sup>(2)</sup>, **Carmelo Cavallo**<sup>(3)</sup>, **Silvia Pianezze**<sup>(2)</sup>, **Vincenzo Lo Turco**<sup>(1)</sup>, **Angela Giorgia Potorti**<sup>(4)</sup>, **Giuseppa Di Bella**<sup>(4)</sup>, **Luigi Liotta**<sup>(3)</sup>

<sup>1)</sup> Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy

<sup>2)</sup> Center of Technology Transfer, Fondazione Edmund Mach, 38098 San Michele all'Adige, Italy, Italy

<sup>3)</sup> Dep. of Veterinary Sciences, University of Messina, 98168 Messina, Italy, Italy

<sup>4)</sup> Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy., Italy

\*Corresponding author - E-mail: felitrenta@unime.it

"Nero dei Nebrodi" pigs are an indigenous breed of Sicily, reared mainly outdoors in the Nebrodi area. Although the presence of these pigs is historically documented [1], there is little information on how the rearing area may contribute to the quality of the meat. As far as we know, no study has evaluated the composition of stable isotopes and mineral elements in "Nero dei Nebrodi" meat as a traceability tool. The aim of this study was correlated *longissimus dorsi* meat from "Nero dei Nebrodi" pigs to the geographical area in which the pigs was reared according to chemical-nutritional parameters, stable isotope composition, fatty acids and sterols profile and mineral elements. The study was carried out in samples from two different geographical areas of north-eastern Sicily: "Mirto" area (inside the Nebrodi National Park) and "Valle del Mela" area (outside the Nebrodi National Park). The analysis was conducted on a total of 20 meat samples from pig females divided into two homogeneous groups: the 'Nebrodi group' (NG) and the 'External Nebrodi group' (ENG). The results showed that 51 out of 80 variables were significantly different ( $p \leq 0.05$ ) between NG and ENG. Chemical-nutritional parameters such as brassicasterol, campesterol and n-7 and n-9 fatty acids, were found significantly higher in the NG samples, due to the rich vegetation endemic to the Nebrodi area [2]. High levels of Pb were found only in the samples from the ENG group, probably due to the different anthropic activities present in the "Valle del Mela" area [3]. Furthermore, the  $\delta^{13}C$ ,  $\delta^{15}N$ ,  $\delta^2H$  and  $\delta^{18}O$  isotopic ratios of defatted meat were statistically different ( $p < 0.01$ ) between NG and ENG animals. This highlights that the change in dietary composition and drinking water consumption affects the quality of the meat and allows differentiation between the samples from the two study sites.

The existence of a link between the meat samples and their geographical origin, a necessary condition for the traceability of this particular product, has been demonstrated. In conclusion, this study could be used as a reliable tool to authenticate the 'Nero dei Nebrodi' breed and to use these correlations for traceability and consumer protection against fraud and commercial disputes.

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**Keywords:** black pig, traceability, stable isotopes, chemical composition, PDO



## C12

### ENSURING TRACEABILITY OF ITALIAN CURED MEAT PRODUCTS TREATED WITH HIGH-PRESSURE PROCESSING USING NEAR-INFRARED SPECTROSCOPY

**Maria Olga Varra**<sup>(1)</sup>, **Sergio Ghidini**<sup>(2)</sup>, **Alessandra Negri**<sup>(3)</sup>, **Riccardo Ghidini**<sup>(3)</sup>, **Riccardo Soldesti**<sup>(3)</sup>, **Adriana Ianieri**<sup>(1)</sup>, **Emanuela Zanardi**<sup>(1)</sup>

<sup>1)</sup> *Department of Food and Drug, University of Parma, Italy*

<sup>2)</sup> *Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy*

<sup>3)</sup> *Certosa Salumi S.p.A. - SterilParma S.p.A, Italy*

\*Corresponding author - E-mail: mariaolga.varra@unipr.it

High-pressure processing (HPP) is widely used in the meat industry as a post-packaging decontamination method, effectively extending the shelf-life and enhancing the safety of ready-to-eat (RTE) meat products. This technology enables European companies to comply with stringent safety regulations in key international markets, such as the USA and Japan, where zero tolerance for *Listeria monocytogenes* in RTE foods is in place, thus facilitating the global export of premium meat products. Currently, the traceability of HPP-treated products relies solely on documentation, highlighting the need for analytical techniques to verify both the products and the processing methods.

This work pioneers the application of near-infrared (NIR) spectroscopy as a rapid and non-destructive approach to verify HPP treatment in traditional cured Italian meat products.

The study encompassed 20 salami (dry fermented sausages), 25 pressed hams (dry-cured, boneless, shaped in rectangular blocks suitable for slicing), and 26 entire hams (dry-cured, boneless, shaped in the classic leg form). Each sample was vacuum-sealed in plastic film and treated by HPP at 600 MPa for 6 minutes using a commercial unit (Hiperbaric, Spain) situated at the Certosa Salumi-SterilParma facility (Parma, Italy). Spectroscopic analysis was conducted before (C), immediately after (T0), and 48 hours post-treatment (T48) using a portable MicroNIR spectrometer (908-1676 nm range, Viavi Solutions, USA). Through packaging, specific surface areas were scanned, and data were analyzed using orthogonal partial least squares discriminant analysis to differentiate between C and T (T0/T48) NIR spectra of samples. The reliability of the discriminant models was rigorously evaluated via 7-fold cross-validation.

Distinct spectral fingerprints for C and T samples of both salami and hams were identified, primarily reflecting changes in protein structure and water state. These unique spectral profiles allowed for the development of discriminant models with exceptional fitting ( $R^2X > 0.98$ ) and predictive abilities ( $R^2Y$  and  $Q^2 > 0.84$ ). Misclassification occurred in only one T pressed ham sample, while a perfect recognition rate (100%) was achieved for C and T entire ham and salami samples.

The analysis of sample distribution in the score plots confirmed that most of the spectral variability primarily stemmed from HPP-induced modifications. While minor spectral changes caused a slight separation between T0 and T48 salami samples following storage, these changes did not lead to overlap with C samples. This indicates that the HPP-induced modifications are retained even after storage, suggesting that the trained models can be applied at different stages of the product's shelf life.

In conclusion, NIR spectroscopy offers substantial promise in enhancing control procedures for food business operators and regulatory bodies, thereby reinforcing traceability and upholding food safety standards for meat products.

**Keywords:** process traceability, chemometrics, NIR fingerprinting, meat products, high-pressure processing

### C13

#### **AUTHENTICITY OF PIEDMONT EXTRA VIRGIN OLIVE OIL: ADVANCED AND NON-TARGETED ANALYSIS AND CHEMOMETRICS TO PROMOTE AN UNDERSTUDIED FUNCTIONAL OIL**

Ambra Bonciolini<sup>(1)</sup>, Giulia Potenziani<sup>(1)</sup>, Virginia Glicerina<sup>(1)</sup>, Vladimiro Cardenia\*<sup>(1)</sup>

<sup>1)</sup> *Department of Agricultural, Forest and Food Sciences, University of Turin, Italy*

\**Corresponding author - E-mail: vladimiro.cardenia@unito.it*

Piedmont, a region in northern Italy, is famous for its hazelnut production. However, the presence of ancient olive trees is an ongoing testimony to the country's long agricultural past, and their genotyping might be valuable as an undiscovered source of olive variation. Furthermore, the quality of virgin olive oil derived from old olive trees in the Piedmont area has received little attention. Currently, due to climate changes, Piedmont produces more than 30 tons of extra virgin olive oil (EVOO). The present study chemically characterized the Piedmont EVOO currently produced and obtained from ancient olive trees. The virgin olive oils were characterized in terms of peroxide value (PV), free acidity (FA), fatty acid (FAMES), sterol composition (STE), total phenolic content (TPC) and volatile organic compounds (VOCs). In addition, non-target screening method with LC-ESI-QTOF was applied. Principal component analysis (PCA) as well as partial least square - discriminant analysis (PLS-DA) was used to recognize qualitative markers. All samples displayed a PV90%. The total sterols content (1669-2124 mg/kg) was mostly constituted of *b*-sitosterol (64-89% of total sterols) followed by  $\Delta$ 5-avenasterol (11-18%), campesterol (4.4-5.1%) and stigmasterol (3.3-4.2%). In addition, detectable amount of obtusifoliol and citrostadienol were found. The main VOCs were (*E*)-2-hexenal, 3-hexen-1-ol, heptanal, 2-heptenal, hexyl acetate, hexanol and terpene compounds such as farnesene and geraniol. The un-target analysis putatively annotated 262 polyphenols; the olive oil was mainly characterized by phenolic acids, in particular hydroxycinnamic acids, flavonoids, tyrosol; while luteolin, epigenin and naringenin were the most recognized flavonoids. However, compared to the current production of EVOO in Piedmont, the oil obtained from ancient trees was mainly recognized for esculetin, diosmetin, quinic acid, and *p*-coumaric acid. In conclusion, the present study establishes a database for minor chemical compounds, especially phenolic compounds and volatile organic compounds. It also represents a starting point for further research on shelf-life and technological factors.

**Keywords:** olive oil, phenols, mass spectrometry, authenticity, volatilomics

### C14

#### FOOD FRAUD OR GMP DEFECTS? A PRAGMATIC APPROACH TO AUTHENTICITY ASSESSMENT OF CITRUS JUICES

**Markus Jungen**\*<sup>(1)</sup>

<sup>1)</sup> SGF International e.V., Germany

\*Corresponding author - E-mail: markus@sgf.org

Volatile commodity prices incentivize food fraud, particularly in *Citrus* juices. Common fraudulent practices include the substitution or addition of juices from different *Citrus* species than those labelled, deviations from good manufacturing practices (GMP), and misrepresentations about the juice's geographical origin. Ensuring the authenticity of *Citrus* juices is essential for consumer trust and fair trade, but distinguishing between food fraud and GMP deficiencies is challenging due to the complexities of conventional *Citrus* juice production technologies.

Which analytical strategies are particularly effective in detecting unauthorised processing operations in *Citrus*? A combined approach of quantitative <sup>1</sup>H-NMR spectroscopy, targeting the albedo marker phlorin, and chromatographic methods analysing numerous phenolic components was utilized to determine whether food fraud, processing deficiencies, or natural defects are present in *Citrus* juices. Using a univariate approach, phlorin content serves as a reliable marker for unauthorized whole-fruit processing. Peel extracts, peel wash, off-line pulp wash, and whole-processed fruit products exhibit significantly higher phlorin levels compared to legally produced citrus juices.

In addition to phlorin, profiles of coumarins, psoralens, polymethoxyflavones, hydroxycinnamates, flavanones, flavones, and flavonols also show potential utility. A pragmatic approach was developed by comparing analytical results from three different (U)HPLC methodologies applied to lemon and lime samples. This approach distinguishes between food fraud and GMP deficiencies by assessing phenolic components and maximum phlorin levels, adhering to the AIJN Code of Practice.

By this a comprehensive chemical evaluation and species-related classification of *Citrus* juices, as well as the post-hoc differentiation of the type and intensity of applied processing technologies is enabled. Utilizing reliable reference sample sets, the new insights into phenolic marker substances and classic major components in *Citrus* juices allow for the separation of food fraud from inadequacies in manufacturing processes and the technological characteristics of standard *Citrus* juice processing.

**Keywords:** authentication, food fraud, fruit juice, phenolics

### C15

#### NMR-BASED METABOLOMICS TO VALIDATE FINGERPRINTS OF DIFFERENT SAUDI HONEYS

Somaiah Almubayedh<sup>(1)</sup>, Amani Albalawi<sup>\* (1)</sup>, Upendra Singh<sup>(2)</sup>, Mariusz Jaremko<sup>(2)</sup>, Abdul-Hamid Emwas<sup>(2)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

<sup>2)</sup> KAUST, Saudi Arabia

<sup>\*</sup>Corresponding author - E-mail: aabalawi@sFDA.gov.sa

A natural, agricultural product made from nectar, honey is nutritional and pharmaceutically valuable. Geographical and botanical origins, as well as other external factors, affect honey's chemical composition. Conditions such as seasonality, environment, processing, and storage play a role. In Saudi Arabia, honey is valued for its nutritional and therapeutic benefits, making it an indispensable part of the diet. Honey quality and authenticity are critical to honey production because of its medicinal properties. Authenticity is concerned with correctly labeling the botanical and geographical origin, whereas quality assures purity during processing. Therefore, NMR and chemometric analysis are effective. Metabolomics is an emerging and rapidly growing research field defined as "the complete set of metabolites/low-molecular-weight intermediates, which are context-dependent, varying according to the physiological, developmental or pathological state of the cell, tissue, organ or organism." NMR is a common method for metabolomics, offering high reproducibility and nondestructiveness. It is also highly quantitative with fast throughput and can automate measurements.

Considering the above, the novelty of this work was conducted by performing metabolomics studies using NMR and chemometrics on Saudi honey. This has been established by applying both 1D and 2D NMR-based metabolomics to validate Saudi honey's botanical and geographical origin. The study revealed that 27 metabolites varied significantly across different honey species, while only five varied across regions. These results confirmed some of the quality, medicinal, and nutritional value of Saudi honey. In addition, they validated the botanical sources and geographical regions of the honey samples. Species classification is more informative than geographical location for identifying honey quality, indicating significant variation within species than across regions.

This discovery under SFDA is significant for honey consumers, as it indicates that honey quality is more variable within species than across locations. Our findings will help promote the production and marketing of local honey. This will encourage local beekeepers to increase their production of honey species with therapeutic and nutritional benefits. The primary results of this study will serve as the basis for guiding the methodology for future investigations of honey authenticity to build a robust Saudi Honey Database.

**Acknowledgement:** Saudi Food and Drug Authority and King Abdulla

## C16

### DETECTION OF PEPPER ADULTERATION USING MALDI-TOF SPECTROMETRY

**Paul Rentz<sup>(1)</sup>, Martine Bergaentzle<sup>(2)</sup>, Morane Lointier<sup>(3)</sup>, Elisa Bard<sup>(3)</sup>, Dalal Werner<sup>\*(3)</sup>**

<sup>1)</sup> Aerial & Université de Strasbourg, CNRS, IPHC UMR7178, France

<sup>2)</sup> Université de Strasbourg, CNRS, IPHC UMR7178, France

<sup>3)</sup> AERIAL, France

\*Corresponding author - E-mail: da.werner@aerial-crt.com

Authenticity of foods and veracity of food labels are currently major concerns for consumers, regulators, and the food industry [1]. While fraud and authenticity issues and more specifically Economically Motivated Adulteration (EMA) [2] are becoming increasingly important in the food industry [3]. A survey carried out in 2016 by the French Directorate General for Competition, Consumer Affairs, and Fraud Control (DGCCRF) revealed that 59% of the pepper products had anomalies, and 19% of them included adulteration [4].

To address food adulteration issue, there is a need for a high-performance, robust method allowing screening of many samples. MALDI-ToF (Matrix-Assisted Laser Desorption-Ionisation technique) spectrometer is a powerful tool that can be used for high-throughput screening to detect and quantify metabolites and other molecules. This technique has advantages of simple and rapid sample preparation, short analysis time, high sensitivity and ability to detect different components in complex mixtures.

The aim of this communication is to present the approach used to explore the potentiality of MALDI-ToF spectrometer for the authenticity testing of pepper.

To this end, samples of authentic *Piper nigrum* (n=40), non-*Piper nigrum* (n=10) and adulterants (n=31) were collected. In addition, blends of *Piper nigrum* and adulterants at adulteration levels of 5 %, 10 %, 25 %, 30 % and 50% (w/w) were prepared. Samples were first cryo-grinded and methanol extracts prepared. An aliquot of 2µL of methanol extract were then placed on a target plate (96 positions) with 2,5-dihydroxybenzoic acid (DHB) as matrix. A dozen replicates per sample were analyzed and spectra were recorded at mass ranging from 50 to 1000 Da. Prior to statistical analysis all spectra were pre-processed and averaged. Principal component analysis (PCA) was used to highlight potential grouping within *Piper nigrum*, non-*Piper nigrum* and adulterant sample populations. To predict *Piper nigrum* adulteration, a one-class classification method Data Driven - Soft Independent Modelling of Class Analogy (DD-SIMCA) was implemented. This latter enabled to create a model using only samples belonging to authentic *Piper nigrum* class and to classify adulterated *Piper nigrum* samples based on how good the model can fit them. *Piper nigrum* samples were grouped and mostly well discriminated from non-*Piper nigrum* and adulterant groups (PCA). DD-SIMCA models built using data spectra of methanol extracts achieved sensitivity at 96% and 96% for specificity. These results constitute a first step in revealing the potential of MALDI-ToF as a suitable tool to detect *Piper nigrum* adulteration.

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**Keywords:** pepper, MALDI-ToF, authenticity, adulteration, chemometric

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## C17

### REGULATORY REGIME AND FOOD FRAUD VULNERABILITY: IMPACT OF BREXIT

Amodya Theekshani Kolamunna<sup>(1)</sup>, Fiona Lalor<sup>\*(1)</sup>, Sinead Furey<sup>(2)</sup>

<sup>1)</sup> University College Dublin, Ireland

<sup>2)</sup> Ulster University, United Kingdom

\*Corresponding author - E-mail: [fiona.lalor@ucd.ie](mailto:fiona.lalor@ucd.ie)

On 24<sup>th</sup> June 2016, the majority of the UK voted to leave the European Union and formally departed the EU on 31<sup>st</sup> December 2020. With this departure, the UK began to diverge from EU law. The objective of this study is to review the risks associated with regulatory divergence and identify the impact of Brexit and subsequent regulatory divergence and inconsistencies on the Irish food and drink industry and potential for food fraud. A systematic literature review was conducted using peer-reviewed articles, government reports, and 9 editions of the EU-UK divergence tracker. As its nearest neighbour and largest trading partner, some of the major divergences that could affect the Irish agri-food sector are the UK's animal export ban, identifying gene editing separately from genetic modification, the new UKCA mark, rules of origin requirements, not banning Titanium Dioxide, removing radioactivity restrictions on food imports from Japan, not following the EU's reduction of permissible Arsenic level by 80%, Windsor Framework, and the Border Target Operating Model. The Windsor Framework has introduced a red lane - green lane process which simplifies customs processes, particularly for Northern Ireland by safeguarding its place in both the EU and UK single markets. The Border Target Operating Model categorizes the food imported into the UK based on the risk and requires pre-notifications and an Export Health Certificate for high-risk SPS goods. The UK landbridge can still be utilized, albeit with increased requirements and documentary checks, potentially leading to higher costs and possible delays. Therefore, Ireland's direct trade with the EU shows a 13% increase in 2021, bypassing the traditional UK landbridge. New trade routes include Dublin-Rotterdam-Zeebrugge, Rosslare-Dunkirk, Rosslare-Bilbao, and Cork-Antwerp-Zeebrugge. With all these new controls and regulatory divergences, Brexit has created an increased risk of food fraud by generating opportunities, motivations, and inconsistencies in control measures. Increased cost, time, and resource consumption associated with the food supply chain can motivate individuals to commit food fraud, and Northern Ireland's position in the EU and UK single markets can be used as an opportunity by the fraudsters. Inconsistencies in regulations and border controls have created weaknesses in control measures. However, even with all those challenges, 95% of Irish food businesses are optimistic about growth, and in 2022, the UK remained Ireland's primary agri-food export destination, with a value of €6.7 billion.

**Keywords:** Brexit, regulatory divergence, Irish food and drink industry, food fraud

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### C18

#### METHOD FOR THE DETERMINATION OF PARAFFIN AND STEARIN/STEARIC ACID FRACTIONS IN BEESWAX BY USING GC/MS

**Chiara Manzinello**<sup>(1)</sup>, **Franco Mutinelli**<sup>(1)</sup>

<sup>1)</sup> *NRL for honey bee health, Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

<sup>\*</sup>*Corresponding author - E-mail: cmanzinello@izsvenezie.it*

Beeswax plays a multifunctional role in the honeybee colony (*Apis mellifera* L.), serving as a building material for the comb (comb wax), which provides essential infrastructure for brood rearing and food storage. It is crucial to use high-quality (genuine and authentic) beeswax in beekeeping to maintain its natural versatility and ensure the well-being of the honey bees.

Furthermore, raw beeswax is utilized in various industrial sectors, including pharmaceuticals, cosmetics, and food (for example, as a food additive, packaging material, and coating), in addition to its primary and significant reuse in beekeeping, where it is processed into comb foundations.

Adulteration of beeswax in beekeeping is a growing global issue. Contamination with paraffin can cause comb collapse, while stearic acid negatively affects brood development in honey bees.

The method described allows for the detection of common adulterants in beeswax, such as paraffin, stearin, and/or stearic acid, using gas chromatography coupled with mass spectrometry (GC/MS) to identify paraffinic n-alkanes and methyl esters of palmitic, oleic, and stearic acids present in beeswax.

A small portion of beeswax (50 mg) is dissolved in a solvent mixture, which is then further diluted (1:500). Part of this diluted solution is subjected to fractionation via solid-phase extraction (SPE). The fraction containing paraffinic n-alkanes is analyzed instrumentally, while the other fraction is further derivatized to produce fatty acid methyl esters. The quantification of paraffinic n-alkanes and fatty acid methyl esters is performed using GC-MS and internal standardization is used for the quantification of analytes.

The methodology obtained satisfactory results in terms of both recovery percentages and relative standard deviations (70-120% and  $\leq 20\%$ , respectively).

Thirty beeswax samples provided by local beekeepers associations were analyzed and the concentrations of the investigated substances were below the LOQ.

This work was carried out as part of an inter-laboratory validation study aimed at evaluating the repeatability and reproducibility of the analytical method promoted by The Food Integrity Unit of the European Commission's Joint Research Centre (JRC).

**Keywords:** beeswax, adulteration, GC/MS

### C19

#### ANALYSIS OF MELAMINE AND CYANURIC ACID IN DAIRY PRODUCTS: INTERLABORATORY TESTS AS A QUALITY TOOL

Julien Leprovost<sup>\*(1)</sup>, Antoine Gorse<sup>(1)</sup>, Anne Tirard<sup>(1)</sup>, François Roussey<sup>(1)</sup>, Abdelkader Boubetra<sup>(1)</sup>

<sup>1)</sup> BIPEA, France

*\*Corresponding author - E-mail: jleprovost@bipea.org*

Melamine and cyanuric acid are low-mass compounds rich in nitrogen. Their fraudulent use to falsify the protein content of dairy products has been at the center of health scandals. Although the lethal toxicity of these compounds is low, their toxicity following chronic exposure is greater. Cyanuric acid, the hydrolysis product of melamine, forms a crystalline complex with the latter, which can be responsible for kidney failure (stones) and urinary failure. The contamination of food products by these compounds is not only the result of fraud: it can also be contamination by melamine as a by-product of phytosanitary treatments (cyromazine), after migration from plastic materials in contact with food, etc. Despite this, neither of these molecules should be found in food.

Quality control is therefore essential, using high-performance analytical measurements. More specifically, as regards powdered milk, these controls are of crucial importance for infant food safety. In this context, BIPEA (Bureau Interprofessionnel d'Etudes Analytiques) has been organizing interlaboratory comparison tests on this specific topic since 2015. The aim is to assess and improve the analytical performance of participating laboratories.

The implementation of the trials, in accordance with standard EN ISO 17043 [1], will be presented. From the production of the samples, spiked with melamine and cyanuric acid, to the statistical processing of the participants' results, in accordance with standard ISO 13528 [2]. The analytical method (HPLC-MS/MS) recommended by ISO 23970 [3] for these measurements will be described. A focus will be given to the results of a typical trial, as well as to the analytical performances resulting from the various campaigns carried out. The many benefits for laboratories of taking part in these tests make these interlaboratory campaigns a powerful quality tool.

[1] ISO 17043:2023, Conformity assessment -- General requirements for proficiency testing, 2023.

[2] ISO 13528:2022, Statistical methods for use in proficiency testing by interlaboratory comparisons, 2022.

[3] ISO 23970:2021, Milk, milk products and infant formula - Determination of melamine and cyanuric acid by liquid chromatography and tandem mass spectrometry (LC-MS/MS), 2021.

**Keywords:** laboratory performance, proficiency testing scheme, melamine, cyanuric acid, food safety

**Acknowledgement:** BIPEA acknowledges all laboratories participating in these PTS.



### C20

#### ORGANIC ACIDS AS MARKERS FOR WINE FRAUDS IDENTIFICATION

Gianina Marian (Toca)<sup>(1)</sup>, Gabriela Rapeanu\*<sup>(1)</sup>

<sup>1)</sup> *Dunarea de Jos University of Galati, Romania*

\**Corresponding author - E-mail: grapeanu@ugal.ro*

Even the importance of organic acids was greatly underestimated in the past, nowadays they are responsible for having anti-inflammatory, antibacterial, and antioxidant qualities in food. Because they are correlated with the final wines' chemical and organoleptic properties, organic acids attracted more attention in recent times. Wine contains acids that contribute to its acidity and freshness, including citric, malic, and tartaric acids. These acids can give a variety of flavors, from fruity to peppery and mineral, to the wine. At some points in the winemaking process, determining the total acidity is a defining criterion to ensure that the value is within the legal limitations. During malolactic fermentation, malic acid is converted to lactic acid, which increases pH and lowers titratable acidity to produce a velvety-tasting wine. Citric acid is considered to have a significant role in the wine-making process. Citric acid content greater than 1 g/L, denotes wine adulteration. The grape variety, the vineyard's location, the grapes' stages of ripeness, and the harvest year all affect the total amount of acids in wine. First, malic acid and then tartaric acid are generated when the grapes ripen. In order to produce balanced wines with a minimum total acidity of 3.5 g/L, tartaric acid is most frequently applied in maximum doses of 1.5 g/L to address the must's lack of acidity. The OIV has approved the addition of tartaric and citric acids as organic acids to wine. One of the main steps in wine authentication is the process of identifying wine fraud by addition of organic acids. The aim of this study was to use classical and modern methods in order to identify the presence of organic acids in wines which can be used as markers for wine frauds identification.

**Keywords:** organic acids, modern methods, wine, authentication, wine frauds

### C21

## HONEY AUTHENTICITY: THE POWER OF ICP-MS TRIPLE QUADRUPOLE TO DETERMINE THE GEOGRAPHICAL AND BOTANICAL ORIGINS OF HONEY

**Laurent Naels**<sup>(1)</sup>, **Jean-François Garnier**<sup>(1)</sup>

<sup>1)</sup> *Thermo Fisher Scientific, France*

*\*Corresponding author - E-mail: laurent.naels@thermofisher.com*

Honey authenticity is a growing concern globally. The falsification of the geographical and botanical origin of honey is a common issue, such as mixing Chinese honey with honey from other countries to pass it off as local, or to incorrectly report the floral origins of certain honeys. To address these concerns, determining the botanical and geographical origin of honey is crucial to ensure its authenticity and quality. Different types of honey have distinct flavors, colors, and nutritional profiles based on the plants from which bees collect nectar. By identifying the botanical origin, consumers can make informed choices based on their preferences and dietary needs. Determining the geographical origin is also vital for traceability and food safety, enabling consumers to know where their honey comes from and ensuring compliance with quality standards and regulations.

The objective of this study is to set-up a workflow for the creation of a reliable database based on quantitative and isotopic analysis of different honey samples and to predict the geographical and botanical origins of unknown honey samples.

For the honey sample preparation, the combination of the Milestone™ easyFILL™ acid dispenser and the ultraWAVE™ Single Reaction Chamber (SRC) microwave digestion system ensures an automatic, complete, and reproducible acid digestion of honey samples.

From an analytical point of view, the main identifying elements of honey authenticity can be present at low concentrations while other elements are predominant in the digested samples. High sensitivity, wide linear range, and robustness are essential for the database accuracy. Triple Quadrupole Inductively Coupled Plasma Mass Spectrometry (TQ ICP-MS) is the elemental analyzer that is best suited for this type of analysis to effectively suppress isobaric and polyatomic interferences for accurate quantitative results and to precisely measure isotopic ratio. The Thermo Scientific™ iCAP™ TQ ICP-MS was used to acquire both quantitative and isotopic analysis.

Minitab® statistical software was selected for this study to combine the data from quantitative and isotopic results, and to automatically create a model based on the known honey samples. The statistical tool thus determines the contributions of each element whether during the quantitative or isotopic analysis and builds a model to establish a prediction on the geographical and floral origins of unknown samples.

122 honey samples with well-known geographical and botanical origins were used to build the model. The complete workflow, including sample preparation, iCAP TQ analytical parameters and performances, and prediction results of unknown honey samples will be highlighted.

**Keywords:** honey, authenticity, elemental composition, profiling, inductively coupled plasma mass spectrometry

## C22

### COMPOSITIONAL PROFILING FOR THE QUALITY ASSESSMENT OF CANADIAN HONEYS

**Mile Shao<sup>(1)</sup>, Asma Mdimagh<sup>(1)</sup>, Lan Liu<sup>(1)</sup>, Stephane Bayen<sup>(1)</sup>, Salwa Karboune<sup>\*(1)</sup>**

<sup>1)</sup> McGill University, Canada

\*Corresponding author - E-mail: salwa.karboune@mcgill.ca

The value of honey, a natural sweetener with long human consumption history, has gained renewed attention in the last decades. This has driven a growing consumer demand for high quality, authentic honeys, particularly monofloral ones. In the present study, the compositional profiles of 163 honey samples from four botanical origins relevant to the Canadian market (buckwheat, clover, goldenrod, and blueberry) were characterized in terms of enzymatic activity and sugar profiling with the aim to develop a robust honey authentication method. The enzymatic activity profiling included the determination of the levels of diastase, invertase, acid phosphatase, glucose oxidase, and catalase activities; while the sugar profiling consisted of the quantification of fructose, glucose, 11 disaccharides and 7 trisaccharides using LC-MS-Qtof. The results showed that clover and goldenrod honeys exhibited higher diastase and invertase activities, with average values of 11.90 DN/4.24 IN and 12.52 DN/5.33 IN, respectively, compared to blueberry (8.832 DN/3.16 IN) and buckwheat (9.92 DN/1.86 IN) honeys. The highest average acid phosphatase (AP) activity was detected in buckwheat honey activity (683.202 mg P/100g), while blueberry honey has the highest average glucose oxidase activity (11.843  $\mu\text{g H}_2\text{O}_2/\text{g/ h}$ ) and average catalase activity (27.629  $\mu\text{g H}_2\text{O}_2/\text{g/ min}$ ), respectively. Fructose, the predominant sugar in all honeys, had an average concentration ranging from 37.0% to 38.1% (w/w). Glucose, the second most abundant sugar, had an average concentration ranging from 32.0% to 34.1% (w/w) across the four floral types. Maltose was identified as the most abundant disaccharide, with an average concentration ranging from 0.94% to 1.12% (w/w), followed by isomaltose at 0.593% to 0.657% (w/w). Sucrose, trehalose, gentiobiose, nigerose, and erlose were also successfully detected and quantified in most of the samples. A multi-targeted analysis of potential predictor variables (e.g. enzyme activities, sugar compounds) in 163 honey samples was conducted to examine their correlation with each other and with the response variables (botanical origin of honey) with the goal to identify the markers for discriminating the botanical origins. AP activity and catalase activity were identified as markers to differentiate clover, blueberry, and buckwheat honey under biplot cluster analysis. Negative correlations were observed between glucose and the F/G ratio, nigerose, isomaltose, gentiobiose, and the overall tetrasaccharide content. Principal component analysis (PCA) was applied to all the quantitative data investigating the correlation between the enzyme and carbohydrate profiling and the botanical origin of the honey, with several enzymes and oligosaccharide found to be potential markers for authentication. A logistic regression model and an XGBoost classifier were successfully employed to predict the floral type of the honeys in the study, achieving accuracies of 84% and 90%, respectively.

**Keywords:** honey, enzymatic activity profiling, carbohydrate profiling, multi-variance analysis

**Acknowledgement:** This study is financially supported by the Natural Sciences and Engineering Research Council of Canada Alliance Project grant (NSERC ALLRP 570864-21) and Canada Foundation for Innovation (John R. Evans Leaders N°36708).

### C23

#### RAPESEED OR NOT RAPESEED? RAPID ANOMALY DETECTION USING RAMAN SPECTROSCOPY COMBINED WITH ONE CLASS CLASSIFICATION

Lena Weckend<sup>(1)</sup>, Michael Pfister<sup>(1)</sup>, Janet Riedl<sup>(1)</sup>, Carsten Fauhl-Hassek<sup>(1)</sup>, Tanja Schwerdtle<sup>(1)</sup>, Marcus Glomb<sup>(2)</sup>, **Felix Wustrack**\*<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

<sup>2)</sup> Martin-Luther-Universität Halle-Wittenberg, Germany

\*Corresponding author - E-mail: felix.wustrack@bfr.bund.de

Detecting anomalous samples in food and feed control is vital for ensuring consumer safety and identifying fraudulent practices. Raman spectroscopy-based fingerprinting is a rapid and non-destructive analytical method that provides an effective solution for this challenge and could be a viable alternative to systems in routine analysis. It can be used to differentiate edible oils based on their botanical origin and to detect atypical products potentially resulting from adulterations. [1, 2] This spectroscopic technique facilitates quick screening and can manage complex sample compositions. When combined with multivariate statistical analysis, it leverages the full spectral data for authentication purposes. The application of one-class classification approaches for authentication offers multiple advantages over multi-class approaches. One of the foremost advantages is that outlying samples are quickly identified and can be flagged as anomalous for further analysis. This ensures that samples with unknown adulterants can be easily detected.

In this study, 138 rapeseed oils from different producers and harvest years have been analysed using a benchtop Raman spectrometer, equipped with a well plate reader, to characterize the target class. Moreover, 23 sunflower oils and 17 soybean oils were used to investigate the discrimination of the botanical origin. In order to examine the detection of adulterations, antioxidants (BHA and BHT) have been utilized as demonstrator substances to represent unknown adulterants within a concentration range of 1-10%. One-class classification successfully detected these adulterations with a specificity of 100% at concentrations of 3% and higher. This is mainly attributable to the presence of exogenous signals for BHA and BHT.

In conclusion, a one-class model for verifying rapeseed oil was developed and allowed to discriminate oils of other botanical origins, to detect anomalous samples with adulterants and can thus make a contribution to consumer safety. This demonstrates the potential of Raman spectroscopy in conjunction with multivariate data analysis for the authentication of edible oils and provides the basis for future applications in routine analysis, employing the developed prediction models.

[1] F. Kwofie et al., Applied Spectroscopy 2020, 74(6).

[2] D. McDowell et al., European Journal of Lipid Science and Technology 2018, 120(7).

**Keywords:** authenticity, one class classification, Raman spectroscopy

## C24

### UNIVARIATE AND MULTIVARIATE QUANTIFICATION AND AUTHENTICATION OF BEER ON THE BASIS OF <sup>1</sup>H-NMR SPECTRA

Sabine Milbert<sup>(1)</sup>, Olga Krenz<sup>(2)</sup>, Teresa M. Müller<sup>(2)</sup>, Johannes Fuchs<sup>(2)</sup>, Leane Lehmann<sup>(3)</sup>, Steffen T. Seifert<sup>\*(2)</sup>

<sup>1)</sup> Food chemistry chair of Julius-Maximilians-University Würzburg and Bavarian Health and Food Safety Authority, Office Würzburg, Germany

<sup>2)</sup> Bavarian Health and Food Safety Authority, Office Würzburg, Germany

<sup>3)</sup> Food chemistry chair of Julius-Maximilians-University Würzburg, Germany

\*Corresponding author - E-mail: [steffen.seifert@lgl.bayern.de](mailto:steffen.seifert@lgl.bayern.de)

German beer enjoys a high reputation, both domestically and internationally. With total sales of 83,762,000 hectoliters and an export volume of 14,326,000 hectoliters in 2023, German beer plays a significant role on the international beer market [1]. <sup>1</sup>H-NMR spectroscopy has proven to be a powerful method for the screening and quantification of food components. This method enables both univariate and multivariate analysis of beer samples and thus fulfils the requirements for efficient analytical authenticity checks.

The aim of this study is to develop a suitable analytical method for routine beer analysis based on <sup>1</sup>H-NMR spectroscopy.

To ensure the spectra are optimal for quantification and subsequent classification of beer, the sample preparation and <sup>1</sup>H-NMR measurement method were optimized for maximal reproducibility of the resulting spectra. In conclusion the pH-adjustment against a pH-reference and the choice of a sufficient water suppression pulse sequence (NOESY-presaturation) proved to be essential.

Univariate quantification of malic acid, citric acid, lactic acid and acetic acid, as well as ethanol was conducted. After peak identification, deconvolution and PULCON-calculation were applied to quantify those components. Analyte-specific factors were determined through spiking experiments to improve accuracy. The results were validated against reference methods. In a small-scale ring-trial against reference methods a sufficient z-score (

Additionally, a multivariate quantification of beer-specific parameters such as original gravity, colour, and bitterness units was carried out. This analysis was based on datasets collected from a variety of beer types/styles. Preliminary results show a good concentration-range wide prediction of the original gravity (RMSE

In addition, preliminary tests for the parallel use of the spectra for multivariate classification, e.g. yeast-type used within brewing process, are shown. As the first part of an ongoing project, these results demonstrate the potential of <sup>1</sup>H-NMR spectroscopy as a powerful tool for comprehensive analysis, authentication and quality control of beer, benefiting both the consumers and the brewing industry.

[1] <https://brauer-bund.de/wp-content/uploads/2024/04/STATIST-2023.pdf>, accessed: 09.07.2024.

**Keywords:** authenticity of beer, <sup>1</sup>H-NMR spectroscopy, quantification, classification, PULCON

### C25

#### FOODSAFER: IS TURMERIC A SUPER-FOOD OR A SUPER-FRAUD?

Simon Haughey\*<sup>(1)</sup>, Wilfred Abia<sup>(1)</sup>, Holly Montgomery<sup>(1)</sup>, Heidi Russel<sup>(1)</sup>, Paul Williams<sup>(1)</sup>, Chris Elliott<sup>(2)</sup>

<sup>1)</sup> National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, N. Ireland BT9 5DL., United Kingdom

<sup>2)</sup> School of Food Science and Technology, Faculty of Science and Technology, Thammasat University, 99 Mhu 18, Pahonyothin Road, Khong Luang, Pathum Thani 12120, Thailand, Thailand

\*Corresponding author - E-mail: s.a.haughey@qub.ac.uk

Turmeric is a highly valued spice recognized for its use as both a culinary ingredient and a superfood supplement, attributed to numerous purported health benefits. Its rising popularity has unfortunately led to instances of fraud, where contaminants like lead chromate and metanil yellow dye are added to enhance its colour, thereby imitating the appearance of curcumin, especially in lower-quality turmeric. This type of fraud is termed economically motivated adulteration (EMA) where the perpetrator can sell low quality material at higher prices. Lead chromate is classified as a human carcinogen, teratogen, and a suspected mutagen. Meanwhile, metanil yellow is a harmful azo dye, and research indicates that prolonged exposure to this dye can result in neurotoxicity, hepatocellular carcinoma, tumour formation, adverse effects on gastric mucin, and lymphocytic leukaemia. Under the auspices of the EU Horizon Europe project, FoodSafeR, rapid analytical techniques, e.g. X-Ray Fluorescence (XRF) and Near-Infrared (NIR) spectroscopy, are being developed and validated with the view to being used for in-field testing in the spice supply chain to test for the presence of these deliberately added contaminants. Samples of turmeric from emerging markets in Tanzania, Cameroon, Ghana and Bangladesh will be analysed and the results presented. Will turmeric be a super-food or a super-fraud?

**Keywords:** turmeric, fraud, NIR, XRF, adulterants

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## C26

### MONOFLORAL HONEY AUTHENTICATION BY LC-HRMS/MS PROFILING

**Roberto Stella**\*<sup>(1)</sup>, **Andrea Massaro**<sup>(1)</sup>, **Carmela Zacometti**<sup>(1)</sup>, **Alessandra Tata**<sup>(1)</sup>, **Giancarlo Biancotto**<sup>(1)</sup>

<sup>1)</sup> *Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

\**Corresponding author - E-mail: rstella@izsvenezie.it*

The purpose of the research was to apply non-targeted metabolomics, based on LC-HRMS technique, for authenticating the botanical origin of monofloral honey. To this aim, 105 monofloral honey samples of different botanical origin (acacia, citrus, eucalyptus, linden, sulla, honey-dew and chestnut) were collected from the Italian beekeepers taking part to the "Goccia d'oro" competition held on 2023. During the competition, the authenticity of the samples was verified by sensory and physicochemical analyses.

This set of samples was splitted in two independent subsets and analysed by LC-HRMS in two different time frames. In the beginning, 70 honey samples were analysed and the resultant outcomes were used as training set to build a classification model based on least absolute shrinkage and selection operator (LASSO). Subsequently, 35 samples were used as external dataset to validate the classification algorithm.

Among the detected and aligned chromatographic peaks collected by the LC-HRMS profiling, sixteen metabolites were selected by LASSO as predictors to classify honey samples. Performance of the classifier were assessed by performing a 5 repeated 5 fold cross-validation and by calculating the overall accuracy that resulted to be 98.86%. The external set of 35 honey samples was then analysed by LC-HRMS and used as validation set. The classification algorithm demonstrated to be fit-for-purpose given that the accuracy was 97.14% with only one sample being misclassified. This research indicates that LC-HRMS profiling is a promising tool to be used to authenticate honey's floral origin and it is worth being validated further with new samples from different harvest season to corroborate our findings.

**Keywords:** monofloral honey, LC-HRMS, honey authenticity

**Acknowledgement:** *This research was supported by the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF), National apicultural subprogram n. 0628067 "Ripartizione delle somme assegnate per l'esecuzione del Sottoprogramma nazionale del Piano apistico nazionale e relativo bando per l'accesso ai finanziamenti per l'annualità 2024". The authors are grateful to Lucia Piana, Giancarlo Naldi and Alberto Contessi from Osservatorio Nazionale Miele for their valuable collaboration.*

## C27

### CREATING A RELIABLE LC-HRMS-BASED METABOLOMICS APPROACH TO ENSURE AUTHENTICITY OF PDO FETA CHEESE FROM RAW MATERIAL TO THE FINAL PRODUCT

**Eleni Nastou<sup>(1)</sup>, Sofia Karanikolopoulou<sup>(1)</sup>, Anastasia Kritikou<sup>(1)</sup>, Panagiotis Loukas Gialouris<sup>(1)</sup>, Nikolaos Thomaidis<sup>\*(1)</sup>**

<sup>1)</sup> *Laboratory of Analytical Chemistry, Chemistry Department, National and Kapodistrian University of Athens, Greece*

*\*Corresponding author - E-mail: ntho@chem.uoa.gr*

Milk and dairy products are vital for the national economy of many countries, especially in the Mediterranean region, such as Italy, and Greece. Due to their high impact on the global market derived from their high nutritional and commercial value, they become targets of fraudulent practices, such as adulteration, and mislabelling. These practices become even more intensive in products that bear indications, as Protected Designation of Origin (PDO). Feta cheese, one of the most popular Greek cheeses worldwide, is a soft white cheese aged in brine, recognized as a PDO product by the European Commission (EC) (IP/02/866, 2002). According to the established European guidelines (EC, 2002a), feta cheese must be produced exclusively from pasteurized sheep milk, or a mixture of sheep and goat milk (the latter up to 30%). To ensure food safety and consumer protection, specific regulations were put in place (Regulation EC/178/2002), and a European Reference Methodology (ERM) was established according to EC No. 273/2008 for the detection of bovine proteins. However, the present regulations do not always guarantee dairy products' authenticity. Therefore, there is an urgent need to develop holistic analytical approaches, to provide fast, and reliable results to reassure dairy products' origin. During the last decades, High-Resolution Mass Spectrometry (HRMS) in combination with advanced chemometrics has revolutionized in food authenticity assessment, especially in dairy products due to the wealth of analytical information provided through the analysis.

In this study, a targeted metabolomics approach exploiting Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) was developed for the investigation of >100 Greek milk samples' metabolite content, belonging to two different animal origins (sheep and goat) and three geographical regions of Greece. These milk samples were used to produce feta cheese samples that were further investigated in terms of their metabolite content. For this purpose, and for the first time on feta cheese, the combination of Reversed Phase Liquid Chromatography (RPLC) and Hydrophilic Interaction Chromatography (HILIC) was fully exploited to achieve maximum metabolic coverage, in both positive and negative ionization mode.

Over 100 metabolites were identified in sheep samples, while more than 80 metabolites in goat samples, belonging to various metabolite classes, such as amino acids and their derivatives, organic acids, nucleotides, etc. Advanced chemometrics were used, such as Partial Least Squares Discriminant Analysis (PLS-DA), to build robust authenticity models that provide information regarding samples' origin, achieving discrimination with high accuracy. Various metabolite markers, found in milk and feta cheese samples, were identified for each group and then correlated between raw material and final product, to reassure the authenticity of feta cheese throughout the production line.

**Keywords:** dairy products, feta cheese, HRMS, authenticity, chemometrics

**Acknowledgement:** *This work took place in the framework of "Authentic Feta" project financed by Submeasure 16.1-16.2 "Establishment and operation of Operational Groups of the European Innovation Partnership for the productivity and sustainability of agriculture", in the context of Action 2 "Implementation of the operational plan of the operational groups for the productivity and sustainability of agriculture" (Project Code: M16ΣYN2-00310). This specific action is implemented within the framework of the Rural Development Program (RDP) of Greece 2014-2020 co-financed by the European Union - European Agricultural Fund for Rural Development (EAFRD).*



## C28

### A METABOLOMICS-BASED MODEL TO ASSESS THE ANTIBIOTIC-FREE LABEL CLAIM IN PORK

Maria Pia Fabrile<sup>(1)</sup>, **Maria Olga Varra**<sup>(1)</sup>, Sergio Ghidini<sup>(2)</sup>, Giovanni Loris Alborali<sup>(3)</sup>, Nicolo Riboni<sup>(4)</sup>, Maurizio Piergiovanni<sup>(4)</sup>, Monica Mattarozzi<sup>(4)</sup>, Federico Scali<sup>(3)</sup>, Maria Medugno<sup>(1)</sup>, Adriana Ianieri<sup>(1)</sup>, Federica Bianchi<sup>(4)</sup>, Maria Careri<sup>(4)</sup>, Emanuela Zanardi<sup>\*(1)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Italy

<sup>2)</sup> Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy

<sup>3)</sup> Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Italy

<sup>4)</sup> Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy

\*Corresponding author - E-mail: emanuela.zanardi@unipr.it

The strategies to monitor the usage of antibiotics in livestock are primarily based on the detection of the parent drug or its metabolites using targeted analytical methods, aimed at evaluating compliance with relevant regulations concerning drug residues. However, the authentication and traceability of meats from antibiotic-free chains pose new issues, and novel analytical tools are needed. The objective of the present study was to verify the possibility of using metabolomics fingerprinting for the distinction between pigs exposed to antibiotic treatment (ABT) and pigs never exposed to antibiotic treatment (ABF). Diaphragm samples were collected in two independent samplings, namely S1 (ABF, n=12; ABT, n= 19) and S2 (ABF, n=5; ABT, n= 10). All samples were randomly extracted according to the Bligh & Dyer [1] protocol to separately recover the metabolome and lipidome. Metabolomic analysis was performed using a binary Acquity UHPLC I-Class system (Waters), coupled with Synapt G2-Si HDMS QTOF mass spectrometer operating in data-independent High-Definition MS<sup>E</sup> acquisition with electrospray ionization in both positive and negative mode [2]. Data were recorded in MassLynx software (v4.2, Waters) and the pre-processing (auto-alignment of signals, peak peaking, deconvolution, and normalization) was conducted by using Progenesis Q1 software (Waters). Partial least squares discriminant analysis (PLS-DA) was applied to the dataset from sampling S1 (training set) (SIMCA 17, Sartorius Stedim Data Analytics AB) and a total of n=86167 selected features to discriminate samples according to antibiotic treatment exposure. The model showed satisfying performance in terms of goodness of fit ( $R^2X = 51\%$  and  $R^2Y = 99\%$ ) and prediction ability ( $Q^2 = 94\%$ ) following internal 7-fold cross-validation. The ability of the model to assign the correct label to candidate pork samples was estimated by using the dataset from sampling S2 as a test for the external validation. Correct classification rates of 80% and 90% for ABF and ABT samples, respectively, were achieved, confirming the accuracy of the model in predicting the class the samples belonged to.

The successful results obtained showed the feasibility of an untargeted metabolomic approach coupled to chemometric techniques to discern antibiotic-free from antibiotic-exposed pigs. The identification of novel biomarkers for authenticity verification purposes may be encouraged.

[1] Bligh EG, Dyer WJ (1959) *Can J Biochem Physiol*, 37, 911-917.

[2] Riboni et al (2023) *J Agric Food Chem*, 71, 15407-154016.

**Keywords:** pigs, untargeted metabolomics, antibiotic-free, high resolution-mass spectrometry

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## C29

### HEAT-STABLE PEPTIDE MARKERS OF BOVINE HEART, KIDNEY, LIVER, LUNG, AND SPLEEN FOR AUTHENTICITY-TESTING OF MINCED BEEF PRODUCTS

Thomas Braunersreuther<sup>(1)</sup>, Dagmar Brüggemann<sup>(1)</sup>, Wolfgang Jira\*<sup>(1)</sup>, Andreas Römpp<sup>(2)</sup>

<sup>1)</sup> Max Rubner-Institut (MRI), Department of Safety and Quality of Meat, Germany

<sup>2)</sup> University of Bayreuth, Chair of Bioanalytical Sciences and Food Analysis, Germany

\*Corresponding author - E-mail: wolfgang.jira@mri.bund.de

Beef is the most expensive of the three most popular types of meat and therefore a plausible target for financially motivated food fraud. Minced and otherwise processed beef products must be considered particularly vulnerable, as processing and seasoning may result in undeclared substitutions remaining unnoticed by the consumer. According to Regulation (EU) No 1169/2011, the term "meat" on food labelling may in the EU only be used to refer to "skeletal muscles of mammalian and bird species [...] with naturally included or adherent tissue". The addition of other tissues, such as beef heart, would have to be declared separately from beef in the list of ingredients of a product. The undeclared substitution of meat with internal organs of animals for financial reasons has been identified as a risk for meat products in the past, and the development of promising approaches for viable detection methods has already shown success. In particular, peptide analysis using LC-MS/MS has shown, using the example of rabbit liver, that it can enable the qualitative and quantitative determination of organ tissue in processed meat products.

The aim of our current work was to identify peptide markers for selected beef organs (heart, kidney, liver, lung, and spleen) and to prove their specificity in order to provide the basis for expanding the analytical possibilities for this type of food fraud. Using a bottom-up proteomics approach, peptide marker candidates were identified for the previously mentioned bovine (*Bos taurus*) organs. The peptides should be specific for the selected target tissues, while not showing cross-selectivity for a number of other relevant food ingredients (i.e. bovine blood, beef and meat from other species, common ingredients for beef burgers). To this end, potential marker peptides were searched for in digests of the target tissues using non-targeted LC-MS/MS analyses. The resulting peptides were subjected to a multi-stage selection process, starting with in silico (BLAST) and experimental specificity tests. Peptides that meet the specificity criteria are finally tested for analytical suitability and heat stability in a beef matrix. Preliminary results include peptide marker candidates for each organ, which still need to be tested for their inter-species selectivity, detectability, and heat stability in the chosen food model. These peptides could enable the development of an analytical method for the detection and differentiation of bovine organs used as ingredients in raw or cooked processed beef products.

**Keywords:** food fraud, beef, organs, LC-MS/MS, proteomics

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### C30

#### THE NEXT GENERATION LC-IRMS FOR HONEY AUTHENTICITY INVESTIGATION

Daniel Felsmann<sup>(1)</sup>, Nils Stoebener<sup>(1)</sup>, Qiong Li<sup>(1)</sup>, Mario Tuthorn<sup>\*(1)</sup>

<sup>1)</sup> Thermo Fisher Scientific, Germany

\*Corresponding author - E-mail: mario.tuthorn@thermofisher.com

LC-IRMS is a state-of-the-art technique for the detection of low-level adulteration of authentic honey with sugar syrups. The new Thermo Scientific™ LC IsoLink™ II IRMS System delivers a reliable, robust and efficient solution for high precision honey fraud detection using compound specific carbon isotope signature of individual sugars.

The next generation LC IsoLink II Conversion Interface is now fully integrated in the innovative Thermo Scientific™ Vanquish™ LC platform. The modular pull-out design is saving space and allows easy accessibility of all system parts without de-stacking for routine maintenance. The new cartridge-based oxidation reactor minimizes flow path blockage and significantly enhances system uptime and productivity. Full LC IsoLink II IRMS System operation is driven by the Thermo Scientific™ Qtegra™ ISDS Software that features complete integration with Chromeleon™ Chromatography Data System Software capabilities. Single software platform setup simplifies workflows, saves time and minimizes errors.

To assess long-term stability, system robustness and data reproducibility, the LC IsoLink II IRMS System has been operated in analytical food testing laboratories for over 2 years, allowing thorough system evaluation and optimization. We report data demonstrating excellent precision and reproducibility of  $\delta^{13}\text{C}$  values for measurements of a laboratory honey standard and commercial honey samples.

**Keywords:** honey, isotopes, sugars, authenticity, fraud

### C31

#### HIGH PRECISION ISOTOPE RATIO-ORBITRAP-MS - A NEW TOOL FOR VANILLIN AND CAFFEINE AUTHENTICATION

**Mario Tuthorn**<sup>\*(1)</sup>, Nils Kuhlbusch<sup>(1)</sup>, Dieter Juchelka<sup>(1)</sup>, Andreas Hilkert<sup>(1)</sup>, Qiong Li<sup>(1)</sup>

<sup>1)</sup> Thermo Fisher Scientific, Germany

\*Corresponding author - E-mail: mario.tuthorn@thermofisher.com

Vanillin and caffeine are widely used in food and beverage products. They can be sourced from natural or synthetic materials, through various production processes. The source of such compounds is of great interest as it carries cost implication for the final product, resulting in great demand for analytical techniques for source differentiation. Until recently, sector field isotope ratio MS and NMR have been the main tools used for these analyses. Here we demonstrate the ability of soft ionization-Orbitrap IRMS to provide high precision isotope ratio determination of intact caffeine and vanillin molecular ions with an option for fragmentation. This offers a unique opportunity for the analysis of intramolecular isotopic information like multiple heavy isotope substitutions (isotope clumping) as well as site specific isotopic information.

In this study we will highlight benefits of using Thermo Scientific™ Orbitrap Exploris™ Isotope Solutions for simultaneous C, H, N and O isotope ratios analysis from intact caffeine and vanillin molecular ions, specifically focusing on sample introduction and efficiency of analysis. Different methodologies and referencing strategies are evaluated to increase accuracy and precision of isotope ratio data. Orbitrap Exploris Isotope Solutions results are compared and benchmarked against international reference materials and the results obtained by sector field MS. Preliminary results of vanillin and caffeine analysis show accuracies and precisions down to ~0.5 ‰ for carbon, 2 ‰ for oxygen, 10 ‰ for hydrogen and 1 ‰ for nitrogen isotope ratios.

**Keywords:** vanillin, caffeine, isotopes, authenticity, orbitrap

## C32

### NON-TARGETED ANALYSIS OF WHISKY USING SPME ARROW AND ORBITRAP EXPLORIS GC 240 MASS SPECTROMETER

**Adam Ladak**<sup>(1)</sup>, **Dominic Roberts**<sup>(1)</sup>, **Giulia Giulia**<sup>(1)</sup>, **Nicholas Warner**<sup>(1)</sup>, **Daniel Kutscher**<sup>(1)</sup>

<sup>1)</sup> Thermo Fisher Scientific, United Kingdom

\*Corresponding author - E-mail: Adam.ladak@thermofisher.com

Whisky is a premium distilled spirit beverage that is produced using long established methods that involve a complex aging process. These processes result in a final product that has unique characteristics, which can sell at a premium retail price due to its high commercial value. It is essential that whisky producers can obtain an accurate and comprehensive chemical profile which is characteristic to their individual product. Such information can then be used to identify adulterated or counterfeit products in order to protect the product and brand reputation. In addition, the chemical profile provides a key component within quality control procedures as it can be used as a reference to detect any changes in the chemical composition between different production batches. This will ensure that the bottled whisky taste and flavour characteristics is a consistent with the consumers' expectations that are expected by the consumer.

The objective of this study was to analyse samples from different geographical regions, ages and types using a full mass range data acquisition to perform both a targeted and non-targeted workflow. Key targeted analytes (2-methyl-1 butanol, 3 methyl-1-butanol, Isoamyl Acetate and 3-Octanone) were analyzed to as a preliminary screening procedure to assess whisky authenticity. Following this, deconvolution of the high-resolution accurate mass data together with spectral matching and statistical analysis was performed for chemical profile identification and differentiation between whisky samples. In this study, an Orbitrap Exploris GC 240 high resolution accurate mass (HRAM) mass spectrometer together with the headspace solid phase micro extraction (HS-SPME) arrow was deployed. Each sample, including one pooled sample, was injected multiple times in a random order and acquired in EI full scan ( $m/z$  40-600) at a mass resolving power of 120,000 (FWHM,  $m/z$  200). Differences in chemical profiles are easily visualized using the statistical tools incorporated into the Thermo Scientific™ Compound Discoverer™ software and a streamlined identification achieved using the Thermo Scientific Flavor and Fragrances HRAM library together with the NIST 2023 nominal mass library. Statistical differences were observed in chemical profiles between different whiskies of Scottish origin, age declaration, and production region within Scotland. Distinguishing between malt and un-malted could be easily made through identification of key chemical markers. In addition, several other compounds known to be flavor/color additives were also Identified chemical markers highlighting the importance of chemical profile identification in whisky authenticity. More details will be presented in the poster.

## C33

### MEAT AND POULTRY ORIGIN WITH ISOTOPE RATIO MASS SPECTROMETRY: A RAPID AND ROBUST SAMPLE PREPARATION METHOD

Alexander Scherl<sup>(1)</sup>, Khadijetou Ahmed Ethmane<sup>(2)</sup>, Pierre Zimmerli<sup>(1)</sup>, Didier Ortelli<sup>(1)</sup>, Patrick Edder<sup>(1)</sup>

<sup>1)</sup> Official Food Control Authority of the State of Geneva, Switzerland, Switzerland

<sup>2)</sup> University of Geneva, School of Chemistry, Faculty of Sciences, Switzerland

\*Corresponding author - E-mail: alexander.scherl@etat.ge.ch

Locally grown meat and poultry has additional economical value due to animal welfare and proximity concerns. The risk of food fraud by selling non-local meat and poultry as local is therefore high. Multi-element stable isotope ratio measurements of defatted content of meat samples were previously shown to be able to discriminate between different geographical origins of beef, lamb and poultry. However, sample preparation, particularly defatting, is long and fastidious. Here, we present an easy, robust and rapid sample preparation method based on muscle protein precipitation prior to isotope ratio measurements.

Poultry tenderloins from various geographical origins were collected at local food stores and slaughterhouses. For  $^{18}\text{O}/^{16}\text{O}$  ratio measurements, 0.6 g homogenized meat was placed in a headspace vial, heated for 15 minutes at 95°C, shaken and transferred into an Isoflow headspace autosampler (Elementar, Langenselbold, Germany). The vials were flushed with 10% CO<sub>2</sub> in He for 3 minutes prior to 8 hours equilibration at 40°C. The headspace CO<sub>2</sub> gas was then injected into a PreciLion mass spectrometer (Elementar, Langenselbold, Germany). For  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  and  $^{34}\text{S}/^{32}\text{S}$  ratio analysis, 4 grams of homogenized meat sample was suspended in 30mL of water and centrifuged at 2700 rcf for 10 minutes, and 15 mL of the resulting solution were collected. Two mL of 10% Na<sub>2</sub>WO<sub>4</sub> and 2mL of HCl 0.67M were added. The solution heated at 80°C until apparition of a precipitate. The precipitate was cleaned with water and methanol and dried under nitrogen. Approximately 5mg of sample were weighed before Dumas combustion in a Vario PYRO cube elemental analyzer (Elementar, Langenselbold, Germany). The obtained gases (N<sub>2</sub>, CO<sub>2</sub> and SO<sub>2</sub>) were injected into the PreciLion mass spectrometer for isotope ratio measurements.

First, the analytical uncertainty was determined on all measured isotope ratios to ensure that the sample preparation method was robust and reproducible. The measured combined standard uncertainty was +/- 0.20‰ for  $\delta^{18}\text{O}$ , +/- 0.15‰ for  $\delta^{15}\text{N}$ , +/- 0.56‰ for  $\delta^{13}\text{C}$  and +/- 0.56‰  $\delta^{34}\text{S}$ . The uncertainties are much smaller than the differences between poultries from different origins, as for example Switzerland, France, Slovenia, Hungary and Brazil. Second, isotopic ratio values for C, N, S and O from over 40 poultry samples showed that  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  were most useful to separate the samples according to their geographical origin. Poultry from Switzerland could clearly be discriminated from Brazil, France, Hungary, Slovenia and Ukraine.

These results show that IRMS combined with a rapid sample preparation method can be used to verify the authenticity of local meat and poultry. Official controls regarding "local food" claims increase consumer's confidence in such labels, prevent frauds, and protect the local farming economy.

**Keywords:** isotope ratio mass spectrometry, poultry, protein precipitation, meat, authenticity

### C34

#### CONFRONTING FOOD FRAUD IN A "GREEN" WAY: DEVELOPMENT AND OPTIMIZATION OF A CHROMATOGRAPHY-FREE METHODOLOGY UTILIZING DART-HRMS FOR THE DETECTION OF EXTRA VIRGIN OLIVE OIL ADULTERATION

**Dimitrios Papelis<sup>(1)</sup>, Sofia Drakopoulou<sup>(1)</sup>, Eleni Nastou<sup>(1)</sup>, Evangelos Gikas<sup>(1)</sup>, Ilona Nordhorn<sup>(2)</sup>, Carsten Baessmann<sup>(2)</sup>, Nikolaos Thomaidis<sup>\*(1)</sup>**

<sup>1)</sup> *Laboratory of Analytical Chemistry, Department of Chemistry, National and Kapodistrian University of Athens, Greece*

<sup>2)</sup> *Bruker Daltonics GmbH & Co KG, Germany*

*\*Corresponding author - E-mail: ntho@chem.uoa.gr*

The need for rapid, efficient and environmental-friendly analytical methods in food analysis is increasingly critical, particularly in the context of authenticity assessment of products like extra virgin olive oil (EVOO). EVOO is considered an integral part of the Mediterranean diet, with numerous health benefits. Therefore, it is particularly susceptible to economically motivated adulteration, being usually substituted with cheaper vegetable oils or olive oils of lower quality. Traditional analytical techniques, such as chromatography coupled with mass spectrometry though widely used to detect these kinds of adulteration, often face limitations due to their time-consuming nature, labour-intensive procedures, and the environmental burden posed by toxic waste generated during the process.

The present study introduces a novel, chromatography-free approach, utilizing Direct Analysis in Real Time (DART) coupled to Quadrupole-Time-of-Flight Mass Spectrometry (QTOF-MS) for EVOO authenticity assessment. Focal point of the study was the development of a comprehensive methodology that incorporates the reliability of High-Resolution Mass Spectrometry (HRMS) workflows and the fast screening of DART. Hence, special attention was paid on the method's optimization to enhance both efficiency and accuracy in EVOO analysis. Initially, a Plackett-Burman design was employed to identify the most significant factors affecting the performance of the DART system, such as gas temperature, grid voltage, sample distance and helium flow. Then, a Central Composite Design was applied to the key factors in order to determine the optimum parameters for the detection and discrimination of oils, in terms of their chemical profile. Different sample spotting techniques were tested, while MS and MS<sup>2</sup> parameters were also adjusted to enhance the sensitivity of the method. Taking a step forward, to ensure that the method was not only effective, but also applicable in a routine-basis, desirability functions were utilized during the optimization process. This approach allowed the simultaneous optimization of multiple response variables, balancing the trade-offs between sensitivity, specificity and analysis time.

The optimized method was implemented in EVOO samples of different cultivar and geographical origin, alongside potential adulterants, including vegetable oils (corn, sunflower, canola, soybean, sesame, linseed) and lower-quality olive oils (pomace, refined, olive oils). Advanced chemometrics was then applied, successfully differentiating authentic and adulterated samples, thus demonstrating the robustness and reliability of the method. Finally, authenticity markers of each oil category were highlighted and successfully identified. In conclusion, the key findings of the study highlighted the potential of DART-HRMS as an emerging and highly promising technique in the field, nicely aligned with the current trends in analysis for reliable, versatile and eco-friendly solutions.

**Keywords:** DART, extra virgin olive oil, HRMS, authenticity, Plackett-Burman design

### C35

#### DETECTION OF FOOD COMMODITIES ADULTERATION USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

**Paul Rogeboz**<sup>(1)</sup>, **Helia Latado**<sup>(1)</sup>, **Julie Zwahlen**<sup>(1)</sup>, **Mathieu Dubois**<sup>(1)</sup>, **Loic Dayon**<sup>(1)</sup>, **Amaury Patin**<sup>(1)</sup>, **Maricel Marin-Kuan**<sup>(1)</sup>

<sup>1)</sup> Nestlé Research, Switzerland

\*Corresponding author - E-mail: Paul.Rogeboz@rd.nestle.com

Food commodities may be exposed to fraudulent activities due to geo-political situations or climate change causing shortage and increase in prices of raw materials [1]. Application of reliable, fast and cost-effective methods to ensure the quality of food commodities is needed [1-3]. Application of HPTLC was identified as a promising tool to answer the current needs for oils and plant-based proteins fraud based on specific chemical markers identification. HPTLC is a sophisticated form of thin layer chromatography with superior and advanced separation efficiency and detection limits and is often an alternative to high-performance liquid chromatography (HPLC) and gas chromatography (GC) improving cost and convenience.

In this study, the feasibility to use HPTLC to assess authenticity of edible oils (1) and plant-based proteins was investigated including natural variability of these raw materials and statistic relevance. Three methods were developed (1) a non-targeted method for the detection of edible oils adulterated with other edible oils generally of lower quality. The adulteration detection consisted of the generation of fingerprint profiling to detect the addition of other edible oils. Second, a targeted method to detect the presence of mineral oil saturated hydrocarbon (MOSH) in food commodities such as edible oil. Finally, a method to detect soy protein concentrate adulteration was developed. The detected adulterants were other types of protein sources such as pea protein, peanut protein, whey protein, and Nitrogen-rich chemicals as melamine combined with cellulose as bulk agent and maltodextrin.

The developed HPTLC methods will be presented as well as their qualification including detection levels. This methodology presents several advantages for the characterization of food commodities, adulteration detection for decision-making, and quality monitoring. Application of the developed HPTLC underlines the capability and versatility of HPTLC to detect fraudulent activities.

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[3] James A Donarski, K.H., and Rosario Romero, Analytical toolbox for food fraud. *Food science & technology: information quarterly of the Institute of Food Science and Technology*, 2020. 34(4): p. 26-30.

**Keywords:** food fraud, adulteration, rapid method, HPTLC



### C36

#### APPLICATION OF CARBON AND NITROGEN ISOTOPE RATIOS IN THE AUTHENTICATION OF LOCALLY GROWN VERSUS IMPORTED POTATOES IN THE CANARY ISLANDS

Raul S. Suarez-Torres<sup>(1)</sup>, Gabriel Santanta-Diepa<sup>(1)</sup>, Samara M. Dionis<sup>\*(1)</sup>, Ricardo Diaz-Diaz<sup>(1)</sup>

<sup>1)</sup> Instituto Tecnológico De Canarias, Spain

\*Corresponding author - E-mail: sdionis@itccanarias.org

The Potato (*Solanum tuberosum* L.) is a staple food in the diet of the Canary Islanders. In this outermost region of the European Union, potato cultivation has an important cultural and economic value. Currently, local production cannot meet the high demand for potatoes, leading to imports from the other countries, with the United Kingdom being the main supplier. This dependence on international markets is necessary to satisfy local demand. However, competition with local production is intensified by inadequate labelling practices in local markets, which can contribute to food fraud and mislabelling issues. In this work, carbon and nitrogen isotope ratios were studied by Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS) to determine their usefulness to verify the authenticity and geographical origin of these crops. A total of 140 potato samples identified as locally grown (Tenerife (n=85) and Gran Canaria (n=33)) and imported (UK (n=22)) were collected from marketplaces in Canary Islands between 2021 and 2023. The samples were processed and freeze-dried for isotopic analysis. The carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotope ratios were determined using the standard delta notation formula (Coplen, 2011), where values are expressed to a reference standard in part per thousand (‰). Statistical analysis was applied to the samples using open-source software. Samples from the Canary Islands were grouped (Tenerife and Gran Canaria) and compared with those from the UK. The one-way ANOVA test shows the significant statistical difference between samples labelled 'Canary Islands' and those labelled 'UK'. Firstly, carbon results showed significant differences, with the mean  $\delta^{13}\text{C}$  data being higher in the Canary Islands samples (-27.15‰) than in the UK samples (-27.95‰). However, the results for nitrogen ( $\delta^{15}\text{N}$ ) do not show significant differences between the Canarian and UK samples due to the high variability of  $\delta^{15}\text{N}$  values for both regions. Although the Canarian samples had a slightly lower mean (4.18‰) compared to the UK samples (5.05‰). This study would permit the isotopic characterisation of potatoes grown in the Canary Islands in order to prevent fraud in marketing and ensure the authenticity of local products in the Canary Islands.

**Keywords:** potato, stable isotope, Canary Islands, EA-IRMS, food authenticity

**Acknowledgement:** This work was supported by European Union / Next Generation EU - INVESTIGO 2022 and 2023

## C37

### ASSESSMENT AND AUTHENTICATION OF EDIBLE INSECTS AVAILABLE AT THE MARKET

**Ondrej Pospisil**\*<sup>(1)</sup>, **Katerina Sebelova**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\*Corresponding author - E-mail: [pospisaf@vscht.cz](mailto:pospisaf@vscht.cz)

The European market with edible insects and insect-based products is continuously growing, until now, four species have been authorised as a novel food: *Tenebrio molitor*, *Acheta domesticus*, *Locusta migratoria* and *Alphitobius diaperinus* (Regulation (EU) 2015/2283). Alike in case of other high nutritional value commodities, fraudulent practices cannot be avoided, therefore effective tools are needed for label control and authentication.

The research mainly focused on untargeted metabolomic analysis and targeted screening of polar metabolites in available insect samples. In total, extracts (aqueous ethanol 1:1, v/v) prepared from 36 authentic insect samples (9 species, including authorized species in the EU, from nine different Czech breeders) and 67 samples of different commercial edible insect-based products were subjected to analysis performed by ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry (UHPLC-HRMS/MS). Multivariate analysis (PCA, PLS-DA) revealed differences in metabolome of tested insect species. The insect-based products clustered with authentic insect samples in PCA and PLS-DA score plots. In total, 31 potential markers of 9 insect species were identified. For example, leucopterin, a compound present in the insect wings, as a marker of adult insects (e.g. *Locusta migratoria* and *Schistocerca gregaria*). The identified markers were incorporated into the in-house metabolomic marker database, which at present contains 51 potential markers, which their applicability was successfully verified on two sets of commercial samples.

The results of this study have identified the challenges for follow-up entometabolomics research, especially for the authentication of edible insects and products thereof.

**Keywords:** edible insects, novel food, entometabolomics, high resolution mass spectrometry, authentication

**Acknowledgement:** *This work was supported by National Agency for Agriculture Research (Project no. NAZV-QK23020101), METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities and specific university research A1\_FPBT\_2024\_006.*

**C38**

### **A SUITE OF FOUR LC-MS/MS ASSAYS FOR ANALYSIS OF PHARMACEUTICAL ADULTERANTS IN DIETARY SUPPLEMENT PRODUCTS**

**John Schmitz**<sup>(1)</sup>, **Lukas Vaclavik**\*<sup>(2)</sup>, **Michael Buhrman**<sup>(1)</sup>, **Grace Bandong**<sup>(1)</sup>, **Jeff Stassi**<sup>(1)</sup>

<sup>1)</sup> Eurofins, United States of America

<sup>2)</sup> Eurofins Food Chemistry Testing, Czech Republic

\*Corresponding author - E-mail: [lukas.vaclavik@ft.eurofinsus.com](mailto:lukas.vaclavik@ft.eurofinsus.com)

There has been increased scrutiny of dietary supplements in recent years. Particularly when dealing with high-value and widely used products, specifically in the genre of weight loss, male sexual enhancement, sports nutrition, and joint wellness and pain management supplements. In each of these categories there are numerous pharmaceutical adulterants of interest that may be added illicitly to develop or enhance the advertised health effect. Weight loss supplements may contain anorectic drugs, such as fluoxetine, phenolphthalein, sibutramine, and others. Male enhancement products are frequently adulterated with phosphodiesterase-5 inhibitors including sildenafil, tadalafil, vardenafil along with their structural analogues. Sports nutrition materials may contain various anabolic steroids, beta agonists, SARMs, or growth hormones. Adulterants potentially present in joint health products include NSAIDs, analgesic agents, or muscle relaxants. In each of these instances it is beneficial to testing labs to have aligned method for extraction, dilution, and analysis. The goal of this project was to harmonize all methodologies for adulterant classes mentioned above while providing reasonably low reporting limits in the range of 1 to 50 ppm in each product. Each analyte class provided its own challenges, including poor ionization, increased matrix effects, need for use of negative ionization mode, isomer coelutions, or poor/excessive retention. We will present data outlining the use of common laboratory reagents and consumables to produce one method for each analyte category and how they are being used together to analyze a variety of relevant dietary supplements representing various dosage forms.

**Keywords:** adulterant, PDE5, weight loss, pain management, supplement

### C39

#### UNTARGETED ANALYSIS WITH LC-HRMS AS A TOOL FOR AUTHENTICATION OF PEPPER

Vojtech Hrbek<sup>(1)</sup>, Karel Wunsche<sup>(1)</sup>, Kamila Stehnova<sup>(1)</sup>, Kamila Bechynska<sup>(1)</sup>, Vit Kosek<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: vojtech.hrbek@vscht.cz*

The authenticity of the product sold is an essential characteristic of its quality. If the product is not authentic, adulteration occurs, the customer is harmed, at least economically, and in the worst case, the adulteration of the product can also have adverse effects on human health. One of the most frequently adulterated commodities are spices, mainly because of their high price. Therefore, a cheaper substitute is often used to adulterate spices, and the product is either diluted or completely replaced with something else.

This study aimed to control the authenticity of whole and ground pepper, which is widely used in the culinary world. There are many types of pepper. Whole black pepper is adulterated with papaya seeds, pepper skins and stalks, buckwheat and millet. Ground black pepper can be adulterated with, cassava starch and rice flour, papaya seed powder and buckwheat flour. In this study, black pepper (*Piper nigrum*) and long pepper (*Piper longum*) were analysed. In addition, adulterants such as buckwheat (*Fagopyrum esculentum*) and papaya seeds (*Carica papaya*) were also included in the experiment.

A sample preparation procedure based on acetonitrile extraction was developed. The sample extracts were analysed by reversed-phase ultra-high performance liquid chromatography in combination with high-resolution mass spectrometry in both ionisation modes. Using data measured in negative ionisation mode appears to be more diagnostic. The measured data were processed using advanced statistical methods. The chosen strategy seems to be very promising and suitable to control the authenticity of both black and long pepper. The statistical processing of the measurement data showed the differentiation of the samples into groups of authentic peppers and individual adulterants. At the same time, statistical processing enabled the selection of characteristic signals for black pepper, long pepper and adulterants. Finally, the analytical strategy could be transferred to a targeted screening of controlled samples.

**Keywords:** pepper, authenticity, liquid chromatography, mass spectrometry

**Acknowledgement:** *The work used [data/tools/services/facilities] provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064).*

### C40

#### METABOLOMIC AND STABLE ISOTOPE SIGNATURES FOR WINE AUTHENTICATION AND CLASSIFICATION

**Marius Gheorghe Miricioiu**\*<sup>(1)</sup>, Oana Botoran<sup>(1)</sup>, Diana Costinel<sup>(1)</sup>, Roxana Elena Ionete<sup>(1)</sup>

<sup>1)</sup> National Research and Development Institute for Cryogenic And Isotopic Technologies, Romania

\*Corresponding author - E-mail: marius.miricioiu@icsi.ro

A complex analysis was conducted to explore the metabolomic content and stable isotope ratios in both red and white wines from national and international grape varieties. A primary challenge was the close geographical proximity of the four agroclimatic regions from which the wines originated. Wines were classified by vintage, variety, and region, offering insights into how these factors shape wine composition. Malic and citric acids were identified as distinctive metabolites in white wines, while being absent in red wines. Principal Component Analysis (PCA) effectively separated wines by vintage and region, particularly through stable isotopes such as  $\delta^{18}\text{O}$ ,  $\delta^{13}\text{C}$ , and deuterium distribution, though it was less effective in distinguishing varieties. Discriminant Analysis (DA) provided better varietal separation, with key metabolites like 2,3-butanediol, galactose caftaric acid, galacturonic acid, formic acid, isopentanol and shikimic acid forming distinct groups. The study revealed a stronger relationship between metabolites and vintage compared to stable isotopes, highlighting the influence of yearly climatic conditions on wine metabolomics. Despite the similar climatic condition across the region, the geographic discrimination it was more evident due organic acids, carbohydrates and phenols than in the case of stable isotopes, but the combination of these two techniques could strengthens the overall classification.

**Keywords:** metabolites, isotopes, wine, region, vintage

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## C41

### INVESTIGATION OF GEOGRAPHIC ORIGIN OF SUSPICIOUS "CYPRIOT" POTATOES FROM THE MARKET BY PERFORMING ISOTOPIC ANALYSES AND CHEMOMETRICS

**Maria Tarapoulouzi**<sup>(1)</sup>, **Charalampos Louka**<sup>(2)</sup>, **Elena Ioannou Papayianni**<sup>(2)</sup>, **Eleni Tzioni**<sup>(2)</sup>, **Chara Savvidou**<sup>(2)</sup>, **Constantinos Damaskinos**<sup>(2)</sup>, **Rebecca Kokkinofta**\*<sup>(2)</sup>

<sup>1)</sup> *General Laboratory of Cyprus, Cyprus*

<sup>2)</sup> *State General Laboratory of Cyprus, Cyprus*

\**Corresponding author - E-mail: sglsnif@cytanet.com.cy*

In Cyprus, potato exports account for 85 to 90% of the total production. "Cyprus red soil potato" is a widely known variety. Given the economic importance of Cyprus' potato production, this study highlights the importance of establishing a database of authentic samples to verify any potatoes labelled as Cypriot. Such a database is critical for potato authentication due to the limited research regarding the geographical origins of potatoes, despite the crop's economic significance. Therefore, this study aims to address this gap by providing a reliable method for determining the true provenance of Cypriot potatoes.

The present study involved 143 raw potato tubers (mainly Spunta variety) from Cyprus to create the authentic database. Based on the database, 18 suspicious potatoes from the Greek market were investigated which were labelled as imported products from Cyprus. Isotopic ratios, including  $^{18}\text{O}/^{16}\text{O}$  in potato water,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$  in freeze dried samples, as well as D/H ratios and  $^{13}\text{C}/^{12}\text{C}$  in ethanol derived after fermentation and distillation. These parameters were examined using advanced techniques such as Isotope Ratio Mass Spectrometry (IRMS) regarding  $^{18}\text{O}/^{16}\text{O}$ ,  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ , as well as Site-Specific Natural Isotopic Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) spectroscopy for D/H ratios. Chemometric analysis of the isotopic measurements took place by applying the chemometric method Partial Least Squares-Discriminant Analysis (PLS-DA).

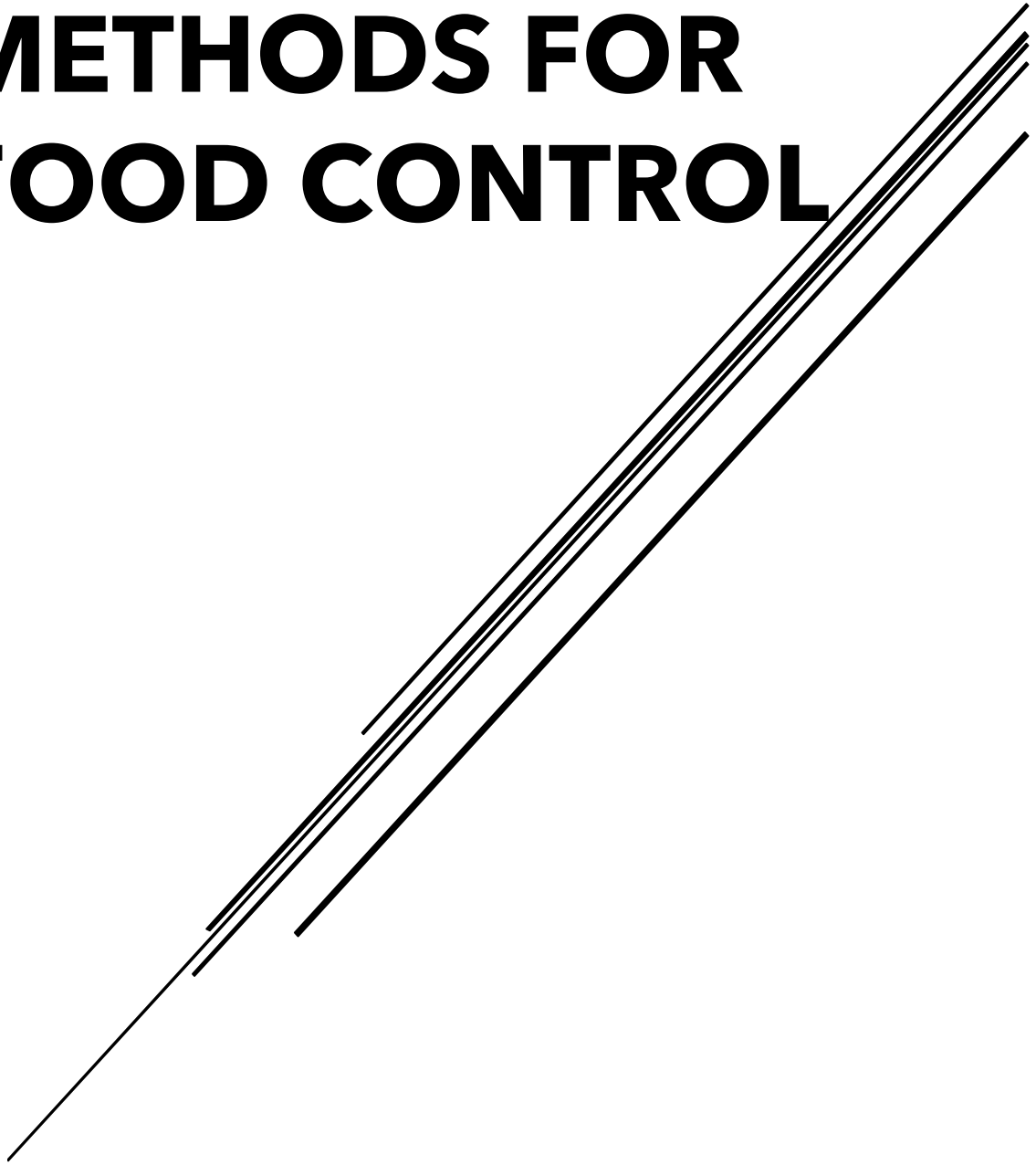
The extracted chemometric model had 95% overall correct classification, and it was found that 7 out of 18 samples were geographically classified as non-Cypriot. The most useful variables of this model found to be  $\delta^{18}\text{O}$  and (D/H)<sub>II</sub>. The study presents a robust method for determining the authenticity of potatoes, particularly those labelled as Cypriot, through the analysis of stable isotopes and chemometric modelling.

In conclusion, the research findings emphasize the significance of this unique isotopic signature, which reflects the influence of Cyprus' specific geological and climatic conditions on the potatoes. It is worth mentioning that this is the first database constructed for local potatoes, which does not only benefit Cypriot agriculture, consumers and producers, but also provides a model that can also be applied to future studies of potato authenticity, highlighting its broader applicability in international markets.

**Keywords:** potato, authenticity, SNIF-NMR, IRMS, isotopes

**Acknowledgement:** *Cyprus Recovery and Resilience Plan 2021-2026.*

# **BIOANALYTICAL METHODS FOR FOOD CONTROL**



## D1

### MILLIFLUIDIC CELL-BASED BIOASSAY AS NEW PROMISING TOOL TO INVESTIGATE FOOD COMPOUNDS BIOACCESSIBILITY

**Rafaella Colombo<sup>(1)</sup>, Mayra Paolillo<sup>(1)</sup>, Adele Papetti<sup>\*(2)</sup>**

<sup>1)</sup> Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy, Italy

<sup>2)</sup> Department of Drug Sciences, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy C.S.G.I., Center for Colloid and Surface Science, Viale Taramelli 12, 27100 Pavia, Italy, Italy

\*Corresponding author - E-mail: adele.papetti@unipv.it

Cell-based bioassays in fluidic devices are promising *in vitro* tools to reproduce *in vivo* flow conditions and interactions of molecules across tissue and cellular barriers. They can be useful in food and pharmaceutical analysis as dynamic platforms for compound high-throughput screening and testing. Different food quality and safety molecule markers (nutrients, bioactives, additives, and contaminants) can be studied to evaluate bioaccessibility, metabolism, and healthy and/or toxic effects [1].

We set-up a new dynamic multi-organ model reproducing the gastrointestinal process by using a commercial millifluidic device (LiveFlow<sup>®</sup>, IvTech). The system was tested with methylglyoxal (MGO), a potential highly reactive and toxic compound, generated both endogenously and in food, that is responsible for the formation of advanced glycation end products (AGEs), involved in age-related diseases (diabetes, cardiovascular and neurological diseases) [2]. Then, we used this platform to investigate the effect of chlorogenic acid (CGA), a food-derived phenolic compound, well-known antiglycative agent able to trap MGO, as confirmed by our results obtained in biochemical assays [3]. The digestion process was reproduced by connecting gastric (GIST-882) and intestinal (Caco-2) cells. MGO and CGA were monitored alone and in mixture at different digestion steps by validated RP-HPLC-DAD methods. In addition, cell viability tests were performed [2,3]. The bioaccessibility results of the two compounds were compared with those obtained by using the InfoGest static digestion method [4] combined with a protocol reproducing the colon phase, completing the digestion process [5]. Differently from the InfoGest reference model, the new system highlighted a role of gastric cells in the digestion and a loss of CGA capacity to trap MGO, confirming how the static and dynamic approaches should be both performed to be predictive of the *in vivo* behaviour/bioactivity of a compound.

The intestinal chamber was also used for testing different polyphenols (caffeic, quinic, and rosmarinic acids, quercetin, rutin) bioaccessibility to validate our new system in comparison to literature data [6].

Millifluidic cell-based bioassays represent an interesting evolution of traditional *in vitro* tests and a future promising alternative to reduce animal testing.

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**Keywords:** food compounds, bioaccessibility studies, dynamic systems, millifluidic cell-based bioassay, digestion model



## D2

### ASSESSING THE AUTHENTICITY AND PURITY OF COMMERCIAL RENNET PRODUCTS THROUGH (META)GENOMICS APPROACHES

**Yari Van Laere<sup>(1)</sup>, Marie-Alice Fraiture<sup>(1)</sup>, Andrea Gobbo<sup>(1)</sup>, Tom Van Nieuwenhuysen<sup>(1)</sup>, Julien Masquelier<sup>(1)</sup>, Sigrid De Keersmaecker<sup>(1)</sup>, Kathleen Marchal<sup>(2)</sup>, Nancy Roosens<sup>(1)</sup>, Kevin Vanneste<sup>\*(1)</sup>**

<sup>1)</sup> *Sciensano, Belgium*

<sup>2)</sup> *UGent, Belgium*

*\*Corresponding author - E-mail: Kevin.Vanneste@sciensano.be*

**Introduction:** Rennet is an ingredient used in cheese production, containing milk-clotting enzyme required for coagulating milk. Rennet products are either from animal, plant or microbial origin. In this study, we analysed rennet products for their authenticity (i.e., evidence of the labelled origin) and purity (i.e., the absence of any undesired biological impurities) using novel and innovative genomics methods. Correct labelling is important to ensure consumers' choices are respected (e.g. vegan or non-vegan rennet). Regarding purity, products are not allowed to contain viable microbial producer organisms or pathogens.

**Materials and Methods:** Twenty-nine commercial rennet products were collected: eleven of animal origin, three of plant origin, and fifteen of microbial origin. These products were analysed using qPCR screening, whole genome sequencing (WGS) of viable microbial isolates and direct metagenomic sequencing (i.e., sequencing all genetic material contained within a sample). qPCR assays were used to screen for the presence of plant-specific action, the *Bacillus subtilis* group and *Rhizomucor spp.* (the latter two are known rennet production organisms). For WGS, isolates were grown under different conditions and underwent Illumina sequencing. Metagenomic sequencing was done using both Illumina and nanopore sequencing. To assess product authenticity, metagenomic data were classified and identified species were compared to the labels. qPCR assays were performed to look for plant and microbial production organisms. To evaluate purity, metagenomic data were used to search for DNA of pathogens, antimicrobial resistance and virulence factor genes. To look for both viable microbial producer organisms and pathogens, the WGS data of the viable isolates were analysed. Cheese was made with rennet products in which viable *B. cereus s. l.* was found.

**Results and discussion:** For the authenticity evaluation, six of the studied rennet products were found to be incorrectly labelled. One product, labelled as being of plant origin, contained more animal than plant genetic material. The five other incorrectly labelled products were labelled as coming from one specific animal but contained genetic material from multiple animals. For the samples labelled as being of microbial or plant origin, DNA from *Rhizomucor spp.* was found in seven samples. Authenticity could not be assessed for nine of the twenty-nine samples because of a lack of genetic material. Regarding purity, metagenomic analyses revealed that two products contained pathogen DNA. Since rennet is often used in conditions ideal for bacterial growth and toxin production, it is important to ensure no viable pathogens are present. Eight products contained viable *B. cereus s. l.* at less than 10 CFU/ml. Viable *B. cereus s. l.* was also found in two of the cheeses. Our findings highlight the effectiveness of genomics methods in identifying both incorrect labelling and impurities.

**Keywords:** authenticity, purity, metagenomics, whole genome sequencing

**Acknowledgement:** *We thank the technicians of the service Transversal activities in Applied Genomics at Sciensano, Belgium for performing the Illumina sequencing.*

## D3

### IDENTIFICATION OF PAPS DERIVING FROM AUTHORIZED INSECTS IN FEED BY HIGH-RESOLUTION LC-MS/MS

Elisabeth Reiter<sup>(1)</sup>, Irmengard Strnad<sup>(1)</sup>, Sara Schlachter\*<sup>(1)</sup>, Stefano D'Amico<sup>(1)</sup>, Evelyn Rampler<sup>(2)</sup>

<sup>1)</sup> AGES - Austrian Agency for Health and Food Safety, Austria

<sup>2)</sup> University of Vienna, Austria

\*Corresponding author - E-mail: sara-sofie.schlachter@ages.at

To ensure the safety of food, also feed hygiene measures (measures and conditions necessary to control hazards and to ensure fitness for animal consumption of a feed) according to regulation EC No. 183/2005 need to be in place. The European Parliament established a feed ban concerning prohibition of mammalian proteins in feeding stuff based on commission decision (EC) No. 94/381 (later repealed by regulation (EC) No. 999/2001) caused by TSE (transmissible spongiform encephalopathy) as a health risk. Since 2007 the prohibition of processed animal proteins (PAPs) has been relaxed gradually and EU regulations (EC) No 893/2017 and 1925/2021 authorized eight insects: black soldier fly (*Hermetia illucens*), house fly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domestica*), banded cricket (*Grylloides sigillatus*), field cricket (*Gryllus assimilis*) and silk moth (*Bombyx mori*) as ingredients in aquaculture and pig and poultry feedingstuff. The official control method as laid down in regulation (EC) 152/2009 annex VI, is a combination of light microscopy (LM) and polymerase chain reaction (PCR). However, LM cannot distinguish between particles derived from authorized and non-authorized insects and an implemented PCR method for the control of insects in feedingstuff is not available so far. This analytical gap can be closed by a targeted LC-MS/MS approach. A LC-MS/MS based qualitative targeted method on peptide level to distinguish between authorized and unauthorized insects was developed. First specific marker peptides for the eight authorized insects were identified based on untargeted discovery proteomics by data dependent (DDA) and data independent analysis (DIA) approaches using a high-resolution UPLC-QToF-MS system. Afterwards, a parallel reaction monitoring (PRM) method will be established and optimized. For this purpose, proteins were extracted by 2 M urea (pH 9.2), alkylated, digested with trypsin and obtained peptides purified by SPE with stage-tips.

For all eight authorized insects specific and selective peptides were identified. To detect also non-authorized insects including contaminations, also peptides of fruit fly (*Drosophila melanogaster*) which occur also in many other insects were identified. The specificity of all peptides was proofed *in silico* by BLAST-analysis using the SwissProt database and experimentally using different sample materials which were commercially available. The results revealed very good results for selectivity of all tested peptides.

**Keywords:** proteomics, high-resolution mass spectrometry, processed animal proteins, parallel reaction monitoring

## D4

### LABEL-FREE RELATIVE QUANTIFICATION OF MAJOR PROTEINS AND TRYPSIN INHIBITOR TYPES IN SOY BY HIGH-RESOLUTION LC-MS/MS

Elisabeth Reiter<sup>(1)</sup>, Stefano D'Amico<sup>(1)</sup>, Sara Schlachter\*<sup>(1)</sup>, Irmengard Strnad<sup>(1)</sup>, Evelyn Rampler<sup>(2)</sup>, Matthias Trimmel<sup>(3)</sup>

<sup>1)</sup> AGES - Austrian Agency for Health and Food Safety, Austria

<sup>2)</sup> University of Vienna, Austria

<sup>3)</sup> Josephinum Research, Austria

\*Corresponding author - E-mail: sara-sofie.schlachter@ages.at

Soybeans are a very important source for animal feed due to their high protein content (up to 40 %). Due to the presence of anti-nutritional factors (ANF) such as trypsin inhibitors a thermal treatment (roasting) of soybeans is required. To ensure consistently high quality and digestibility, processing must be carried out in a controlled manner to maximise the reduction of ANF and preserve valuable components (e.g. amino acids). Soybean treatment is currently controlled in the final product, mostly by analysing the trypsin inhibitor activity (TIA), which is time-consuming and costly. This often leads to overtreatment, undesirable protein damage and loss of valuable amino acids such as lysine and methionine. The lack of data of the precise composition of the different trypsin inhibitor types and their influence on the inhibition activity also contributes to inefficient soybean treatment.

The aim is a prospective control during soybean processing. The use of high-resolution proteomics approaches for characterisation of protein composition is an innovative solution. Differences in protein composition between different cultivars and growing locations, as well as the composition of the different trypsin inhibitors should enable the prediction for the most suitable process parameters thus guarantee a proper treatment resulting in high quality of soybean products for feed and food production.

For this purpose, the protein profiles of raw and treated soybeans (different periods of time and temperatures) were analysed. The milled soybeans were defatted using n-hexane and proteins extracted using 50% isopropanol, 1 M urea, 0.1 M TRIS and 0.5% DTT, pH= 8.2. Extracted proteins were digested with trypsin and obtained peptides purified by SPE with stage-tips. A label-free relative quantification of the proteins glycinin G1 - G5,  $\beta$ -conglycinin beta subunit 1 - 2,  $\beta$ -conglycinin alpha subunit 1 - 2, 7S globulin 1 - 2,  $\beta$ -amylase and the trypsin inhibitors Kunitz-type trypsin inhibitor KTI 1 - 3, Bowman-Birk type proteinase inhibitor C-II and D-II was performed based on data independent acquisition (DIA) approaches using a high-resolution UPLC-ESI-QToF-MS system. For each protein specific peptides were identified, and specificity was proofed *in silico* by BLAST-analysis using the SwissProt database. Apomyoglobin of horse heart was used as internal standard for the relative quantification.

There are clear differences in the proteome profile depending on the variety and differences in the heat stability of the individual proteins. The relative contents of the types of trypsin inhibitors match with the results of values for determination of trypsin inhibitor activity.

**Keywords:** high-resolution mass spectrometry, trypsin inhibitors, proteomics, label-free relative quantification

## D5

### DIRECT & REAL-TIME HEADSPACE ANALYSIS OF ESSENTIAL OILS BY SICRIT-WATERS ACQUITY™ QDA™ MS DETECTOR

Jan Bucek<sup>\*(1)</sup>, Ciara Conway<sup>(1)</sup>, Jan-Christoph Wolf<sup>(1)</sup>

<sup>1)</sup> Plasmion GmbH, Germany

\*Corresponding author - E-mail: [jan.bucek@plasmion.de](mailto:jan.bucek@plasmion.de)

In this study, a direct analysis method for essential oils was developed using SICRIT® ionization technology coupled with a portable single quadrupole mass spectrometer (Waters ACQUITY™ QDa™ Detector). The technique, which requires no sample preparation and offers a rapid analysis time of under 2 minutes, demonstrated high accuracy in classifying various essential oils and differentiating between similar oils from different manufacturers. The method's effectiveness in quality control and authenticity verification was validated using chemometric data analysis with Waters Live ID software. This approach presents a promising, efficient alternative to traditional methods like GC-MS, allowing for high-throughput analysis and on-site evaluation of essential oil quality, provenance, and potential adulteration.

Essential oils, complex mixtures primarily consisting of terpenes and phenylpropanoids, are widely used across industries. Due to their high cost and the risk of adulteration, accurate analysis of their composition and origin is crucial. Traditional methods, while effective, are time-consuming and require extensive sample preparation. The newly developed SICRIT®-MS method provides a rapid, non-destructive alternative that simplifies the analysis process. Experimental results using 13 different essential oils demonstrated the system's capability to distinguish between oils from different brands and geographic origins with high precision. The PCA-based chemometric analysis yielded high classification accuracy, confirming the method's potential for quality control and fraud detection in the essential oil industry.

Furthermore, we can additionally apply this exact methodology to many other terpene-dense samples that can easily be differentiated between each other, such as tea leaves, cooking oils, and even cannabis samples, without any sample preparation.

What this study concludes, along with several similar approaches, is that the SICRIT®-MS approach is a cost-effective, reliable solution for the rapid analysis of essential oils, surpassing traditional techniques in efficiency and ease of use.

**Keywords:** essential oils, rapid MS, quality control, food analysis, SICRIT

## D6

### YEAST-BASED ASSAY FOR THE DETECTION OF THYROIDAL ACTIVITY IN FOOD AND FOOD CONTACT MATERIAL

Martin Jähne<sup>\*1)</sup>, Karina Hettwer<sup>r(2)</sup>, Kapil Nichani<sup>i(2)</sup>, Kirsten Simon<sup>(1)</sup>, Steffen Uhlig<sup>(2)</sup>

<sup>1)</sup> *New\_Diagnostics GmbH, Germany*

<sup>2)</sup> *QuoData GmbH, Germany*

*\*Corresponding author - E-mail: martin.jaehne@new-diagnostics.com*

Endocrine disrupting chemicals (EDCs) are well known for their potential to cause adverse effects at extremely low concentrations (ng to pg per liter). Moreover, it has been proven that a permanent exposure of EDCs from food contact materials (FCM) and food ingredients interfere with the natural human endocrine system and cause serious diseases. Hence, the sensitive and reliable detection of EDCs in different types of samples is of an urgent necessity for the establishment of appropriate measures to minimize the risk of exposure. The use of effect-based in vitro methods (EBM) for the determination of hormonal effects in addition to analytical methods contributes beneficial information for a comprehensive risk assessment. Yeast based assays performed in multiwell plates are a convenient way to measure endocrine effects in extracts of food and FCM samples. One alternative and promising approach is the combination of high-performance thin layer chromatography (HPTLC) and a fluorescent yeast EBM, called effect-directed analysis (EDA). With this method, the sample is first separated into fractions before applying the yeast biosensor. Possible inhibitory effects on the yeast biosensor are significantly reduced by the previous separation step. For the detection a TLC-Scanner measures the fluorescence of the different spots on the HPTLC plate.

The detection of estrogen-active substances as the most prominent group of EDCs has been the major challenge in the last decades, and various bioassays have also been developed and described for EDA applications. The focus is now also on the detection and identification of other endocrine activities mediated by androgens, glucocorticoids, thyroids, etc. Here, we describe a versatile yeast-based assay which uses a recombinant fluorescent *S. cerevisiae* biosensor that can be used in both a 96well assay and an EDA to identify substances that activate the human thyroid receptor  $\beta$ . An appropriate and stable expression strain of *S. cerevisiae* was used for the integration of different plasmids which carry genes for a coactivator, the gene for the human thyroid receptor  $\beta$ , modified regulation elements and an optimized red fluorescent protein. In the microplate assay the fluorescence can be measured directly in the plate after around 20 hours of exposure with substances which activate the thyroid receptor  $\beta$ . Alternatively, organic extracts from food, coatings and packaging material can be loaded on a HPTLC plate which is subsequently covered with the yeast biosensor suspension followed by an incubation step in a humid chamber. Fluorescence is measured with a common fluorescence multiplate reader. This assay variant is an interesting and simple approach for the detecting of thyroid receptor  $\beta$  mediated activity in organic extracts when separation with EDA, which requires specialized laboratory equipment, is not feasible. The validation of the different assay variants is currently in progress.

**Keywords:** effect-based bioassay, thyroidal activity

# **CIRCULARITY & SUSTAINABILITY**



## E1

### UTILIZING WASTE FROM THE AGRI-FOOD CHAIN FOR THE PRODUCTION OF ACTIVE PACKAGING

**Francesco Romaniello**\*<sup>(1)</sup>, **Consolato Schiavone**<sup>(1)</sup>, **Andrea Mario Rossi**<sup>(1)</sup>, **Andrea Mario Giovannozzi**<sup>(1)</sup>, **Gabriele Alo**<sup>(2)</sup>, **Pierangela Rovellini**<sup>(3)</sup>, **Paola Fusari**<sup>(3)</sup>, **Antonio Perna**<sup>(3)</sup>, **Chiara Portesi**<sup>(1)</sup>

<sup>1)</sup> National Metrology Institute of Italy - INRiM, Italy

<sup>2)</sup> University of Turin, Italy

<sup>3)</sup> Innovhub SSI, Italy

\*Corresponding author - E-mail: f.romaniello@inrim.it

Each year, according to the FAO (Food and Agriculture Organization of the United Nations), 1.3 billion tons of food are discarded during various stages of the agri-food chain, from the cultivation of agricultural products to leftovers from cooked food. Traditional strategies for valorizing food waste include composting, formulating animal feed, producing energy or biofuels such as biogas, bioalcohols and manufacturing biomaterials. An alternative approach aims to harness biologically active components still present in agri-food waste, such as phenolic acids, tannins, and flavonoids, for high-value applications [1].

Italy is the second-largest producer of olive oil in the world after Spain, with an annual production of 315,000 tons of extra virgin olive oil. However, olive oil production generates by-products rich in antioxidant molecules: leaves, pomace, and olive stones. This research aimed to develop a prototype of active packaging with antioxidant properties, utilizing waste from the olive oil production chain [2].

For this purpose, antioxidant molecules were extracted using a green method based on solid-liquid extraction in ethanol. The extracts were characterized using HPLC-DAD at a wavelength of 280 nm, typical for polyphenols, and semi-quantified as tyrosol. Among the by-products, olive stones were found to be the richest in antioxidants with a value of 120 g/kg, followed by leaves (40 g/kg) and pomace (10 g/kg). Afterwards, the polyphenols of olive stones were characterized by HPLC-HRMS/MS. The antioxidant capacity was evaluated using the DPPH assay, with comparable results: olive stones had the highest antioxidant activity with an EC<sub>50</sub> (half maximal effective concentration) of 97.1 mg/L, while pomace 778.7 mg/L, compared to a reference value of 1.6 mg/L for gallic acid. The olive stones extract was then used to produce a prototype of active packaging. A commercial cellulose acetate substrate was functionalized with the previously prepared olive stone extract, creating an active coating. The experimental packaging was tested on a model food: minced beef containing 50% fat, by monitoring the oxidation of fatty acids in the meat. The marker used was malondialdehyde (MDA), with the oxidation percentage calculated. The olive stone-based packaging was compared with a reference functionalized with gallic acid and with non-functionalized cellulose acetate. The oxidation percentage calculated at 10 days based on MDA was 47% for the plain cellulose acetate, 18% for the olive stone packaging, and 2% for the gallic acid.

The extracts of olive stones demonstrated excellent antioxidant capabilities, proving to be a low-cost alternative to synthetic antioxidants for application in active packaging. In this way, we can contribute to the implementation of circular economy strategies and enhance the sustainability of the agri-food sector, in line with the EU priorities and the aims of the European Metrology Network for Safe and Sustainable Food.

**Keywords:** circular economy, food packaging, olive oil, sustainable food

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## E2

### RECOVERY OF CAROTENOIDS AND EXTRACTABLE POLYPHENOLS FROM GRAPEFRUIT PEELS BY MICROWAVE-ASSISTED EXTRACTION WITH BIOBASED SOLVENTS AND SUBSEQUENT SUSTAINABLE EXTRACTION OF NON-EXTRACTABLE POLYPHENOLS

**Gloria Dominguez-Rodriguez\***<sup>(1)</sup>, Maria Concepción Garcia<sup>(1)</sup>, Maria Castro-Puyana<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>

<sup>1)</sup> University of Alcalá, Spain

\*Corresponding author - E-mail: gloria.dominguezr@uah.es

Valorization of food waste rich in bioactive compounds is one of the most relevant topics in biorefinery and circular economy [1]. Grapefruit peels have been reported to contain a higher amount of bioactive compounds, such as phenolic compounds or carotenoids, compared to the edible part [2]. These bioactive compounds can be recovered using conventional and advanced extraction techniques; however, a significant fraction of certain bioactive compounds, such as phenolic compounds, remain retained in the extraction residue [3]. To obtain non-extractable polyphenols (NEPs) more sustainable and selective methodologies have to be developed. Thus, the main aim of this work was to propose sustainable strategies enabling the extraction of phenolic compounds and carotenoids from grapefruit peels using microwave-assisted extraction (MAE) combined with biobased solvents, and the subsequent recovery of NEPs using three approaches: ultrasound-assisted extraction (UAE) combined with enzymatic-assisted extraction (EAE), and UAE and MAE combined with natural deep eutectic solvents (NaDES).

A mixture design combining MAE (50°C, S/L ratio: 1:16, 6 min) with  $\gamma$ -valerolactone ( $\gamma$ -VAL), ethyl acetate (EtOAc), and cyclopentyl methyl ether was carried out to study the influence of biobased solvent composition on the polyphenols and carotenoids extraction. Total phenolic content (Folin-Ciocalteu method), total proanthocyanidin content (vanillin and DMAC assays), total carotenoid content, the antioxidant capacity (DPPH and the inhibition of hydroxyl radical assays), and the anti-inflammatory capacity (egg albumin denaturation assay), were used as response variables. Results indicated that the optimized MAE combined with EtOAc: $\gamma$ -VAL (20:80, v/v) allowed to obtain extracts with the highest polyphenol and carotenoid contents with antioxidant and anti-inflammatory activities than combined with ethanol/water (55:45, v/v) solvent. Besides, NEPs were subsequently extracted from the residue remaining after applying the optimized MAE strategy, and results were compared with the obtained by UAE-EAE using a protease and polygalacturonase enzyme, UAE-NaDES, MAE-NaDES, and alkaline and acid hydrolysis. UAE-EAE and MAE-NaDES extraction, using choline chloride:lactic acid (1:2) with 50% water provided the extracts with the highest antioxidant and anti-inflammatory NEPs. The extracts exhibited a similar phenolic profile, determined by HPLC-DAD-QTOF-MS, where naringin highlighted as the majority compound. However, a forced stability study showed that UAE-EAE extraction provided higher protection against the degradation of antioxidant phenolic compounds than MAE-NaDES extraction, while MAE-NaDES extraction presented superior protection against the degradation of anti-inflammatory compounds.

[1] R. Reynoso-Camacho, et al. Food Chem, 350, 129239 (2021).

[2] V. Singh, et al. J Food Meas Charact, 15, 2531-2539 (2021).

[3] G. Dominguez-Rodríguez, et al. Food Chem, 339, 128086 (2021).

**Keywords:** biobased solvent, phenolic compounds, carotenoides, microwave-assisted extraction, non-extractable polyphenols

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### E3

## SUSTAINABLE RECOVERY OF PROTEINS FROM LIME PEELS USING SUBCRITICAL WATER EXTRACTION COMBINED WITH ENZYME-ASSISTED EXTRACTION

**Rosa María Palma-Manrique<sup>(1)</sup>, Maria Luisa Marina<sup>\*(2)</sup>, Maria Concepcion Garcia<sup>(2)</sup>, Maria Castro-Puyana<sup>(2)</sup>**

<sup>1)</sup> Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain

<sup>2)</sup> Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km.33.600, 28871 Alcalá de Henares (Madrid), Spain & Universidad de Alcalá. Instituto de Investigación Química Andrés M. del Río. Ctra. Madrid-Barcelona Km.33.600, 28871 Alcalá, Spain

\*Corresponding author - E-mail: mluisa.marina@uah.es

Citrus industry generates a great amount of waste such as peels or seeds which are a rich source of bioactive substances (proteins, phenolic compounds, carotenoids, etc.). These residues are mainly discarded resulting in major economic and environmental problems and in the loss of these valuable compounds. For these reasons, it is necessary to develop sustainable methodologies allowing to revalorise this waste to recover high-added value compounds and promote a circular economy. Protein extraction from lime peels has scarcely been investigated. The only methodology reported was developed by our research group using pressurized liquid extraction (PLE). Nevertheless, the protein recovery obtained by this method from peels of limes of two different geographical origins was 66-69 % [1].

The aim of this work was to develop sustainable strategies based on green techniques to increase the efficiency of the extraction of proteins from lime peels. Two techniques, non-explored before for this purpose, were investigated: enzyme-assisted extraction (EAE) and subcritical water extraction (SWE). In the case of EAE, seventeen polysaccharidase enzymes were firstly evaluated, under suitable conditions (pH 5.0, 50 °C, and 5 h), to assist in the extraction of lime peel proteins. Despite some enzymes enabled to achieve high extraction yields, extraction times were very long. In order to improve the extraction, EAE using the enzymes showing the higher extraction efficiency was combined with SWE. SWE was maximized by using a three-level factorial experimental design. Under optimal conditions, it was possible to recover 63% of proteins using just SWE, while the simultaneous combination of EAE with *Viscozyme Wheat HT* enzyme and SWE led to a 95% protein recovery in a significantly shorter time (30 min). The combination SWE-EAE, which avoids the use of organic solvents, showed a higher protein recovery compared to the use of PLE with ethanol-water as solvent [1].

[1] A. Sánchez-Elvira, E. Hernández-Corroto, M.C. García, M. Castro-Puyana, M.L. Marina, Food Chemistry 458 (2024) 140139.

**Keywords:** lime peel, protein, sustainable extraction

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### E4

#### CHARACTERIZATION OF HYDROLYSATES FROM LIME PEELS PROTEIN EXTRACTS. PEPTIDE AND POLYPHENOL IDENTIFICATION BY UHPLC-HRMS

Rosa Maria Palma-Manrique<sup>(1)</sup>, Esther Gomez-Mejia<sup>(2)</sup>, Maria Castro-Puyana<sup>(3)</sup>, Maria Concepcion Garcia<sup>(3)</sup>, Maria Luisa Marina<sup>\*(3)</sup>

<sup>1)</sup> Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain

<sup>2)</sup> Departamento de Química Analítica, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040, Madrid, Spain.

<sup>3)</sup> Universidad de Alcalá. Departamento de Química Analítica, Química Física e Ingeniería Química. Ctra. Madrid-Barcelona Km.33.600, 28871 Alcalá de Henares (Madrid), Spain & Universidad de Alcalá. Instituto de Investigación Química Andrés M. del Río. Ctra. Madrid-Barcelona Km.33.600, 28871 Alcalá, Spain

\*Corresponding author - E-mail: mluisa.marina@uah.es

Citrus fruits are rich in nutrients, phytochemicals and bioactive compounds that have beneficial effects on human health. Non-edible part of citrus fruits (50-55% of total fruit weight) such as peels are also a rich source of high-added value substances like proteins [1], polyphenols, vitamins, etc. Proteins are important molecules from a nutritional perspective, but they are also interesting due to their ability to release bioactive peptides after being consumed and hydrolysed by gastrointestinal enzymes.

In this work, two enzymes (Alcalase and Thermolysin) were used to hydrolyse proteins in lime peel extracts obtained using subcritical water extraction combined with enzyme-assisted extraction. The hydrolysates were characterized through the evaluation of diverse bioactivities (antihypertensive, antimicrobial, and antioxidant). To assess the antihypertensive activity, the potential of the extracts to inhibit angiotensin-converting enzyme was investigated. For antimicrobial activity, the minimum inhibitory concentration was studied against the bacteria *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Antioxidant capacity was determined by the evaluation of the capacity of extracts to: (i) reduce oxidizing compounds, (ii) scavenge free radicals (ABTS<sup>+</sup>), and (iii) inhibit the formation of hydroxyl radicals (OH<sup>·</sup>). Peptides present in the most active hydrolysates were identified by UHPLC-Q-TOF-MS/MS. Results demonstrated that, in addition to peptides, other bioactive compounds, including polyphenols, could be identified in hydrolysates. These compounds could be co-extracted with proteins and contribute to the observed bioactivity.

[1] S. Suri, A. Singh, P.K. Nema, S. Malakar, V.K. Arora, A. *Food Bioscience*, 48 (2022) 101789.

**Keywords:** enzymatic hydrolysis, bioactivity, peptide identification, polyphenol identification

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### E5

#### SUSTAINABLE EMERGING TECHNIQUES FOR A SEQUENTIAL EXTRACTION OF BIOACTIVE COMPOUNDS FROM CITRUS PEELS

**Gloria Dominguez-Rodriguez**\*<sup>(1)</sup>, Victor M. Amador-Luna<sup>(2)</sup>, Maria Castro-Puyana<sup>(3)</sup>, Elena Ibanez<sup>(2)</sup>, Maria Luisa Marina<sup>(3)</sup>

<sup>1)</sup> Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain & Laboratory of Foodomics, Institute of Food Science Research, CIAL, CSIC, Nicolás Cabrera 9, 28049 Madrid, Spain., Spain

<sup>2)</sup> Laboratory of Foodomics, Institute of Food Science Research, CIAL, CSIC, Nicolás Cabrera 9, 28049 Madrid, Spain., Spain

<sup>3)</sup> Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain & Universidad de Alcalá, Instituto de Investigación Química "Andrés M. del Río" (IQAR), Campus Universitario, Ctra. Madrid-B, Spain

\*Corresponding author - E-mail: gloria.dominguezr@uah.es

Citrus industry generates a large amount of waste that could be a sustainable source of bioactive compounds. However, the strategies developed for its complete exploitation are limited. Thus, a sequential sustainable extraction for the recovery of bioactive compounds from grapefruit, lime, and lemon peels using supercritical fluid extraction (SC-CO<sub>2</sub>) and ultrasound-assisted extraction (UAE) combined with natural deep eutectic solvents (NaDES) is proposed in this work. After evaluating the potential of different NADES, the results demonstrated that the use of Choline Chloride:Tartaric acid for grapefruit and lemon peels and Choline Chloride:Glycerol for lime peels with 50% water enabled to obtain, in combination with UAE, the extracts with the highest contents of phenolic compounds. On the other hand, the potential of SC-CO<sub>2</sub> as pretreatment of citrus peels before UAE-NaDES was investigated. Although UAE-NaDES without sample pretreatments enabled to obtain the extracts with higher bioactivity and higher peak areas for phenolic compounds determined by HPLC-DAD-QTOF-MS, its combination with SC-CO<sub>2</sub> gave rise to an increase in the extraction of minority phenolic compounds which in some cases could not be recovered using the direct UAE-NaDES extraction. Particularly, naringin was the majority phenolic compound identified in grapefruit peel extract while hesperidin was the majority in lime and lemon peel extracts. Moreover, the analysis of SC-CO<sub>2</sub> extracts highlighted that they showed an anticholinergic capacity much greater than the extracts obtained by direct UAE-NaDES extraction and an interesting terpenoid composition determined by GC-Q-MS. Limonene was identified as the main compound in grapefruit peel extract while sitosterol and neryl acetate were the majority compounds in lime and lemon peels, respectively. The sequential strategy developed for the recovery of bioactive terpenoids by SC-CO<sub>2</sub> followed by the extraction of phenolic compounds by UAE-NaDES was suitable for a more holistic exploitation of citrus peels in a biorefinery context.

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[2] P. Sharma et al. Valorization of citrus peel waste for the sustainable production of value-added products. *Bioresource Technology*, 351, 127064 (2022).

**Keywords:** supercritical fluid extraction, ultrasound-assisted extraction, natural deep eutectic solvents, phenolic compounds, terpenoids

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### E6

## CLOVAMIDE EXTRACTION THROUGH UNCONVENTIONAL TECHNIQUES FROM COCOA BEAN SHELLS

**Vincenzo Disca**<sup>(1)</sup>, **Fabiano Travaglia**<sup>(1)</sup>, **Chiara Carini**<sup>(1)</sup>, **Jean Daniel Coisson**<sup>(1)</sup>, **Gian Carlo Cravotto**<sup>(2)</sup>, **Marco Arlorio**<sup>(1)</sup>, **Monica Locatelli**<sup>(1)</sup>

<sup>1)</sup> Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale "A. Avogadro", Italy

<sup>2)</sup> Department of Drug Science and Technology, Università di Torino, Italy

\*Corresponding author - E-mail: vincenzo.disca@uniupo.it

Cocoa (*Theobroma cacao* L.) beans have established their prominence in the global market, prized for their delightful flavor and continuously escalating demand [1,2]. Cocoa holds by-products and waste potentially useful as a source of bioactive compounds; among them, cocoa bean shells (CBSs), deriving from roasting and pre-roasting processes, are one of the major wastes generated from the cocoa productivity chain. CBSs are particularly rich in dietary fibers and phenolic compounds of which clovamide, a derivative of caffeic acid and an amide isostere of rosmarinic acid, is known for its antioxidant and anti-inflammatory properties [3]. The extraction of bioactive compounds from food by-products is one of the most important research areas for nutraceutical, pharmaceutical and food industries.

This research aimed to evaluate the efficiency of Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE), either alone or in combination, on cocoa bean shells (CBSs). These techniques were compared with conventional methods, such as under simple magnetic stirring and Soxhlet apparatus. Following the initial characterization of the gross composition of CBSs, the total polyphenol content and radical scavenging activity of extracts from both raw and defatted cocoa bean shells were examined. Clovamide and the major polyphenolic compounds were subsequently quantified using reverse-phase high-performance liquid chromatography with diode-array detection (RP-HPLC-DAD). The use of MAE and UAE resulted in similar or superior clovamide extraction compared to traditional methods; however, the concentration of individual polyphenols varied depending on the extraction method used. Notably, combining MAE and UAE at 90°C produced the extract with the highest antiradical activity.

Concluding, these outcomes highlight the efficiency of MAE and UAE techniques to obtain clovamide-rich extracts from CBSs confirming this cocoa by-product as a valuable biomass for the recovery of antioxidant compounds, also in view of a possible industrial scale-up.

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**Keywords:** by-products, antioxidant, polyphenols, ultrasound-assisted extraction, microwave-assisted extraction

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### E7

#### INVESTIGATION OF A WHEAT-BASED PHARMACEUTICAL WASTE PRODUCT TO ANALYZE ITS PROPERTIES AND EXPLORE POTENTIAL NOVEL APPLICATIONS

**Lidia Ciriaco**<sup>\*(1)</sup>, **Antonio Paolillo**<sup>(1)</sup>, **Ivana Ledenko**<sup>(1)</sup>, **Vincenzo Armini**<sup>(1)</sup>, **Sonia Lombardi**<sup>(1)</sup>, **Barbara Maglione**<sup>(2)</sup>, **Paolo Grieco**<sup>(3)</sup>, **Luana Izzo**<sup>(1)</sup>

<sup>1)</sup> FoodLab, Department of Pharmacy, University of Naples "Federico II", Via Domenico Montesano, 49, 80131 Naples, Italy, Italy

<sup>2)</sup> Farmaceutici Damor, Via Emilio Scaglione, 27, 80145 Naples, Italy, Italy

<sup>3)</sup> Department of Pharmacy, University of Naples "Federico II", Via Domenico Montesano, 49, 80131 Naples, Italy, Italy

\*Corresponding author - E-mail: lidia.ciriaco@unina.it

*Triticum vulgare* is an annual herbaceous plant belonging to the genus *Triticum*, family Poaceae. It's commonly known as wheat and its products are the main food source in developing countries. According to FAO data, the global production of soft wheat is approximately 750-780 million tons per year. The aqueous extract of *Triticum vulgare* is obtained from the germ and it is involved in tissue repair processes: it stimulates chemotaxis and fibroblast maturation (increased fibroblast index). During the production process of this aqueous extract, a significant quantity of solid and liquid waste is produced and to date no purpose has been found for these wastes. The aim of this study is to analyze the properties of the waste obtained from wheat processing, in order to valorize these products, in line with the principles of the circular economy: Reduce, Reuse, and Recycle. In detail, the liquid and solid waste derived from the processing of wheat extract were analyzed and characterized; it was performed an in-depth physiochemical analysis using ultra-high-performance liquid chromatography coupled with high-resolution Orbitrap mass spectrometry (UHPLC Q-Orbitrap HRMS) to assess the bioactive compounds. The data obtained showed that the most abundant phenolic compounds present were ferulic acid for solid waste and dihydroferulic acid for liquid waste, respectively. In the solid extract, ferulic acid comprises up to 55.5% of the total identified polyphenol content with a concentration of 89.782 µg/g; while in the liquid extract, dihydroferulic acid represents the 85% of the total. Comparing all the total pattern of bioactive compounds, the liquid waste showed a significant decrease in the concentration of active compounds, up to 95.4%, establishing that solid waste holds a greater recovery value compared to liquid waste. The evaluation of antioxidant activity was performed by using two spectrophotometric assays, ABTS and FRAP. The Folin-Ciocalteu method was employed to determine the total phenolic content (TPC). In particular, the TPC value reported is 1.771 mg GAE/g for solid waste and 0.105 mg GAE/ml for liquid waste, confirming the solid waste as the richest in total polyphenols. This study highlights the potential of utilizing these waste products as raw materials in various production processes, considering their chemical and physical characteristics as well as the presence of valuable bioactive compounds. Further in-depth studies are required to validate their applications, especially as significant components in cosmeceutical products, owing to their abundant ferulic acid content, which is a recognized antioxidant commonly employed to shield the skin against harm caused by free radicals.

**Keywords:** wheat, waste management, circular economy, bioactive compounds, antioxidant activity

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### E8

#### **EVALUATION OF SELECTED QUALITY PARAMETERS OF MUFFINS ENRICHED WITH UPCYCLED INGREDIENTS, A REVIEW**

**Ewa Biazik<sup>(1)</sup>, Alicja Kulik<sup>(2)</sup>**

<sup>1)</sup> *Wroclaw University of Economics and Business, Poland*

<sup>2)</sup> *Student Scientific Club & Safety Management Systems in Organization&UEW, Poland*

*\*Corresponding author - E-mail: ewa.biazik@ue.wroc.pl*

A sustainable economy requires that current production reinstall agricultural by-products via the reformulation of typical food recipes. This concept, called upcycling, constitutes an important innovative practice in the food sector contributing to food waste reduction. Poland is one of the leading producers and exporters of bakery products in the EU, and the domestic market is characterized by rapid development. In the last decade, the structure of the bakery industry has changed significantly as a result of opposing trends in two main areas: a decrease in the volume of bread production and an increase in other bakery products such as donuts or muffins. Food product quality, including that of muffins, affects consumer acceptance and is determined by attributes such as taste, freshness or nutritional value, and certain cues, for example color, structure, shape or appearance.

The aim of the study is to provide a review of recent studies which describe methods of quality evaluation for upcycle bakery products, especially muffins. The study employs following databases: Web of Science, Scopus, and Google Scholar.

This study summarizes current research about quality-attribute evaluation methods for upcycling muffins that contain unmarketable ingredients, for example cauliflower leaves, apple skin powder, or upcycled sunflower flour. The literature indicates that upcycled muffins often contain low quality ingredients, including food waste, and some of the features of final products may be difficult to improve. However, certain quality attributes such as nutrition value can increase.

In conclusion, while upcycling is an increasingly popular factor in the campaign against food wastage, improving some upcycle muffin features may be challenging, and this requires further research with the use of advanced analytical techniques.

**Keywords:** upcycled food products, quality evaluation, upcycled food evaluation methods

## E9

**A BIOREFINERY PROCESS FOR THE EXTRACTION OF TERPENES AND PHENOLIC COMPOUNDS FROM EUCALYPTUS GLOBULUS LABILL. AND SALVIA OFFICINALIS L.**

**Enrico Romano**<sup>(1)</sup>, **Gloria Dominguez-Rodriguez**<sup>\*(2)</sup>, **Mattia Spano**<sup>(1)</sup>, **Luisa Mannina**<sup>(1)</sup>, **Elena Ibanez**<sup>(3)</sup>, **Alejandro Cifuentes**<sup>(3)</sup>

<sup>1)</sup> Sapienza University of Rome, Italy

<sup>2)</sup> University of Alcalá, Spain

<sup>3)</sup> Laboratory of Foodomics, Institute of Food Science Research, CIAL, CSIC, Spain

\*Corresponding author - E-mail: gloria.dominguezr@uah.es

Supercritical fluid extraction (SFE) has become one of the most used techniques for the extraction of volatile compounds such as bioactive terpenoids while pressurized liquid extraction (PLE) has been widely employed for the recovery of more polar bioactive compounds like phenolic compounds from different plant matrices [1]. Moreover, Natural Deep Eutectic Solvents (NaDES) has emerged as sustainable solvents that provide higher extraction efficiencies on the recovery of phenolic compounds compared with conventional extraction solvents such as ethanol/water [2]. However, there are no studies about the combination of PLE with NaDES for the recovery of phenolic compounds from *Eucalyptus* and *Salvia*. Thus, the aim of this work was to develop a sequential extraction process consisting of an SFE extraction for the recovery of bioactive terpenoids followed by a PLE extraction combined with NaDES from the residue of SFE to obtain bioactive phenolic compounds from *Eucalyptus* and *Salvia* in order to maximize the value of these two plants.

The SFE extraction allowed the recovery of terpenoids with high anticholinergic and antioxidant capacities. Particularly, *Salvia* extract showed greater antioxidant and anticholinergic capacities than *Eucalyptus* extract. Besides, a total of 33 and 38 terpenoids were tentatively identified by GC-MS in *Eucalyptus* and *Salvia*, respectively. Phytol was the majority terpenoid detected in *Eucalyptus*, while Manol was the majority terpenoid observed in *Salvia*, according to their peak areas.

Subsequently, the residue of SFE was submitted to a PLE extraction combined with NaDES composed of choline chloride:glycerol (1:2) with 56.7% of water. DPPH and ORAC results indicated that PLE-NaDES *Eucalyptus* extract has the greatest antioxidant capacity compared with *Salvia* PLE-NaDES extract. Regarding the anticholinergic properties, PLE-NaDES *Salvia* extract showed the highest activity, even higher than SFE extracts.

Besides, a tentative identification of phenolic compounds obtained by PLE-NaDES was carried out by UHPLC/Q-TOF-MS/MS.

The results show that the developed process for the recovery of terpenoids by SFE followed by PLE-NaDES for the extraction of polyphenols could be an interesting sustainable alternative to be used as a biorefinery process for plants such as *Eucalyptus globulus* Labill. and *Salvia officinalis* L.

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**Keywords:** supercritical fluid extraction, pressurized liquid extraction, natural deep eutectic solvents, terpenoids, phenolic compounds

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### E10

## THE HEPARIN REVOLUTION: ENZYMATIC SWINE LIVER EXTRACTION FOR NEXT-LEVEL HEMODIALYSIS

**Maria Martingo**\*<sup>(1)</sup>, Sara Baptista-Silva<sup>(1)</sup>, Manuela Pintado<sup>(1)</sup>, Ines Cruz<sup>(2)</sup>

<sup>1)</sup> Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Portugal

<sup>2)</sup> Grupo Primor / Primor Charcutaria-Prima, Portugal

\*Corresponding author - E-mail: mmartingo@ucp.pt

**Introduction:** The prevalence of chronic kidney disease (CKD) has been rising steadily over the years, posing an escalating public health challenge. Heparin is a high molecular weight sulphated glycosaminoglycan, which exerts its anticoagulant effect by inhibiting the activity of thrombin and other proteins involved in blood coagulation. This property makes heparin essential in medical procedures that require anti-coagulation, such as haemodialysis in CKD. Hereupon, heparin was enzymatically extracted from the swine liver to functionalize chitosan-based membranes for hemodialysis application. **Methodology:** The liver was crushed, and enzymatic hydrolysis was carried out using alcalase 1% (W/V) during 6h. The hydrolysed solution was subsequently processed through a filtration system equipped with a 10 kDa cut-off membrane and quantified by HPLC. A hybrid chitosan-based membrane with polyvinyl chloride (PVC) was prepared in a ratio of 1:2, respectively. A proof-of-concept phase for hemodialysis, focusing on the semi-permeability parameters was performed using urea and albumin indicators (urea 37 mg/dL and albumin 8 g/dL). A membrane with 6x1 cm was used in Gilson Minipuls 3 peristaltic pump. The membrane was functionalized with heparin (6.67 mg/mL) by dipping procedures for further dialysis studies. **Results:** This work proposes a green and sustainable method of heparin extraction from swine liver and developed heparin-functionalized membranes for greener and biological dialysis systems. The membranes permeated 70% urea and retained 100% of albumin. **Conclusion:** This method demonstrates an efficient approach for heparin extraction from liver tissue and shows its potential in a vital biomedical application.

**Keywords:** liver, sustainability, heparin, chitosan, hemodialysis

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### E11

#### ALTERNATIVE HIGH-QUALITY PROTEIN INGREDIENTS AS KEY NITROGEN SOURCE FOR PROBIOTICS

**Maria Leonor Teixeira Pinto de Castro**<sup>(1)</sup>, Sandra Borges<sup>(1)</sup>, Ana Rosa<sup>(2)</sup>, André Almeida<sup>(3)</sup>, Manuela Pintado<sup>(1)</sup>

<sup>1)</sup> Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina - Laboratório Associado, Escola Superior de Biotecnologia, Portugal

<sup>2)</sup> SEBOL - Comércio e Indústria do Sebo, S.A, Portugal

<sup>3)</sup> I.T.S. - Indústria Transformadora de Subprodutos S.A, Portugal

\*Corresponding author - E-mail: mlcastro@ucp.pt

Microbiological culture media are essential for studying microbial growth in laboratory settings. A key and costly component of these media is the nitrogen source which is crucial for supporting bacterial metabolism and growth. The valorization of food by-products and the use of new sustainable protein sources (e.g. insects) is a priority. Animal by-products are routinely dumped in landfills without adequate treatment, resulting in environmental impact. Recent research suggests that proteins, hydrolysates, and peptides can boost probiotic development. This study aimed to characterize and evaluate the potential of alternative protein hydrolysates as substitutes for the traditional protein used in microbiological culture media. Three protein hydrolysates were obtained from pork (PH) and fish (FH) by-products and black soldier fly larvae (IH). These hydrolysates were characterized regarding protein content (Kjeldahl method), peptide profile (HPLC-SEC), free amino groups (TNBS), and mineral content (ICP-OES). Bioactive properties were also evaluated, such as antioxidant activity by ABTS and ORAC. The growth analysis of probiotics *Lactobacillus casei* 01 and *Lacticaseibacillus rhamnosus* LGG was studied for three concentrations of the protein hydrolysates (0.5, 1 and 2.5% w/v) replacing traditional protein used in Man Rogosa Sharpe broth (MRS), i.e. peptone and yeast extract (2.5% w/v). The hydrolysates had a high crude protein content between 60-90%, consisting mainly of peptides.

**Keywords:** protein hydrolysates, alternative protein sources, industry by-products, microbiological nutrient media, probiotics

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## E12

### VALORIZING GRAPE POMACE AS INGREDIENT OF BEVERAGES WITH FUNCTIONAL PROPERTIES

**Alessio Buono**<sup>\*1)</sup>, **Francesco Romaniello**<sup>(2)</sup>, **Simona Lucia Bavaro**<sup>(3)</sup>, **Loredana Loredana Tarraran**<sup>(3)</sup>, **Giovanna Giovinazzo**<sup>(3)</sup>, **Carmela Gerardi**<sup>(3)</sup>

<sup>1)</sup> University of Turin, Italy

<sup>2)</sup> INRIM, Italy

<sup>3)</sup> CNR ISPA, Italy

\*Corresponding author - E-mail: [alessiobuono54@gmail.com](mailto:alessiobuono54@gmail.com)

The wine industry generates a wide variety of waste; of the 75 million tons of grapes produced annually, 80% is destined to different wine production. The grape pomace still contains a large amount of phytochemicals, especially polyphenols such as anthocyanins, flavonoids and. Italy is one of the main wine producers in the world; ISTAT (Italian national statistical institute) estimates a production of 4 billion liters of wine every year, that corresponds to 4 tons of grape pomace. From the perspective of a circular economy, the richness of these residues makes it desirable to take advantage of them to later incorporate as functional ingredients in food and beverages formulations. To add value to these byproducts, pomace from two red Italian grape, Lambrusco cv and Negroamaro cv, and a white grape, Fiano cv, were stabilized by drying and beverages were then produced by infusing the resulting powders in acidified water.

The first step involved the antioxidant activity (AA), total phenols (TP) and total anthocyanins (TA) characterizations of the beverages along with an initial characterization of the polyphenol profiles; preliminary tests were performed using a spectrophotometer, respectively AA, TP and TA were measured in trolox, acid gallic and oenin equivalent.

Preliminary results show an AA which are, for Fiano, Negroamaro and Lambrusco beverages respectively:  $7.34 \pm 0.30$  (mmol TE/L),  $2.93 \pm 0.11$  (mmol TE/L),  $12.36 \pm 0.44$  (mmol TE/L). These results were correlated with TP content (Fiano, Negroamaro and Lambrusco, respectively):  $446.24 \pm 9.45$  (mgGAE/L),  $175.91 \pm 7.17$  (mgGAE/L),  $893.36 \pm 15.7$  (mgGAE/L) and TA content  $3.17 \pm 0.09$  (mgOeninE/L),  $16.34 \pm 0.75$  (mgOeninE/L),  $500.25 \pm 7.0$  (mgOeninE/L).

Individual polyphenols were identified using an UHPLC-HRMS system (Q-Exactive Plus Orbitrap) to determine the molecules responsible for the antioxidant power. The molecules were identified based on HRMS and MS/MS experiments. Finally, these 3 beverages underwent a simulated digestion following the INFOGEST protocol, which is an in vitro model static digestion. For all the samples, two variations of INFOGEST were used to simulate digestion of an elder individual and an adult one. Moreover, the antioxidant activity of aqueous extracts of pomace of Lambrusco, Negroamaro and Fiano were analyzed after the application of INFOGEST digestion protocol. The study of the digestates allows us to monitor the variation of antioxidant activity and their composition during the different digestion phases both in elder and adult digestion.

Our results demonstrate that grape pomace could eventually be used in the production of antioxidant-functional foods. As expected, both the antioxidant activity and the levels of total polyphenols decrease in the digested samples compared to the initial beverages; however, they remain appreciable. The next step is to evaluate the possibility of testing the beverage digestate in cellular models to assess their cellular absorption.

**Keywords:** grape pomace, polyphenols, HPLC-MS/MS, characterization, digestomic INFOGEST

### E13

#### **BIOBASED PACKAGING FOR W-3 FORTIFIED BISCUITS: AROMA QUALITY STABILITY OVER TIME**

**Eloisa Bagnulo<sup>(1)</sup>, Erica Liberto<sup>\*(2)</sup>, Chiara Cordero<sup>(2)</sup>, Monica Locatelli<sup>(3)</sup>**

<sup>1)</sup> *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Italy*

<sup>2)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>3)</sup> *Dipartimento di Scienze del Farmaco, Università del Piemonte Orientale, Italy*

*\*Corresponding author - E-mail: erica.liberto@unito.it*

Functional foods, nutraceuticals and dietary supplements as part of a controlled lifestyle can provide opportunities to reduce health risk factors and address the need for prevention and control of diet-related chronic diseases to save healthcare resources [1].

The development of functional foods that target the needs of specific consumer groups (especially young children and the elderly) is motivated by specific market demands and needs [2].

Environmental sustainability is also an important aspect, driven by the design and use of innovative, biodegradable and recyclable packaging that is also able to maintain the flavour and nutritional quality of the product [3].

The aim of this work is to evaluate the quality and the evolution over time of the aromatic fraction of biscuits functionalized with oils enriched with omega-3 fatty acids and packaged in innovative and more sustainable packaging (BB961, NK, NKME, NKPLA). The evaluation was carried out from T0 to T5 and comparative analyses were performed on the stability of the same samples stored in the conventional packaging and the original recipe stored in the innovative packaging.

For the characterisation of the compounds contained in the volatile fraction of the biscuits, the technique of online headspace analysis in conjunction with gas chromatography with mass spectrometry as detector (HS-SPME-GC-MS) was used.

The analysis of the aromatic properties and their stability over time when packaged in different packages showed that the biscuit recipes formulated with the blend of N6 oils (10% hemp, 90% EVO) are less accustomed to the development of oxidation compounds than those with the N29 blend (20% hemp, 40% EVO, 40%OO) [4-5].

Empty packaging has its own profile of volatile organic compounds, with components such as propylene glycol, acetone and toluene being relatively common. Depending on the type of formulation, some packages have slightly different interactions (NK and NKME). The material BB961 is the least inert of all. It can even be said that this packaging tends to absorb compounds from the biscuit that give it characteristic aromatic notes and instead release compounds that characterise the packaging material. In contrast, the situation is more favourable for the other two types of packaging. In particular, the NKPLA packaging seems to have a tendency to adsorb those components that can be associated with the lipid oxidation of the biscuit.

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**Keywords:** sustainable packaging, functional foods, aroma quality stability, gas chromatography, nutraceuticals

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### E14

#### SUSTAINABLE PACKAGING EDIBLE FILMS WITH ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES FROM DIFFERENT VEGETABLE WASTES: A COMPARATIVE STUDY

**Nicoleta Corina Predescu**\*<sup>(1)</sup>, **Gheorghe Valentin Goran**<sup>(1)</sup>, **Carmen Daniela Petcu**<sup>(1)</sup>, **Camelia Papuc**<sup>(2)</sup>

<sup>1)</sup> Faculty of Veterinary Medicine of Bucharest, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independentei, District 5, 050097, Romania

<sup>2)</sup> Academy of Romanian Scientists (AOSR), 54 Splaiul Independentei, District 5, 050094, Romania

\*Corresponding author - E-mail: corina.predescu@usamvb.fmvb.ro

Vegetable waste, a significant byproduct of the food industry, is rich in bioactive compounds such as polyphenols, betalains, vitamins (like vitamin C), minerals (Ca, P, Zn), and fibre such as pectin. Common fruits and vegetal wastes, including peels, seeds, and pulp, are often discarded despite their high nutritional and bioactive content. Repurposing fruit waste into packaging films promotes a circular economy, where fruit waste is converted into valuable resources. These biodegradable films naturally break down after use, reducing their environmental footprint.

This study aims to compare the biologically active compounds, antioxidant, antimicrobial properties, and sensory attributes of packaging edible films derived from red apple (*Malus domestica*) and red beet (*Beta vulgaris*) wastes, alone or in combination with blueberry (*Vaccinium myrtillus*) waste.

The red apples, red beet, and blueberry waste materials were sourced from a juice processing facility near Bucharest, Romania. The waste materials were ground, then blended to obtain a smooth paste and heated for 15 minutes at 82 °C, mixing once again, and pouring the mixture into the oven tray, letting edible films dry in the oven. The total polyphenol, anthocyanins, betalains concentrations, and antioxidant properties were determined using spectrophotometric methods. The antimicrobial property was tested against *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecium*, *Bacillus cereus*, and *Salmonella enteritidis*. Waste fruit sensory attributes were tested in the laboratory with the help of 10 panellists.

The highest polyphenol concentration was found for the edible film that contained blueberry wastes 101±6.45 mg GAE/100 g film. Betalains were present in higher concentrations in the film obtained with the addition of red beet 273±18.32 mg/100 g film. All edible films showed antimicrobial activity against all the tested microbial strains. Regarding the sensory properties, the colour was better preserved for red beet-containing films than the rest; the texture was preferred for the films without blueberry. The taste was appreciated for edible film based on apple waste. The antioxidant and antimicrobial properties of these edible films are essential for preventing unwanted odours, tastes, texture, and reduced nutritional value. The antimicrobial properties inhibit the growth of harmful microorganisms, extending shelf life and ensuring food safety.

This study highlights the potential of agricultural byproducts for sustainable packaging through a comparative analysis of films developed from red apple, red beet, and blueberry wastes. With their properties, these films provide a natural and eco-friendly solution for preserving food while minimizing environmental impact, biodegradable packaging materials, and addressing the global challenges of food waste and plastic pollution.

**Keywords:** sustainable packaging, packaging edible films, vegetable waste, circular economy, antioxidant and antimicrobial properties

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### E15

#### PHYSICOCHEMICAL CHARACTERIZATION OF INDUSTRIAL AND AGRICULTURAL BY-PRODUCTS FOR SUSTAINABLE BIOFERTILIZER DEVELOPMENT IN ORGANIC RASPBERRY CULTIVATION

Oana Romina Botoran<sup>(1)</sup>, Elisabeta-Irina Geana<sup>(1)</sup>, Corina-Teodora Ciucure<sup>(1)</sup>, Andreea Maria Iordache<sup>(1)</sup>, Claudia Sandru<sup>(1)</sup>, Marius Gheorghe Miricioiu<sup>(1)</sup>, Roxana Elena Ionete<sup>(1)</sup>

<sup>1)</sup> National Research and Development Institute For Cryogenic And Isotopic Technologies, Romania

\*Corresponding author - E-mail: oana.botoran@icsi.ro

By repurposing industrial and agricultural by-products, the study seeks to improve soil fertility and contribute to circularity and sustainability within organic agricultural practices. The biofertilizer aims to neutralize soil acidity, improve nutrient availability, and mitigate the mobility of heavy metals in contaminated soils, promoting soil health and crop productivity. The slow release of nutrients from agricultural by-products and the liming effect of steel slag highlights the potential for sustained soil improvement. This study investigates the physicochemical characterization of raw materials such as steel slag, dolomite, cement dust, grape pomace and wine lees for their potential use in biofertilizer production aimed at organic raspberry cultivation. The research assesses key parameters, including polycyclic aromatic hydrocarbons (PAHs), acid herbicides, and heavy metals (As, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sr, V, Zn), alongside the analysis of water-soluble extracts of calcium, magnesium, sodium, potassium, and sulfates, and the total elemental composition of these nutrients. This research supports the use of biofertilizers as a sustainable alternative to conventional chemical fertilizers, offering eco-friendly solutions to soil degradation, heavy metal contamination, and nutrient depletion. By integrating waste materials from the steel, cement, and wine industries, the study advocates for a closed-loop economy, reducing environmental pollution while enhancing crop production. The findings have significant implications for the development of sustainable agricultural practices that align with global goals for resource efficiency, environmental health, and economic viability.

**Keywords:** soil, fertility, by-products, biofertilizer, agriculture

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### E16

#### ENHANCING BIOMASS YIELD AND ELEMENTAL COMPOSITION OF ALFALFA (MEDICAGO SATIVA L.) PLANTS VIA BIOSTIMULANT APPLICATION COMBINED WITH FOLIAR FERTILIZATION

**Ebenezer Ayew Appiah**\*<sup>(1)</sup>, Erika Kutasy<sup>(1)</sup>

<sup>1)</sup> University of Debrecen, Hungary

\*Corresponding author - E-mail: ayew.appiah@agr.unideb.hu

Optimizing alfalfa biomass yield production and elemental composition through innovative agronomics practices is crucial for sustainable agriculture productivity. A study was conducted at the University of Debrecen Hungary, to examine the effect of biostimulants supplemented with foliar fertilizer on alfalfa biomass yield and elemental composition. The experiment was arranged in a randomised complete block design (RCBD) with two biostimulant treatments and a foliar fertilizer plus control replicated three times. Data collected were subjected to analyses of variance using Genstat, where significantly different means were separated at a probability of 5% using the least significant difference. Our findings show that the impacts of biostimulant application with or without foliar fertilizer significantly affected alfalfa biomass yield and elemental composition. The result indicated that ticho immum combined with ino green significantly maximized biomass yield with 20862 kg/ha followed by fonix and tricho Immum alone with 18454 and 17360 kg/ha while the control treatment had the least biomass yield. Comparing the treatment applied against the control shows that the combined treatment of tricho immum and ino green increased biomass yield by 34.8% while 19.2% and 12.2% increases were observed for fonix and tricho immum alone. Biostimulant combined with or without foliar fertilizer affected the elemental composition of zinc (Zn), titanium (Ti), and iron (Fe) except for sulphur (S), phosphorus (P), and molybdenum (Mo). It revealed that tricho immum alone significantly increased Zn (11%), Fe (3.72%), S (7%), P (0.33%), and Mo (16%). The result indicates that the treatments applied improved nutrient uptake and enhanced plant vigor, as evidenced by increased biomass accumulation and optimized elemental composition. Therefore our study suggested that the use of biostimulants along with foliar fertilizer could help to improve biomass yield production and elemental composition in alfalfa production, thereby benefiting agricultural sustainability and livestock feedstock.

**Keywords:** biostimulants, foliar fertilizer, biomass, yield, alfalfa, elemental composition

**Acknowledgement:** *The authors of this paper gratefully acknowledge the enormous support by the Tempus Public Foundation (Hungary) within the framework of the Stipendium Hungaricum Scholarship programme and also the members of the departments that helped to shape this effort*

# **DATA HANDLING & ARTIFICIAL INTELLIGENCE IN FOOD ANALYSIS**



## F1

### MINERALS AND AMINO ACIDS PROFILE OF BEE POLLEN FROM A SELECTED APIARY IN SOKOTO STATE, NIGERIA

**Muhammad Sirajo**\*<sup>(1)</sup>

<sup>1)</sup> *Department of Chemistry, Sokoto State University, Nigeria*

*\*Corresponding author - E-mail: msmabera75@gmail.com*

Bee pollen is an indispensable apicultural product in many diets due to its high nutritional and medicinal value. The research investigated its minerals and amino acids content using standard methods of analyses. The results revealed that Potassium is the most abundant macro element in the fruit ( $360.56 \pm 4.92$ mg), followed by calcium ( $245.69 \pm 3.12$  mg), phosphorus ( $197.76 \pm 1.87$  mg), magnesium ( $98.46 \pm 1.08$  mg), and sodium ( $27.63 \pm 2.51$  mg). On the other hand, iron is the most abundant micro element ( $14.44 \pm 1.75$  mg), followed by manganese ( $6.97 \pm 0.77$  mg), zinc ( $4.33 \pm 0.98$  mg), copper ( $3.50 \pm 0.12$  mg), and nickel ( $2.51 \pm 0.09$  mg). Of the essential amino acids, leucine, lysine and aromatic (phenylalanine and tyrosine) are the predominant acids, while glutamic, aspartic and alanine were found to be the major non-essential amino acids. The essential amino acids were above the recommended level by Food and Agricultural Organization/World Health Organization (FAO/WHO) for both adults and pre-school children aged 2-5 years. The present investigation indicates the potential use of bee pollen as rich source of many important nutrients that appear to have a very positive effect on human health. Bee pollen is an indispensable apicultural product in many diets due to its high nutritional and medicinal value. The research investigated its minerals and amino acids content using standard methods of analyses. The results revealed that Potassium is the most abundant macro element in the fruit ( $360.56 \pm 4.92$  mg), followed by calcium ( $245.69 \pm 3.12$  mg), phosphorus ( $197.76 \pm 1.87$  mg), magnesium ( $98.46 \pm 1.08$  mg), and sodium ( $27.63 \pm 2.51$  mg). On the other hand, iron is the most abundant micro element ( $14.44 \pm 1.75$  mg), followed by manganese ( $6.97 \pm 0.77$  mg), zinc ( $4.33 \pm 0.98$ mg), copper ( $3.50 \pm 0.12$  mg), and nickel ( $2.51 \pm 0.09$  mg). Of the essential amino acids, leucine, lysine and aromatic (phenylalanine and tyrosine) are the predominant acids, while glutamic, aspartic and alanine were found to be the major non-essential amino acids. The essential amino acids were above the recommended level by Food and Agricultural Organization/World Health Organization (FAO/WHO) for both adults and pre-school children aged 2-5 years. The present investigation indicates the potential use of bee pollen as rich source of many important nutrients that appear to have a very positive effect on human health.



## F2

### A TECHNOLOGICAL FRAMEWORK FOR ENHANCED FOOD QUALITY CONTROL AND TRANSPARENCY IN FOOD SUPPLY CHAINS

**Paraskevas Bourgos**<sup>(1)</sup>, **Amalia Ntemou**<sup>(1)</sup>, **Ioanna Drigkopoulou**<sup>(1)</sup>, **Eleni Provopoulou**<sup>(1)</sup>, **Pantelis Lappas**<sup>(1)</sup>, **Spyros Evangelatos**<sup>(1)</sup>

<sup>1)</sup> Netcompany-Intrasoft, Luxembourg

\*Corresponding author - E-mail: Paraskevas.Bourgos@netcompany.com

Global food security and supply chain integrity are facing rising concerns, making robust technological solutions crucial. Netcompany-Intrasoft (NCIS) introduces a pioneering technological framework designed to address critical issues within global food supply chains, including food fraud, sustainability, traceability, and transparency.

The framework integrates advanced blockchain technology, a digital food product passport as well as fosters evidence-based decision making through AI and ML for preventative interventions and actionable planning.

At the core of this initiative is the implementation of blockchain technology. Blockchain's decentralized and immutable nature ensures data integrity and traceability across the supply chain. This infrastructure facilitates real-time data accessibility, enabling all participants to access trustworthy information. This transparency is crucial for fraud prevention, as it creates an auditable, tamper-proof record of transactions and processes. By maintaining a secure ledger, blockchain technology fosters a trustworthy environment for all stakeholders, from farmers to consumers.

Complementing the blockchain infrastructure is the development of a digital food product passport. This innovative tool provides detailed, tamper-proof information about food products at every stage from farm to table. The digital passport includes critical data such as origin, production methods, transportation conditions, and quality metrics. This level of detail not only enhances traceability but also empowers stakeholders throughout the food value chains (e.g., producers, retailers, authorities, consumers) to make informed decisions. The digital passport interface is user-friendly, ensuring all stakeholders can easily access and understand the information provided.

An AI-enabled Early Warning and Decision Support System is integrated into the framework to further enhance its capabilities. This system harnesses predictive analytics to identify and mitigate risks associated with food fraud and security proactively. By analyzing historical and real-time data, the AI system can predict potential threats and provide actionable insights to prevent issues. This proactive approach significantly improves decision-making processes, ensuring the reliability of food quality and safety.

In conclusion, this novel technological framework represents a significant advancement in the management of global food supply chains and aims to tackle critical issues of food fraud, security, sustainability, traceability, and transparency.

**Keywords:** blockchain, digital food product passport, AI/ML, food supply chain, traceability

**Acknowledgement:** *The efficacy and adaptability of this technological framework are exemplified through its application in several Horizon Europe projects—FOODGUARD (GA No 101136542), WATSON (GA No 101084265), ALLIANCE (GA No 101084188), Data4FOOD2030 (GA No 101059473), and FOODDATAQUEST (GA No 101134138). These projects demonstrate how the framework's innovations are tailored to meet specific challenges within the food industry. NCIS has played a key role in deploying these technologies, showcasing its commitment to support safer, more transparent food value chains.*

## F3

### NOVEL MACHINE LEARNING PIPELINES FOR THE DISCOVERY OF FOOD AUTHENTICITY MARKERS VIA NON-TARGETED LC-MS ANALYSIS: HONEY AS A CASE STUDY

**Stephane Bayen**<sup>(1)</sup>, **Shawninder Chahal**<sup>(1)</sup>, **Lei Tian**<sup>(1)</sup>, **Shaghig Bilamjian**<sup>(1)</sup>, **Ferenc Balogh**<sup>(2)</sup>, **Lorna De Leoz**<sup>(3)</sup>, **Tarun Anumol**<sup>(3)</sup>, **Daniel Cuthbertson**<sup>(3)</sup>

<sup>1)</sup> McGill University, Canada

<sup>2)</sup> John Abbott College, Canada

<sup>3)</sup> Agilent Technologies, United States of America

\*Corresponding author - E-mail: stephane.bayen@mcgill.ca

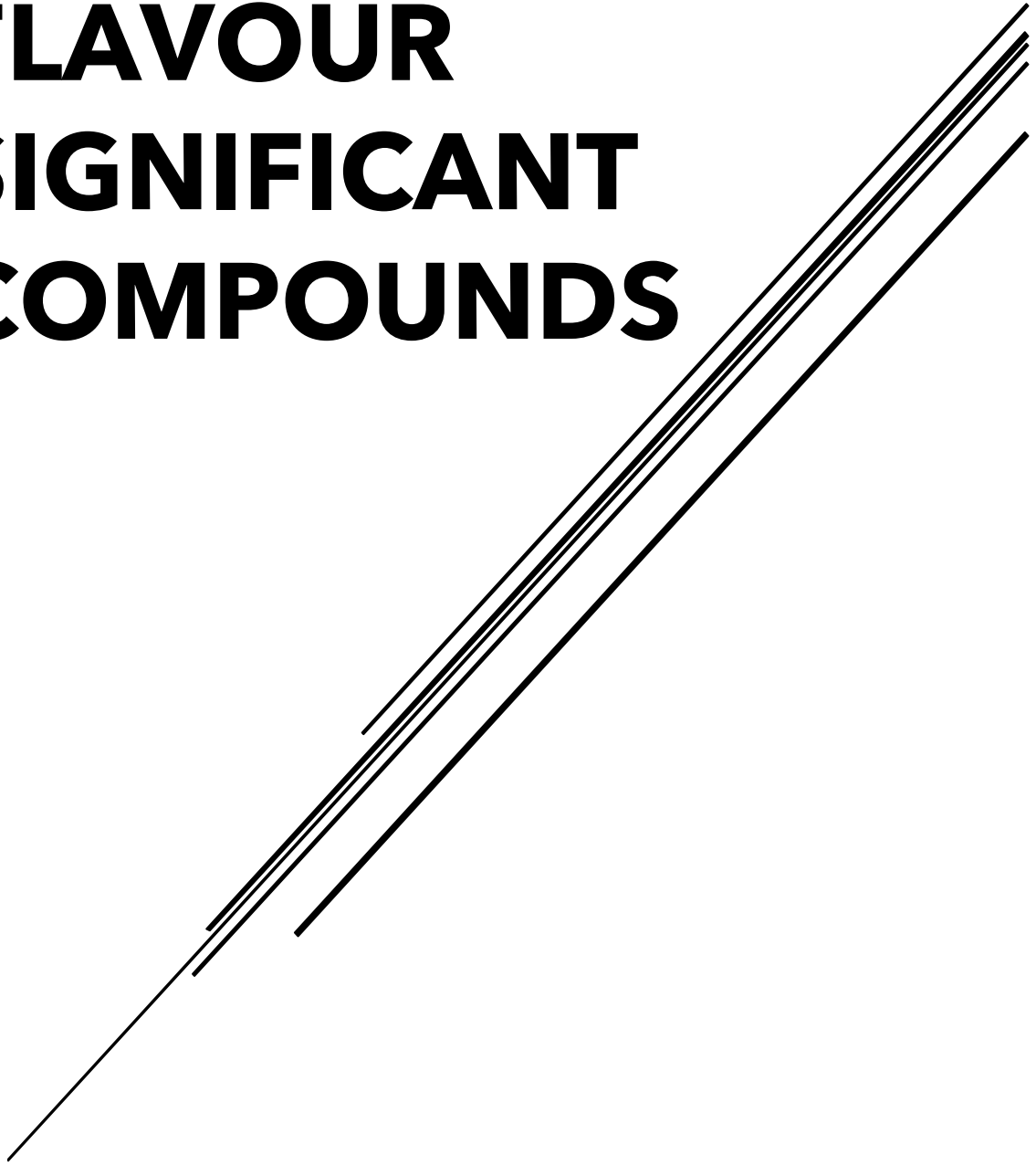
Modern food authentication methods are required to protect consumers and support food professionals. Recent advances in analytical sciences, notably in high-resolution mass spectrometry (HRMS) and large data evaluation, have opened the door to the comprehensive chemical fingerprinting of food, and signals for thousands of molecules can be recorded simultaneously for a single sample. However, not all of these chemical signals are relevant to the authentication task, and automated machine learning pipelines are required to identify authenticity markers (1 or 2 analytical targets) and the authentic profiles (>2 analytical targets) of different types of food.

In the present study, we used the botanical origin of honey as a case study for the application of HRMS for food authenticity purposes due to its global use and status as one of the most adulterated foodstuffs. Several hundreds of honey samples, self-reported as monofloral blueberry, buckwheat, clover, goldenrod, linden, or others, were collected in Canada and these samples were analyzed using a "dilute-and-shoot" method with liquid chromatography (LC) coupled with HRMS. This resulted in the collection of hundreds of gigabytes of data. We then developed various automated machine learning pipelines capable of performing feature selection; optimizing, training, and validating machine learning models; and subsequently visualizing the results. For example, a recursive feature elimination pipeline was developed to optimize the honey chemical fingerprint for multiclass machine learning classifiers, and the LC-HRMS data set (consisting of 2028 features) was reduced to only 54 key features (candidate markers) sufficient to classify honeys based on their floral origin and to detect mislabelled honey samples. We also developed fast pipelines for the discovery of binary markers, threshold markers, and interval ratio-markers (ratio of two compounds within a unique interval in a specific honey type). This work demonstrates an end-to-end approach to mine the honey metabolome for novel authenticity markers and can readily be applied to other types of food.

**Keywords:** food authentication, high-resolution mass spectrometry, honey, machine learning, authenticity markers

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**FLAVOUR & OFF-  
FLAVOUR  
SIGNIFICANT  
COMPOUNDS**



## G1

### IMPROVING TASTE AND FLAVOR IN DAIRY PRODUCT THROUGH MILK ANALYSIS OF FREE FATTY BY MID-INFRARED (MIR) SPECTROMETRY

Octave Christophe<sup>\*(1)</sup>, Romain Reding<sup>(2)</sup>, Julie Leblois<sup>(3)</sup>, Denis Pitttois<sup>(4)</sup>, Cedric Guignard<sup>(4)</sup>, Frederic Dehareng<sup>(1)</sup>

<sup>1)</sup> Walloon Agricultural Research Centre (CRA-W), Belgium

<sup>2)</sup> CONVIS, Luxembourg

<sup>3)</sup> Eleveo asbl, Belgium

<sup>4)</sup> Luxembourg Institute of Science and Technology (LIST), Luxembourg

\*Corresponding author - E-mail: o.christophe@cra.wallonie.be

The dairy sector deals with a recurring issue: a taste alteration due to degradation of fat, commonly called lipolysis. Lipolysis happens after the milking, through the physical shocks induced by freezing, pumping, transfer and storage of the milk. Physical break of fat globules makes triglycerides accessible to enzymes and degraded into free fatty acids (FFA). Among them, the volatile short chain FFA lead to organoleptic issues through undesired tastes.

An easy quantification of these individual short chain FFA, responsible of taste alteration, is very difficult. Historically, the lipolysis was quantified with the BDI methods by the measurement of the fat acidity. On the other hand, the analysis of a wide range of FFA is now possible by Gas Chromatography coupled with tandem mass spectrometer (GC-MS/MS). This analysis is time consuming, expensive and difficult to apply for routine analysis on a large set of samples. In order to bring a new way of preventive and corrective action for dairies and farmers, this project aims to develop predictive models based on milk mid Infrared spectroscopy (FT-MIR) to quantify FFA.

For this purpose, milk samples from 4 different countries were collected and analyzed by MIR spectroscopy as well as GC-MS/MS. The different models provided moderate  $R^2$  for long-chain FFA and relatively low  $R^2$  for short-chain FFA. Indeed, most of short chain FFA were under the limit of quantification. The lack of short-chain FFA concentration was solved by testing different mechanical induced lipolysis without interfering with the MIR spectrum. Among them, time milk homogenization has demonstrated a clear increase of short chain FFA value leading to better predictive models. More than 750 analyses were collected (classical and mechanical induced lipolysis included) to setup this model. Five different machine learning algorithm (principal component regression (PCR), partial least square regression (PLS), Kernel ridge regression (KRR), Elastic-Net Regression (ENR) and Support Vector machine Regression (SVMR)) were performed for each free fatty acid on the entire dataset. These different algorithm exhibit for each free fatty acid performances  $R^2$  is ranged from 0.4 to 0.8 for the different FFA (C4 to C18).

**Acknowledgement:** The authors would like to thank the EIP Agri Program supported by the government of the Grand Duchy of Luxembourg for funding this research.

# FLAVOUR & OFF-FLAVOUR SIGNIFICANT COMPOUNDS

## G2

### PHYSICOCHEMICAL PROPERTY AND SENSORY ATTRIBUTES OF NUT-BASED MILK COFFEE

Woorin Lee<sup>(1)</sup>, Kwang-Geun Lee<sup>\*(1)</sup>

<sup>1)</sup> Dongguk University, South Korea

<sup>\*</sup>Corresponding author - E-mail: kwglee@dongguk.edu

In this study, the aim was to create nut-based milk utilizing four different types of nut-based milk—almond, cashew, hazelnut, and walnut—in conjunction with Arabica (*Coffea arabica*) espresso coffee. Furthermore, the research aimed to assess and compare the physicochemical properties and sensory characteristics of the different nut-based milk coffees. In the roasted nut vegan coffee samples, the content of oleic acid, linoleic acid, and linolenic acid increased significantly ( $p < 0.05$ ). The nut-based vegan coffee sample contained a large amount of benzaldehyde and 1-methylpyrrole-2-carboxaldehyde ( $p < 0.05$ ), and the commercial milk coffee sample had a high proportion of 5-methyl furfural and furfuryl alcohol ( $p < 0.05$ ). As a result of sensory evaluation, the cashew nut milk vegan coffee sample showed the highest preference along with the nut-based milk coffee samples ( $p < 0.05$ ). The findings provide a foundational resource for understanding the physicochemical and sensory attributes of nut-based milk coffee and their correlation with overall preference.

**Keywords:** plant-based milk, volatile compounds, sensory attributes, fatty acid content, antioxidant activity

**Acknowledgement:** This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (grant number NRF, RS-2024-00336432).

## G3

### COMPARISON AND DIFFERENTIATION OF FLAVOR PROFILES IN VEGAN FOOD AND THEIR NON-VEGAN PRECURSORS BY A NOVEL GC-HRMS TECHNOLOGY

Marleen Vetter<sup>(1)</sup>, Eliška Ceznerova\*<sup>(1)</sup>, Sonja Klee<sup>(1)</sup>, Steffen Bräkling<sup>(1)</sup>, Arnd Ingendoh<sup>(2)</sup>

<sup>1)</sup> TOFWERK AG, Switzerland

<sup>2)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: [eliska.ceznerova@tofwerk.com](mailto:eliska.ceznerova@tofwerk.com)

Increased awareness of health, environmental issues, and animal welfare has led to growing public interest in vegan products. Consequently, the market for meat- and dairy-free alternatives is expanding rapidly. One market strategy involves imitating popular non-vegan products, which has the advantage that customers are already familiar with the product and may be inclined to switch to a vegan alternative with a similar taste and appeal. Characterizing flavor and aroma profiles is particularly important in developing these types of products. Since a large part of flavor and aroma perception arises from volatile compounds, GC-HRMS is often used for analysis. However, in non-target studies, conventional EI (electron ionization) combined with NIST library searches can suffer from ambiguous identification of unknowns due to less specific fragmentation, missing molecular ion signals, or absence in reference libraries. We present a novel GC-HRMS method that simultaneously uses both chemical ionization (CI) for exact molecular mass assignment and EI for NIST-searchable fragment spectra in a single GC run.

For this study, 10 g of vegan cheese from New Roots AG and its supposed non-vegan equivalent (Appenzeller Classic) were incubated for 10 minutes in a headspace vial. Each cheese was sampled in triplicate at 60°C for 30 minutes using a Restek Polyacrylate SPME fiber (BGB Analytik, Switzerland). The GC-HRMS setup includes a high-resolution TOF analyzer (ecTOF, TOFWERK AG, Switzerland) operating simultaneously with a standard 70 eV EI source and a medium-pressure CI source, with an automated selection of different CI reagent ions ( $N_2H^+$ ,  $H_3O^+$ ,  $NH_4^+$ ). This setup enables the adjustment of reactant selectivity and the degree of fragmentation for various compound classes of interest.

Using both EI and CI information in a single run allows for highly confident compound identification and quantification in complex matrices. Clear differences in common flavor compounds such as butanoic acid and 2/3-methyl butanoic acid were observed between the two types of cheese. Several peaks of interest for both the vegan and non-vegan equivalents were selected at different retention times to evaluate profile differences. Statistical tools like volcano plots revealed potential markers for both samples. Several examples illustrate the advantages of incorporating CI alongside traditional EI in a single GC-HRMS analysis to enhance reliability, particularly when dealing with complex samples. The ecTOF not only easily identifies commonly investigated flavor compounds of interest but also distinguishes other volatile and semi-volatile compounds that may play crucial roles in flavor development or later processing steps.

**Keywords:** GC-HRMS, flavor and off-flavor compounds, EI & CI, non-target, food quality and authenticity

## G4

### THE CHEMISTRY BEHIND CHANGES IN COFFEE FLAVOR DURING STORAGE IN COMPOSTABLE AND STANDARD CAPSULES

Giulia Strocchi<sup>(1)</sup>, Eloisa Bagnulo<sup>(1)</sup>, Gloria Pellegrino<sup>(2)</sup>, Carlo Bicchi<sup>(1)</sup>, **Erica Liberto**\*<sup>(3)</sup>

<sup>1)</sup> Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Italy, Italy

<sup>2)</sup> Luigi Lavazza S.p.A., Italy

<sup>3)</sup> Department of Drug Science and Technology, University of Turin, Italy

\*Corresponding author - E-mail: erica.liberto@unito.it

Rationale: Coffee quality is influenced by a series of genetic precursors, environmental factors, and post-harvest processes [1-3]. In this scenario, the storage of roasted&ground coffee plays a role. Even if it is a dehydrated product, oxygen and moisture affect its stability and thus its shelf life [2,4-6]. The selection of packaging materials is crucial for maintaining quality, and polymer-aluminium multilayer composite packaging materials are generally used to meet these requirements. However, especially in the broad market of coffee capsules, this packaging has an impact on the environment [7]. Some companies are looking for ways to balance flavor quality and sustainability by replacing aluminium-polymer packaging with compostable alternatives. This paper focuses on investigating a flavoromics approach to explore the chemistry behind changes in coffee flavor during storage.

Methods: Different commercial blends (P and B, 100% Arabica and I 50/50% Arabica/Robusta) of coffee capsules in different packaging, namely standard (multilayer foil with aluminium barrier) (and eco-capsules, batches and were analysed. Samples were stored under stress conditions (65% RH and 45°C) and monitored over a period from T0 to T180 days for the standard caps and from T0 to T90 days for the eco caps. The chemical data of the investigated coffee samples were obtained by analyzing both volatile and non-volatile profiles (i.e. lipids and phenolic fractions including alkaloids) using HS-SPME-GC-MS, HPLC-UV/DAD, moisture, pH, acidity, peroxide value and p-anisidine were measured. Sensory tests were carried out by an expert panel.

Results: A series of volatile compounds were detected aged samples, including 3 VOCs exhibiting pungent, rancid and acidic notes and appearing in sensory unacceptable samples independently from blends, batches and packaging. The phenolic fraction is very stable over time regardless of packaging and mixture. The evolution of the free fatty acids (FFAs) was correlated with the peroxides, p-anisidine, acidity values, and VOCs. pH decreases and moisture increase is measured in particular with compostable capsules. The results show that the different blends behave differently over time in the standard packaging than in the eco-capsules. The latter have a shorter shelf life due to moisture adsorption, which triggers hydrolytic and oxidative reactions.

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**Keywords:** R&D coffee, flavor quality, storage, packaging

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## G5

### FOODOMICS APPROACH FOR THE CHARACTERIZATION DEFECTIVE COCOA LIQUORS IN ENSURING COCOA QUALITY

**Eloisa Bagnulo**<sup>(1)</sup>, **Cristian Bortolini**<sup>(2)</sup>, **Alessandro Guglielmetti**<sup>(2)</sup>, **Irene Chetschik**<sup>(3)</sup>, **Carlo Bicchi**<sup>(4)</sup>, **Chiara Cordero**<sup>(5)</sup>, **Erica Liberto**\*<sup>(5)</sup>

<sup>1)</sup> *Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Italy*

<sup>2)</sup> *Soremartec Italia S.r.l. (Ferrero S.p.a. group), Italy*

<sup>3)</sup> *ZHAW School of Life Sciences and Facility Management, Switzerland*

<sup>4)</sup> *Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Italy*

<sup>5)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

\*Corresponding author - E-mail: [erica.liberto@unito.it](mailto:erica.liberto@unito.it)

*Theobroma cacao* is a worldwide comfort food with numerous benefits, including potential cardiovascular effects and positive influence on mood [1]. Political tensions in cocoa production regions and climate changes severely impact cocoa yield and quality, causing premature ripening, fermentation irregularities and mold growth in beans. These factors can also alter the chemical flavour profile of cocoa products, affecting market value and the choices of manufacturers and consumers [2]. This study aims to characterize cocoa defects for effective quality control [2-3]. Guided by company panel sensory characterization, different Good and Bad samples were analyzed using several advanced analytical techniques within a foodomic approach. We investigated several chemical fractions contributing to cocoa flavour and emerging off-notes. Chromatographic targeted fingerprint were processed using multivariate analysis to identify chemical differences between defective samples and quality standards. Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS) provided an overall profile of volatile fraction in cocoa samples, but no significant differences between the two groups were found. We then adopted a targeted approach focusing on fractions affecting the main defective sensory descriptors identified by the panel: bitter, astringent, sour, and earthy. Chemical fingerprint analysis showed significant differences between Good and Bad samples only for the volatile acidic fraction. For the non-volatile fraction, we determined the Total Phenolic Content (TPC) and through Liquid Chromatography with Ultraviolet Diode-Array Detection (LC-UV-DAD) we analysed the fractions responsible for bitter and astringent taste (polyphenols and methylxanthines). No significant results were highlighted in discrimination of defective samples due to a "seasonality" effect that influence data interpretation caused by harvest batches periods and high sample variability. We then adopted a sensomic approach, analyzing the most representative cocoa samples of the two groups using Gas Chromatography-Olfactometry-Mass Spectrometry (GC-O-MS) to enable olfactory identification of main cocoa odorant compounds [3]. Results identified chromatographic regions with off-notes in defective samples, particularly a strong moldy odor and aromatic imbalance. Next step will involve the Comprehensive two-dimensional Gas Chromatography-Time Of Flight Mass Spectrometry (GC×GC-TOF MS) analyses to precisely identify this defect-causing molecules.

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**Keywords:** foodomics, food quality, cocoa flavour, sensomics

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### G6

#### COMPARATIVE VOLATILOMIC STUDY OF GRANA PADANO-TYPE CHEESE USING DIFFERENT RENNET TYPES

Manuela Giordano<sup>(1)</sup>, Vladimiro Cardenia<sup>(1)</sup>, Marta Bertolino<sup>(1)</sup>, Daniela Ghirardello<sup>(1)</sup>, Giuseppe Zeppa<sup>\*(1)</sup>

<sup>1)</sup> *Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy*

<sup>\*</sup> *Corresponding author - E-mail: giuseppe.zeppa@unito.it*

The development of low molecular weight volatile organic metabolites (VOCs) in cheeses is the result of a number of chemical processes, including proteolysis, lipolysis and glycolysis, occurring throughout the production and ripening stages. These processes, driven by microbial and enzymatic activities, are fundamental to the development of flavor, aroma and texture of cheese. Moreover, milk coagulation, a crucial step in cheese production, requires the addition of coagulating enzymes. In some Italian PDO hard cheeses, the use of animal rennet is mandatory. However, the production of dairy animal rennet has recently declined due to the limited availability of calf stomachs, and increased demand for vegetarian alternatives.

The present research aimed to determine the volatile organic compounds (VOCs) during the ripening (up to 9 months) of Grana Padano-Type hard cheese, produced with either animal rennet (A) or vegetable rennet (V) (a plant extract of *Cynara cardunculus* L.) using milk collected during the summer (S) or winter (W) seasons. Through volatilome analysis by SPME-GC/MS, this study provides insight into the impact of rennet type and season of milk production on the VOC profile, thereby affecting the aroma and sensory characteristics, as well as the overall quality of the hard cheese.

In summer production, the use of animal rennet resulted in a higher VOCs content, approximately three times that of the vegetable rennet ( $p < 0.05$ ) in both the core and sub-crust of the cheese. Carboxylic acids were identified as the main VOCs, pointing to a reduced lipolytic activity in the vegetable rennet samples. In winter production, the performance of animal rennet was only confirmed in the core of the product, while at the sub-crust level the use of vegetable rennet resulted in the formation of more VOCs.

Although vegetable rennet was able to develop a lower aromatic component than animal rennet, it was interesting to note that the maximum VOCs development was reached after 7 months of maturation. This suggests that the vegetable rennet hard cheese samples seem to have a faster biochemical metabolism, indicating that a shorter ripening period might be sufficient for vegetable rennet cheeses. Furthermore, at 9 months of curing period, for both winter and summer production, the qualitative-quantitative VOCs profile of Grana Padano-Type cheeses made with vegetable rennet were comparable to those made with animal rennet.

**Keywords:** GC-MS, hard cheese, rennet type, VOCs

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## G7

### AROMATIC FINGERPRINTING OF VALUABLE PRUNUS SEED OILS FOR THEIR CHARACTERIZATION THROUGH HS-SPME-GC-MS AND GC-IMS ANALYSIS OF VOCS

**Francesca Carra**\*<sup>(1)</sup>, **Lorenzo Cecchi**<sup>(2)</sup>, **Vincenzo Disca**<sup>(1)</sup>, **Yassine Jaouhari**<sup>(1)</sup>, **Matteo Bordiga**<sup>(1)</sup>, **Marco Arlorio**<sup>(1)</sup>

<sup>1)</sup> Department of Pharmaceutical Sciences, Food Chemistry, Biotechnology and Nutrition Unit, Università degli Studi del Piemonte Orientale, Italy

<sup>2)</sup> Department of Agricultural, Food, Environmental and Forestry Science and Technology (DAGRI), Università degli Studi di Firenze, Italy

\*Corresponding author - E-mail: francesca.carra@uniupo.it

Volatile Organic Compounds (VOCs) play a crucial role in determining oils' sensory attributes, influencing their aroma profiles both positively and negatively. In oils, VOCs embrace a wide range of chemical families including aldehydes, ketones, alcohols, esters, hydrocarbons and acids, each conferring specific sensory characteristics. The flavor profile, key determinant of oils' acceptability and quality, is influenced by VOCs, that may originate from the raw materials deriving from different cultivar and geographical origin, or be formed during extraction and subsequent processing steps including deodorization and storage through lipid oxidation pathways. Refining processes, especially deodorization, often involve high-temperature and high-vacuum conditions to remove undesirable flavors. These processes can also impoverish the oil of valuable VOCs, altering sensory attributes and potentially forming degradation compounds [1].

Peach kernel oil (PKO) and apricot kernel oil (AKO), derived from seeds have caught attention for their nutritional and cosmetic properties [2]. Rich in unsaturated fatty acids, vitamins and bioactive compounds, these oils are highly valued in food and skincare applications. Their aroma fingerprint is crucial for their market acceptability and quality.

Headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) and gas chromatography coupled with ion mobility spectrometry (GC-IMS) are useful to identify/quantify VOCs, providing detailed volatile fingerprints of the oils [3]. Aldehydes, that can indicate oxidative degradation, and acrolein, a toxic byproduct used as marker of thermal degradation are usefully detected.

This work aims to characterize the volatile profile of three different apricot and three different peach seed oils using HS-SPME-GC-MS and GC-IMS. The 2D fingerprints obtained by GC-IMS permitted to compare VOC profiles in non-commercial cold-pressed PKO and AKO, identifying key flavor compounds. The most complex VOC's profile for these samples was obtained from peach oil, a finding supported by HS-SPME-GC-MS analysis. Both samples subjected to deodorization showed higher total volatile contents, 78.92 and 78.85 mg/kg respectively for AKO and PKO, due to elevated levels of hydrocarbons and aldehydes. Aldehyde contents were 28.16 and 30.49 mg/kg, respectively for AKO and PKO, as expected from the refining treatment. This process also reduced benzaldehyde, formed by the oxidation of benzyl alcohol, absent in both treated samples. Acrolein (2-propenal) was showed in commercial samples in trace. Highest concentration of benzaldehyde and vanillin were showed by non-commercial cold-pressed PKO, contributing to the typical "almond" aroma. These findings offer valuable insights for food and cosmetic industries to select and process apricot and peach oils to achieve desired sensory attributes.

[1] G. Song et al., 2018.

[2] A. Sharma et al., 2019.

[3] M. Chang et al., 2020.

**Keywords:** volatile organic compounds, prunus seed oil, HS-SPME-GC-MS, oil quality

**Acknowledgement:** Dr. Carrà holds a PhD career grant supported by Next Generation EU-MUR. This work is part of the project NODES which has received funding from the MUR - M4C2 1.5 of PNRR funded by the European Union - NextGenerationEU (Grant agreement no. ECS00000036).

### G8

#### EVALUATING BASIL VARIETIES: SENSORY, CHEMICAL, AND VISUAL ANALYSES FOR PESTO INDUSTRY APPLICATIONS

**Giulia Tapparo**<sup>(1)</sup>, **Erica Liberto**<sup>(2)</sup>, **Cosetta Galamini**<sup>(3)</sup>, **Gianluca Cucco**<sup>(3)</sup>, **Marta Bertolino**<sup>(1)</sup>

<sup>1)</sup> *Dipartimento di Scienze Agrarie, Forestali e Alimentari, Università degli Studi di Torino, Italy*

<sup>2)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>3)</sup> *Fratelli Saclà S.p.a., Italy*

\*Corresponding author - E-mail: [giulia.tapparo@unito.it](mailto:giulia.tapparo@unito.it)

Basil (*Ocimum spp.*, L.), known for its aromatic properties, is cultivated worldwide and is a cornerstone of Mediterranean cuisine. *Ocimum basilicum* L. is an important ingredient in the famous Pesto alla Genovese sauce and it is recognised by the EU as a Traditional Speciality Guaranteed (TSG). Pesto sensory quality, critical for the food industry, is determined by its organoleptic properties, which include colour, aroma, taste and trigeminal/chemesthetic perception. These properties are influenced by a complex interplay of factors, including genetic, ontogenetic and morphogenetic variations in basil, biotic elements and abiotic factors. Cultivar and variety also play a key role in defining the quality characteristics of basil, and their characterisation can lead to the identification and improvement of stable, high quality cultivars with excellent flavour and yield, as well as the selection of basil cultivars suitable for commercial use. To date, the organoleptic properties of basil have been analysed independently of its chemical composition.

The aim of this study was to establish correlations between sensory and visual analyses and specific chemical components of some basil cultivars grown in different Italian regions during their entire seasonal cultivation, in order to obtain a comprehensive objective characterisation. Headspace solid phase microextraction coupled with gas chromatography mass spectrometry (HS-SPME-GC-MS) was used to analyse the volatilome of different varieties of *Ocimum basilicum* L., while colour determination was made using a stand-alone spectrophotometer to quantify colour and monitor its changes in both raw materials and finished products, from the harvesting stage through the production process to the final product. During the sampling of the raw material, several important data were recorded, including the month of harvesting, the geographical area, the number of mowings previously carried out, the abundance of foliage and stems in relation to the total weight, the state of the plant, the presence of inflorescences, as well as other visual and olfactory characteristics. The integration of these data provided a more accurate and complete overview. The application of machine learning to the data collected was used to identify high quality raw materials, define differences at the molecular level between the different varieties analysed and in order to link sensory aspects to chemical composition.

**Keywords:** *Ocimum basilicum* L., quality, profiling, variety characterization, pesto sauce

# FLAVOUR & OFF-FLAVOUR SIGNIFICANT COMPOUNDS

G9

## VOLATILE OFF-FLAVOURS IN PLANT PROTEIN CONCENTRATES

**Maria Filatova**\*<sup>(1)</sup>, **Michal Stupak**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

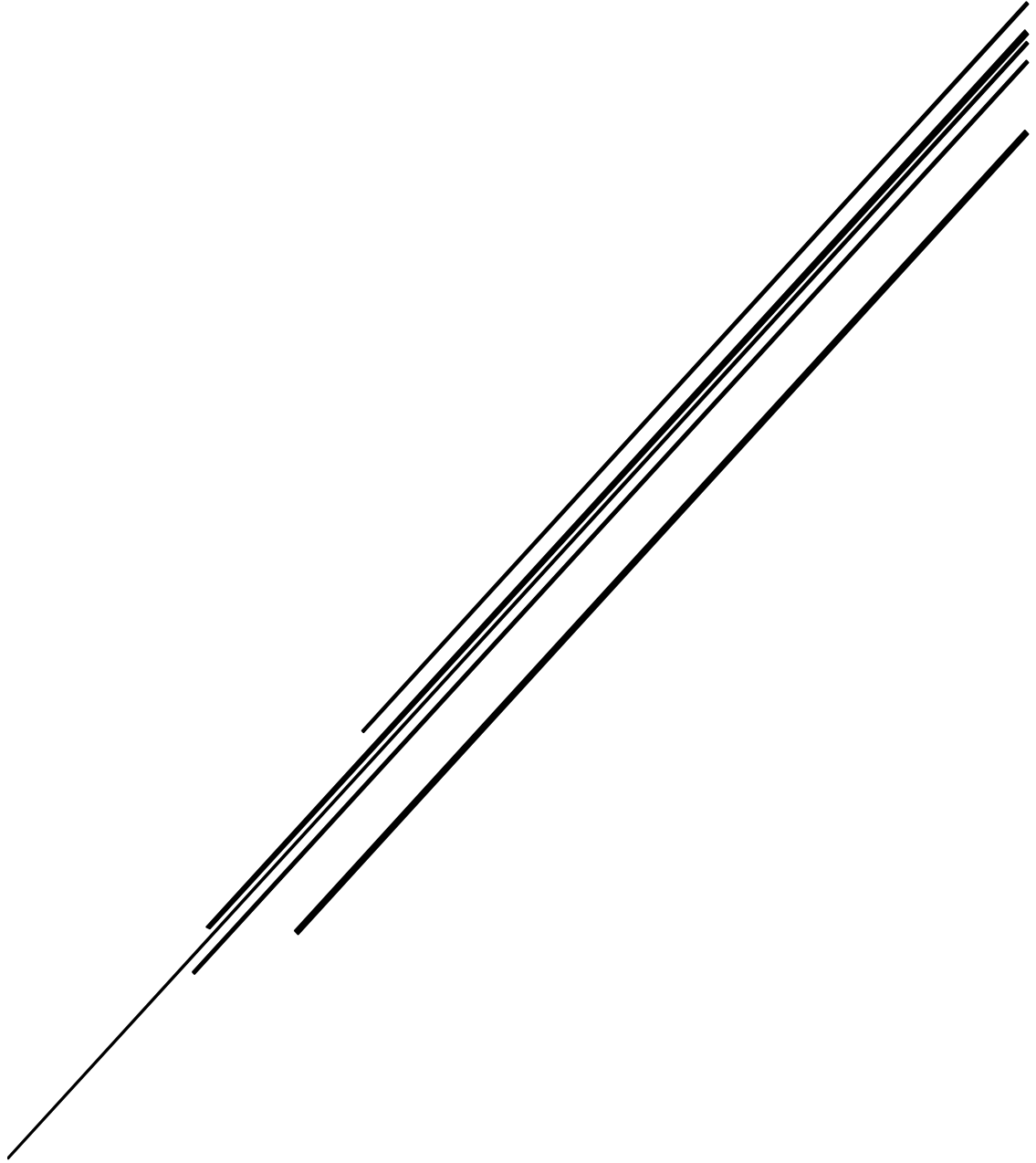
*\*Corresponding author - E-mail: maria.filatova@vscht.cz*

Plant-based proteins are becoming increasingly popular as the food industry addresses the growing global population and the rising demand for sustainable and nutritious protein sources. However, their use is often challenged by an undesirable aroma, typically described as “beany”, “oily” or “green”. These off-notes can originate from plant metabolism or be produced during processing and storage, encompassing a wide range of chemical classes. Since aroma significantly influences consumer acceptance and eating habits, it is crucial to enhance understanding of the key factors contributing to the off flavour in plant proteins. In this pilot study, solid-phase microextraction followed by gas chromatography coupled to high-resolution mass spectrometry (SPME-GC-HRMS) was applied to analyse volatile profiles of 12 samples of commercial plant-based protein concentrates including soy, pear, rice, sunflower and cannabis proteins. The predominant volatiles identified in these products were aldehydes and furans, including hexanal, pentanal, 2-ethylfuran and 2-pentylfuran. Additionally, minor volatiles such as  $\alpha$ -pinene,  $\beta$ -pinene, and  $\beta$ -phellandrene were found in all protein samples except soy protein. Only few volatiles were detected in the sunflower protein. Based on the collected data, dominant volatile compounds were tentatively identified for each type of plant-based protein. Finally, the identified volatiles were semi-quantified, and the results were compared across different protein types.

**Keywords:** plant proteins, volatile off flavour, gas chromatography, high resolution mass spectrometry

**Acknowledgement:** *This work was supported by INTER-COST LUC24117 project (Protein concentrates with favourable sensory properties obtained from oilseeds by ‘green’ technology)*

# FOOD ADDITIVES



## FOOD ADDITIVES

### H1

#### **IDENTIFICATION AND DETERMINATION OF SYNTHETIC COLORS IN FINE BAKERY WARES (CAKES) SOLD IN RIYADH CITY, SAUDI ARABIA: A COMPARATIVE STUDY BETWEEN THE SPECIFICATIONS LAID OUT IN THE GULF STANDARDIZATION ORGANIZATION AND THE EUROPEAN UNION**

**Mazen AlFayez**\*<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: mmfayez@sfda.gov.sa

The aim of this study was to identify and determine of synthetic colors in Fine Bakery Wares (cakes) Sold in Riyadh City, Saudi Arabia and comparing the differences in meeting the specifications laid out in the Gulf Standardization Organization (GSO) and the European Union directive. A hundred and three different samples were purchased from 25 different fine bakery brands in Riyadh city, Saudi Arabia. The samples were Red velvet cake, cupcake, cube cake, mini cake, cheese cake, role cake, and push-up cake. The analysis was performed using reversed phase High Performance Liquid Chromatography with UV detector (HPLC-DAD). The results of the analysis were compared against the Gulf standard (GSO 2500/2015) and the European standard (94/36/EC). Eighty samples (78%) were positive for the presence of food colorants, while 23 samples (22%) were negative for food colorants. Nineteen samples (18%) were non-complied according to the Gulf standard (GSO 2500/2015), while 37 samples (36%) were non-complied to the European standard (94/36/EC). The following synthetic colorants were tested Tartrazine (E102), Quinoline Yellow (E104), Sunset Yellow (E110), Carmoisine (azorubine) (E122), Ponceau 4R (E124), Erythrosine (E127), Allura red AC (E129), Patent Blue V (E131), Indigo carmine (indigotine) (E132), Brilliant Blue (E133), Green S (E142), Fast Green FCF (E143), Brilliant black (E151) and Brown HT (chocolate brown HT) (E155). The detection frequency was (19%), (N.D), (3%), (10%), (N.D), (3%), (36%), (N.D), (2%), (22%), (N.D), (N.D), (1%) and (3%) respectively. In addition, unknown oil soluble color was detected (1%).

**Keywords:** colors

## H2

### DEVELOPMENT OF A HPLC-MS/MS MULTI-METHOD FOR THE ANALYSIS OF FOOD ADDITIVES WITH SPECIAL FOCUS ON SWEETENERS

**Svenja Schneider**<sup>(1)</sup>, **Sabrina Darge**<sup>(1)</sup>, **Susanne Esslinger**<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: [svenja.schneider@bfr.bund.de](mailto:svenja.schneider@bfr.bund.de)

Around 320 food additives, including sweeteners, are approved in the EU and may be used for technological purposes or to improve the organoleptic properties of food. According to Article 27 (1) of Regulation (EC) No 1333/2008, member states should systematically monitor the intake levels and use of food additives on the basis of a risk-based approach.

According to EU Recommendation 2023/965, analytical occurrence data of food additives should be collected [1]. In the BfR MEAL study, a wide range of sweeteners were determined in low calorie or sugar-free soft drinks, and the majority of the soft drinks analyzed contained more than one sweetener, including aspartame, cyclamate and steviol glycosides [2]. According to the EFSA opinion, the exposure of aspartame, acesulfame K and saccharin is particularly relevant in the food categories "flavored fermented milk products" and "soft drinks" for infants, children, adolescents and the elderly [3]. For this reason, it makes sense to develop analytical multi-methods that enable the analysis of additive combinations in different foods. The multi-methods should cover as many food additives in different concentrations as possible, include uniform and simple sample preparation for different foods, enable rapid analysis and provide reliable results [4].

High-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) was chosen because of its high sensitivity and selectivity for the quantitative determination of sweeteners in various foods. The initial focus of the method development was on soft drinks and fermented milk mix products. The aim of this work is to develop one or more HPLC-MS/MS multi-methods in negative and positive ionization mode to include other food additives such as colorings and preservatives at a later stage. In particular, the different polarities of the analytes were taken into account and evaluated by using reversed-phase HPLC and hydrophilic interaction chromatography. To date, a total of 26 sweeteners, including 7 sugar substitutes and 9 steviol glycosides, are included in the method.

[1] EU Recommendation 2023/965 of the Commission from 12<sup>th</sup> of May 2023.

[2] German Federal Institute for Risk Assessment. Opinion No. 006/2023 BfR from 07<sup>th</sup> of February 2023.

[3] European Food Safety Authority (EFSA). EFSA supporting publication 2020; EN-1913, 52 pp.

[4] Detry, P. *et al.* Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment 39, 1349-1364.

**Keywords:** food additives, HPLC, MS/MS, sweeteners, multi-method

## H3

### PHYSICO-CHEMICAL AND SENSORY CHARACTERIZATION OF FRUIT JUICES ENRICHED WITH TANNINS

**Daniela Ghirardello**<sup>(1)</sup>, **Manuela Giordano**<sup>(1)</sup>, **Giuseppe Zeppa**<sup>(1)</sup>, **Samuele Giovando**<sup>(2)</sup>, **Michele Battaglia**<sup>(2)</sup>

<sup>1)</sup> *Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy*

<sup>2)</sup> *Silvateam Spa, Italy*

*\*Corresponding author - E-mail: daniela.ghirardello@unito.it*

The global fruit and vegetable juice market is experiencing significant growth, largely due to evolving consumer preferences that favour natural and organic products.

As a result, there is a growing emphasis on the development of functional or nutraceutical beverages with health-promoting properties and good shelf-life. An aid, in this sense, can come from the addition of natural, particularly plant-based, antioxidants which can prove advantageous in enhancing the stability of the product, even during pasteurisation and extended storage. The addition of plant polyphenols, with notable antioxidant properties, could improve the nutritional profile of juices while also facilitating their stabilization.

The present study examined the physico-chemical and sensory properties of eight commercial tannins in comparison with ascorbic acid on different fruit juices.

The study was conducted on homogeneous batches of six juices (raspberry, blueberry, apple, pear, peach and apricot) from a local producer. Prior to bottling and pasteurisation, ascorbic acid or tannins were added to the juices. A preliminary sensory test was conducted for each juice to identify the tannin that would allow the highest dose of addition (0.5 g/L) while maintaining overall product acceptability. The dose of ascorbic acid capable of expressing equivalent antioxidant capacity was then estimated for each tannin. A non-treated batch was used as the reference for each juice. The juices were subjected to analysis immediately following the bottling process (prior to and after pasteurisation) and over a four-month of storage, with samples collected and analysed monthly. During this period, the juices were stored at 20 °C.

Total polyphenol content, DPPH radical scavenging activity, CIE L\*a\*b\* chromatic coordinates, pH and sensory differences with a Duo-Trio test were evaluated.

Our results showed that fruit juices with added tannins had a higher antioxidant capacity than untreated juices, as hoped, but lower than or in some cases equivalent to the antioxidant capacity of juices with added ascorbic acid. During storage, the antioxidant capacity varied rather little, with different trends for the different samples, but generally showing a substantial stability of the products.

Tannins enrichment resulted in colour variations, but only in some cases the difference was perceptible to the human eye.

In terms of sensory properties, there were no significant differences between the samples in most of the comparisons, particularly for juices with a pronounced sensory impact, such as raspberry and blueberry juices.

The study showed that ascorbic acid can be replaced by commercial tannins without any notable impact on the sensory profile of the products. Furthermore, this substitution maintains the antioxidant potential of the products while also preserving the nutritional benefits associated with phenolic compounds.

**Keywords:** functional fruit juices, antioxidants, tannins, ascorbic acid

**Acknowledgement:** *The authors thank the "Silvateam Spa", San Michele Mondovì (CN - Italy) for kindly providing the tannins. Furthermore, thanks are extended to the agricultural cooperative, "Il Frutto Permesso", Bibiana (TO, Italy) whose processing facilities were used to prepare the juices.*



# FOOD ADDITIVES

## H4

### ADDED PHOSPHATES ANALYSIS IN FOOD: A NON COMPLETELY SOLVED ISSUE YET

Josep Calderon<sup>\*(1)</sup>, Sonia Abuin<sup>(1)</sup>, Soledad Soler<sup>(1)</sup>, Matilde Alvarez<sup>(1)</sup>, Víctor Pavon<sup>(1)</sup>, David Samblas<sup>(1)</sup>

<sup>1)</sup> *Agència de Salut Pública de Barcelona (ASPB), Spain*

*\*Corresponding author - E-mail: jcaldero@aspb.cat*

Added phosphates in food serve several important functions as texture improvement, moisture retention, emulsification, preservation and colour enhancement. Nevertheless, while phosphates are essential nutrients, excessive intake from food additives can pose health risks, especially for individuals with kidney issues.

The maximum permitted content of these additives (E338 - E452, phosphoric acid, orthophosphates, diti and polyphosphate) is regulated by EU 1333/2008, for those foods in which their use is allowed. The problem lies in finding a reliable method of analysis, which stabilizes the compound and can differentiate the added phosphate content from the endogenous content of the sample itself. In fact, there is not an official method for measurement of 'added' phosphates yet.

There are approximate methods that use certain theoretical tabulated values, with which it is not easy to calculate a reliable concentration of added phosphates, the validation of the method is more complicated as well as its accreditation (ISO 17025) and its use in official control.

This study aims to develop and validate 2 different methods of analysis for added phosphates, showing the advantages and limitations. One based on ion chromatography for the most used (E450, E451 and E452) and another that include all kind of added phosphates, based on the phosphate/protein ratio using ICP-MS for total phosphate determination and Kjeldahl for protein content.

Those methods are currently being used in the Public Health Agency of Barcelona (ASPB) within official control programs and it has been included in the scope of the accreditation following ISO/IEC 17025 requirements.

**Keywords:** added-phosphates, method validation, polyphosphates analysis in food

### H5

#### EXPANDING THE BACTERIOPHAGE ARSENAL FOR ALICYCLOBACILLUS SPOILAGE PREVENTION IN THE FOOD INDUSTRY

Helena Ferreira<sup>(1)</sup>, Ines Carvalho Leonardo<sup>(1)</sup>, Ana Patricia Quendera<sup>(1)</sup>, Maria Teresa Barreto Crespo<sup>(1)</sup>, Carlos Sao-Jose<sup>(2)</sup>, **Frederic Bustos Gaspar**\*<sup>(1)</sup>

<sup>1)</sup> iBET, Instituto de Biologia Experimental e Tecnológica & Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

<sup>2)</sup> PhaBRIC lab, Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal

\*Corresponding author - E-mail: fgaspar@ibet.pt

The food and beverages industry faces numerous spoilage events, leading to food waste, product recalls, economic losses, and the erosion of consumer confidence. Part of these spoilage events are due to the production of off-flavour that have been correlated to the presence of isolates from the *Alicyclobacillus* (ACB) genus. ACB threatens the agri-food industry since they resist acidic environments, form endospores, endure pasteurisation, and germinate during shelf-life. Traditional methods are ineffective against ACB and require new approaches.

Novel biopreservation systems using bacteriophages are solutions that fulfil both the industry's and the consumer's requirements. They are targeted, effective, natural, and green technologies, compliant with organic and vegan claims, have no effect on taste, colour, or smell, and do not require labelling. Although a temperate phage was recently described [1], no strictly lytic phage for the biocontrol and biopreservation of spoilage ACB in food and beverages has yet been found.

In this study, a phage capable of infecting ACB isolates was isolated when an environmental sample of grapevine soil was enriched with *Alicyclobacillus acidoterrestris* DSM 3922<sup>T</sup> [2]. Subsequent purification and clonal amplification cycles were performed using a different *A. acidoterrestris* strain, isolated from a spoiled fruit-based product. The isolated phage was cryopreserved and phenotypically characterised for pH stability, thermal stability, and bacteriolytic activity toward different ACB isolates. Molecular characterisation using next-generation sequencing was used for taxonomy elucidation and functional analysis to ensure its safe use in the food industry.

In addition to improving the scarce biopreservation arsenal towards ACB, this newly isolated phage will contribute to future phage-based applications. These may include direct or indirect use of the phage in (i) ACB detection methods, (ii) molecular tools for bacterial gene editing, or (iii) as a source of antimicrobial lysins.

[1] <https://doi.org/10.3390/genes14061303>.

[2] <https://doi.org/10.1093/g3journal/jkac225>.

**Keywords:** Alicyclobacillus, bacteriophage, spoilage, biopreservation, agri-food industry

**Acknowledgement:** This work was funded by FCT/MCTES (Portugal) through national funds to iNOVA4Health (UIDB/04462/2020 and UIDP/04462/2020), the Associate Laboratory LS4FUTURE (LA/P/0087/2020), and the phages4ACB project (2022.04043.PTDC). ICL acknowledges FCT for PhD scholarship 2020.08210.BD.

### H6

#### EXPLORING THE ANTIBACTERIAL POTENTIAL OF A GEOBACILLIN-26 HOMOLOG FROM *ALICYCLOBACILLUS ACIDOTERRESTRIS* DSM 3922T: A NOVEL APPROACH TO COMBAT SPOILAGE IN FOOD PRODUCTS

Ines Carvalho Leonardo<sup>(1)</sup>, Ana Patrícia Quendera<sup>(1)</sup>, Helena Ferreira<sup>(1)</sup>, Maria Teresa Barreto Crespo<sup>(1)</sup>, Carlos Sao-Jose<sup>(2)</sup>, Frederic Bustos Gaspar<sup>\*(1)</sup>

<sup>1)</sup> iBET, Instituto de Biologia Experimental e Tecnológica & Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Portugal

<sup>2)</sup> PhaBRIC lab, Research Institute for Medicines (iMed.Ulisboa), Faculdade de Farmácia, Universidade de Lisboa Lisboa, Portugal, Portugal

\*Corresponding author - E-mail: fgaspar@ibet.pt

*Alicyclobacillus* (ACB) spp. are Gram-positive bacteria linked to spoilage events in the food industry, namely in fruit-based products. Some of these bacteria produce off-flavours and odours, compromising product quality. ACB's resilience in acidic environments and during pasteurisation is due to its thermoacidophilic nature and spore-forming ability.

Different strategies to eliminate ACB from food matrices have been explored, including bacteriocins. Bacteriocins are ribosomally synthesised peptides/proteins produced by bacteria, with antimicrobial activity against other bacteria, usually closely related to the producer strain. Despite promising findings on ACB inhibition by bacteriocins from other Gram-positive bacteria, only a few from thermophilic bacteria have been thoroughly characterised [1].

In previous work, a homolog of geobacillin-26 was annotated in the *A. acidoterrestris* DSM 3922<sup>T</sup> genome [2]. Geobacillin-26, produced by *Geobacillus stearothermophilus*, is a recently characterised heat-labile, high molecular weight antibacterial protein classified as a class III bacteriocin, with a narrow antibacterial spectrum targeting other thermophilic bacteria [3].

This study aimed to further characterise the geobacillin-26 homolog in *A. acidoterrestris* DSM 3922<sup>T</sup>. The supernatant from *A. acidoterrestris* DSM 3922<sup>T</sup> exhibited inhibitory activity against other *A. acidoterrestris* isolates. Proteomic analysis confirmed the presence of the geobacillin-26-like protein in the supernatant. Subsequently, the protein was heterologously expressed in *Escherichia coli* to determine its antibacterial activity against various ACB species, including isolates from food products. The stability of the recombinant geobacillin-26-like protein in different conditions was also evaluated.

This work demonstrates that naturally occurring antibacterial agents, like bacteriocins and this geobacillin-26 homolog, hold significant potential for use in industries concerned with ACB contamination of food matrices.

[1] <https://doi.org/10.4315/0362-028X.JFP-12-496>.

[2] <https://doi.org/10.1093/g3journal/jkac225>.

[3] <https://doi.org/10.1016/j.ijbiomac.2019.09.047>.

**Keywords:** agri-food industry, *Alicyclobacillus*, spoilage, bacteriocin, biopreservation

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## FOOD ADDITIVES

### H7

#### QUANTITATIVE LC ANALYSIS OF RARE SUGARS IN FOOD

**Vadim Kraft**<sup>(1)</sup>, **Annika Malz**<sup>(1)</sup>, **Uwe Oppermann**<sup>(1)</sup>

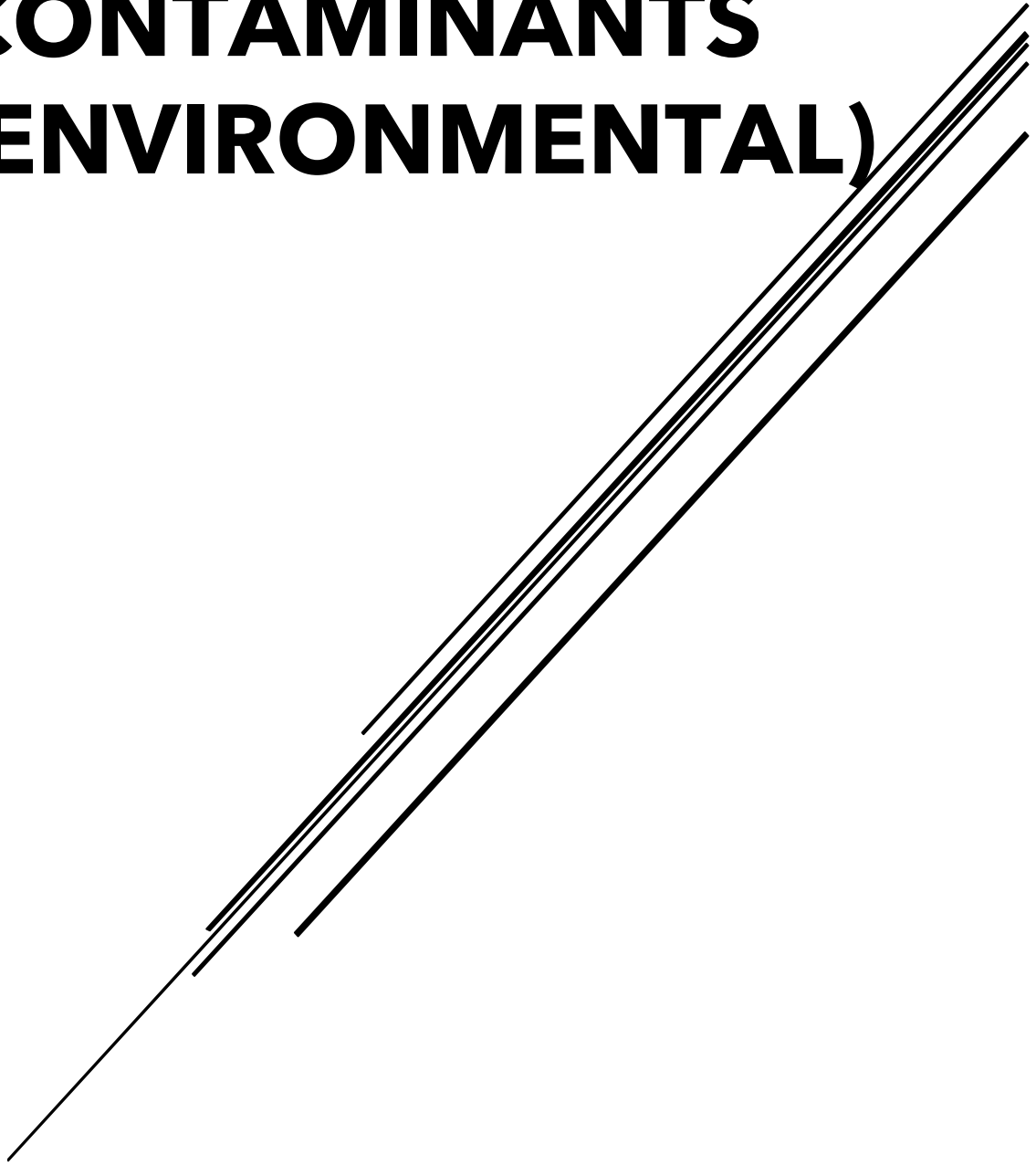
<sup>1)</sup> Shimadzu Europa GmbH, Germany

\*Corresponding author - E-mail: vkr@shimadzu.eu

Sugar and sweet foods are culturally significant, enhancing flavors and providing energy, often associated with celebrations. However, health effects vary; excessive sugar consumption is linked to obesity and diabetes, making moderation essential. Natural sugars from fruits are healthier options. Rare sugars, found in small amounts in certain foods, offer unique benefits and can help reduce caloric intake or manage blood sugar, although they are less common than traditional sweeteners. In the EU, rare sugars are regulated under the Novel Foods Regulation, requiring safety assessments by the European Food Safety Authority (EFSA) and clear labelling of ingredients and health claims. Accurate quantification of rare sugars is important for ensuring safety compliance, labelling accuracy, health claim verification, market research, nutritional studies, and fostering consumer trust. Overall, reliable measurement supports safety, compliance, and advances in nutritional science. To develop an effective method, three sweetened commercial products containing Psicose were analyzed. Additionally, Tagatose, Glucose and Sucrose were included in the analysis, as they were present in some of the samples. The chosen methodology utilized a HILIC column, with detection achieved through an ELSD LT-III system (evaporative light scattering detection).

**Keywords:** rare sugars, liquid chromatography, ELSD

# **FOOD CONTAMINANTS (ENVIRONMENTAL)**



### I1

## PRELIMINARY RISK EXPOSURE ASSESSMENT OF CONTAMINANTS OF EMERGING CONCERN THROUGH THE CONSUMPTION OF TAP WATER

Luis Muniz-Bustamante\*<sup>(1)</sup>, Noelia Caballero-Casero<sup>(1)</sup>, Soledad Rubio<sup>(1)</sup>

<sup>1)</sup> University of Córdoba, Spain

\*Corresponding author - E-mail: t52mubul@uco.es

The ongoing overproduction of chemicals results in the release of thousands of compounds into the environment. Research has shown that wastewater effluents contain tens of thousands of organic pollutants, underscoring the challenge of completely removing all contaminants in wastewater treatment plants. Chemical and toxicological data are limited or unknown for many of these compounds, classifying them as chemicals of emerging concern (CEC). These effluents are discharged into various water bodies, where the presence of CECs has also been detected. Since these water bodies are often sources for drinking water production, which involves targeted methods to remove specific organic pollutants, CECs can end up in tap water.

CECs exhibit a wide range of polarity and chemical properties, rendering traditional extraction techniques, such as solid phase extraction or liquid-liquid extraction inefficient for the multi-extraction of CECs. Furthermore, while traditional target approaches allow the detection and determination of known chemical compounds based on their correspondence with analytical standards, suspect and non-target approaches, mainly based on liquid chromatography high-resolution mass spectrometry (LC-HRMS), allow to detect and identify thousands of chemicals without standards.

To address the limitations of traditional extraction and identification methods for CECs, this study suggests using supramolecular solvents (SUPRAS) for extracting CECs from tap water, followed by analysis with LC-HRMS. Tap water samples (n=53) from 12 countries worldwide were analysed using the developed suspect screening workflow. Twenty-nine CECs from categories such as personal care products, stimulants, surfactants, industrial chemicals, plasticizers and cleaning products, were identified in the samples. The concentration of chemicals identified with a confidence level above 2b was semi-quantified, and a tentative risk assessment of human health was performed. Some identified compounds had hazard quotient (HQ) values above 1, indicating a potential risk to human health. These findings demonstrate that combining SUPRAS with suspect screening analysis can effectively discover new contaminants and human exposure sources.

**Keywords:** tap water, contaminants of emerging concern, new source of exposure, LC-HRMS, risk exposure assessment

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### 12

## FLUORINATED FEATURES IN FRESHWATER FISH: UNCOVERING UNKNOWN PFAS USING NON-TARGETED SCREENING AND MASS BALANCE EVALUATION

**Bas Van Dijk**\*<sup>(1)</sup>, Rosalie Nijssen<sup>(1)</sup>, Ruben Kause<sup>(1)</sup>, Federico Padilla Gonzalez<sup>(1)</sup>, Stefan Van Leeuwen<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: bas1.vandijk@wur.nl

Per- and polyfluoroalkyl substances (PFASs) are a large group of persistent substances that are omnipresent in the environment and food chain. Targeted LC-MS/MS analysis is widely applied, but generally covers only a small number (20+) of the PFASs. Comprehensive PFAS analysis overcomes this limitation. Combustion Ion Chromatography (CIC) can provide insights into extractable PFASs (measured as total organic fluorine), providing a total mass. When the total organic fluorine content and LC-MS/MS results are not in agreement, a mass balance gap exists, suggesting the presence of unknown/unidentified PFASs. A non-targeted screening (NTS) method by LC-HRMS can be used to detect the missing PFASs. In this study, we applied this comprehensive approach to fillets and livers of contaminated freshwater fish (eel) samples, finding a gap in mass balance and identifying new PFASs using the NTS method.

Eel samples were collected as part of the Dutch national monitoring program. The fillet and liver samples were extracted with methanol, followed by clean-up using Strata-X-AW SPE. The cleaned extracts were measured using LC-HRMS (Orbitrap IQ-X Tribrid Mass Spectrometer). Full-scan MS1 detection was used at a mass range of 130-1250 m/z. In addition, top n data-dependent MS2 (ddMS2) high-resolution spectra were recorded in parallel with low-resolution ddMS2 ion-trap spectra with a cycle time of 1 s.

The data was processed using Compound Discoverer with a customized PFAS workflow. The dataset was filtered using various techniques. The mass defect over expected carbon ratio (MD/C) and mass over carbon ratio (M/C) played a major role in distinguishing the PFAS features from other features, by reducing the number of signals from over 10,000 to approximately 300. The Kendrick Mass Defect (KMD) and the use of in-house and online libraries made identifying some of the selected features possible.

Several PFAS sub-groups were detected in the samples. The presence of PFCAs (carboxylic acids) and PFASs (sulfonic acids) was expected based on earlier targeted analysis of eel samples. However, previously unreported PFAS groups, such as FTs (telomer sulfonic acids), H-PFCAs (hydrogen substituted carboxylic acids), FASAs (sulfonamides), PFECAs (ether carboxylic acids) and FASAAs (sulfonamidoacetic acids) were also identified. Additionally, the pesticide fipronil and its metabolite fipronil sulfone (both containing two CF<sub>3</sub> groups) were annotated. Level 1 identification was achievable for approximately 30 PFASs. Lower confidence levels were achieved for other compounds, due to the lack of reference standards and library limitations, some of which were semi-quantified, further reducing the mass balance gap. This study demonstrates that comprehensive PFAS approaches, including total organic fluorine analysis and NTS, can uncover previously unknown PFASs in environmental and food samples.

**Keywords:** PFAS, total organic fluorine, LC-HRMS, non-targeted screening

**Acknowledgement:** The Dutch Ministry of Agriculture, Nature and Food Quality is gratefully acknowledged for financial support of the study (KB-37-001-024). The technicians taking care of sampling and chemical analysis, are gratefully acknowledged.

### I3

## DIETARY EXPOSURE ASSESSMENT TO INORGANIC ARSENIC AND SMALL ORGANOARSENIC SPECIES IN THE EUROPEAN POPULATION

Jose Angel Gomez Ruiz\*<sup>(1)</sup>

<sup>1)</sup> European Food Safety Authority (EFSA), Italy

\*Corresponding author - E-mail: joseangel.gomezruiz@efsa.europa.eu

The EFSA CONTAM Panel recently conducted dietary exposure assessments to inorganic arsenic (iAs) and small organoarsenic species [(monomethylarsonic acid MMA(V) and dimethylarsinic acid DMA(V)] in the European population as part of the risk assessments carried out on these substances (EFSA CONTAM Panel, 2024a,b).

For iAs, the highest mean dietary exposure at the lower bound (LB) was estimated in 'Toddlers' (0.30 µg/kg bw per day), and in both 'Infants' and 'Toddlers' (0.61 µg/kg bw per day) at the upper bound (UB). At the 95th percentile, the highest exposure estimates (LB-UB) were 0.58 and 1.20 µg/kg bw per day in 'Toddlers' and 'Infants', respectively. Overall, the main contributors to the dietary exposure were 'Rice', 'Rice-based products', 'Grains and grain-based products (no rice)' and 'Drinking water'. Foodstuffs for the young population also made a relevant contribution in the dietary exposure to iAs in this age group. Breastfeeding in infants would lead to much lower exposure to iAs compared to the consumption of rice-based formulae. A Reference Point (RP) of 0.06 µg iAs/kg bw per day (BMDL05) was derived based on a case-control study on skin cancer. In adults, the Margin of Exposure (MOE) ranged between 2 and 0.4 for average consumers and between 0.9 and 0.2 at the 95th percentile exposure. The EFSA CONTAM Panel concluded that dietary exposure to iAs raises a health concern in the European population (EFSA CONTAM Panel, 2024a).

For small organoarsenic species, the highest DMA dietary exposure was estimated in 'Toddlers', with LB-UB mean exposures of 0.13–0.16 µg As/kg bw per day and LB-UB 95th percentile exposures of 0.40–0.48 µg As/kg bw per day. 'Rice' and 'Fish meat' were the main contributors to DMA exposure across population groups and, additionally, 'Food products for young population' for part of the young population ('Infants', 'Toddlers'). For MMA, the highest exposures were estimated for high consumers of 'Fish meat' in 'Infants' and high consumers of processed/preserved fish in the 'Elderly' age class, in both cases with MMA estimates of 0.34 µg As/kg bw per day. A BMDL10 of 0.6 mg As/kg bw per day (based on decreased body weight in rats resulting from diarrhoea) and a BMDL10 of 9.7 mg As/kg bw per day (based on increased total urinary bladder tumour incidence in male rats) were identified as RP for DMA(V) and MMA(V), respectively. For DMA(V), MOEs were below 10,000 in many cases across dietary surveys and age groups, in particular for some 95th percentile exposures; the EFSA CONTAM Panel concluded that the dietary exposure to DMA(V) raises a health concern in the European population. No health concerns were identified in the European population from their dietary exposure to MMA(V).

The EFSA CONTAM Panel is currently working on the risk assessment of other organoarsenic compounds, e.g., arsenobetaine, arsenosugars, arsenolipids; this risk assessment is expected to be published in early 2025.

**Keywords:** risk assessment, dietary exposure, inorganic arsenic, small organoarsenics, European population

**Acknowledgement:** To the EFSA WG on Arsenic in Food and to the EFSA Scientific Panel on Contaminants in the Food Chain as responsible of the risk assessments conducted for inorganic arsenic and small organoarsenic species in food.



### 14

#### REGENERATION OF MAGNETIC NANOSTRUCTURED MATERIALS FOR REPEATED USES TO REMOVE CYANOTOXINS FROM WATER

**Alejandro Cao**<sup>(1)</sup>, **Natalia Vilarino**\*<sup>(1)</sup>, **Lisandra De Castro Alves**<sup>(2)</sup>, **Jose Rivas**<sup>(2)</sup>, **Yolanda Pineiro**<sup>(2)</sup>, **Luis Rodriguez-Santos**<sup>(1)</sup>, **M. Carmen Louzao**<sup>(1)</sup>, **Sandra Raposo-García**<sup>(1)</sup>, **Luis M. Botana**<sup>(1)</sup>

<sup>1)</sup> *Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain*

<sup>2)</sup> *Departamento de Física Aplicada, Facultad de Física, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain*

\*Corresponding author - E-mail: natalia.vilarino@usc.es

Water may contain numerous contaminants that can harm both human and animal health, including naturally occurring cyanotoxins. The presence of these toxins has increased in recent years due to climate change and eutrophication. While water treatment stations are essential before consumption, no existing method can completely remove cyanotoxins from water. Therefore, cyanotoxins are currently considered a public health threat considering that they may cause acute and chronic intoxication in humans. A reusable method for toxin removal is investigated as complementary to existing water treatment procedures. Toxin removal is achieved by adsorption to magnetic nanostructured composites that have mesoporous carbon as adsorption material to remove microcystins (MCs) or activated carbon to remove cylindrospermopsin (CYN) and anatoxin-A (ATX-A). In this study effectiveness of these nanostructured particles for toxin adsorption from water for several adsorption/desorption cycles and their performance in water samples from a drinking water treatment plant was evaluated.

Adsorption experiments consisted in the incubation for 120 min of nanostructured particles with water samples that contained a known concentration of the toxins. Then the toxin concentration remaining in solution was quantified by UHPLC-MS/MS to calculate toxin removal efficiency. Optimal particle regeneration for further adsorption was obtained by a 60 min treatment with 75% acetonitrile for both materials. Activated carbon and mesoporous carbon nanostructured composites removed cyanotoxins during 9 adsorption/desorption cycles without significant loss of efficiency. Cyanotoxin removal by mesoporous and activated carbon particles was also tested in samples collected from a drinking water plant at two different points after the coagulation/flocculation/sedimentation process, and no differences were observed versus Milli-Q water controls. In addition, toxicity of water incubated with these materials for 120 min was tested with zebrafish embryos. No toxic effect was detected in embryos cultured for 5 days in water pre-exposed to whole or pulverized particles.

In conclusion, the use of magnetic nanostructured composites with activated carbon or mesoporous carbon as adsorption materials can be used to remove cyanotoxins from water at different stages of processing through a water treatment plant. Reusability improves cost-effectiveness of this method, while the absence of toxic effects in zebrafish embryos supports its safety for humans and the environment.

**Keywords:** magnetic nanoparticles, cyanotoxins removal, drinking water safety

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### 15

#### OCURRENCE OF QUATERNARY AMMONIUMS IN FOODSTUFFS OF ANIMAL ORIGIN: 3RD FRENCH TOTAL DIET STUDY

Kahina Slimani\*<sup>(1)</sup>, Annaëlle Chailleux<sup>(1)</sup>, Dominique Hurtaud-Pessel<sup>(1)</sup>

<sup>1)</sup> ANSES, France

\*Corresponding author - E-mail: kahina.slimani@anses.fr

French total diet studies (TDS) are carried out at a national scale and have the primary objective to monitor the exposure of populations to chemical substances present in foods prepared "as consumed" and to address the issue of nutritional and health risk assessment. For the third TDS currently in progress, the French authorities have decided to include the measurement of quaternary ammonium biocides for the first time in order to generate occurrence data to assess the French population's exposure to this class of contaminants.

In fact, Quaternary Ammonium Compounds (QACs) are biocides substances widely used as disinfectants, preservatives and pest control products, as well as antifouling and taxidermy products. They can be present in the composition of disinfection products for professional and domestic use (e.g. Benzalkonium chlorides (BACs), dialkyldimethylammonium chlorides (DDACs), alkyldimethyl(ethylbenzyl) ammonium chlorides (ADEBACs)), but also in products used as wood preservatives (alkyltrimethylammonium chlorides (TMAC)). Due to their wide use, particularly in the agri-food industry, these biocidal substances can unintentionally end up in foodstuffs, following their use during the disinfection of surfaces but also in food storage areas. In 2023, maximum residue levels (MRL) have been re-evaluated, but only apply to BACs and DDACs. MRLs are set at 100 µg/kg except for products of plant origin. However, to date, there are no MRLs for processed foods. This demonstrates the importance of having occurrence data for all food categories.

To achieve this objective, analytical methods have been developed to quantify 18 QACs in foodstuffs of animal origin (dairy products, meat and seafood products) from the TDS sampling. QACs were extracted from sample with a mixture of acetonitrile and ethyl acetate. For meat and seafood products, a dispersive purification step with primary and secondary amines (PSA) has proved useful for removing interferents from the matrix. Finally, the extract was reconstituted in methanol. The analysis was performed by isotope-dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Thermo® TSQ Vantage triple quadrupole operating in positive electrospray mode. The method has been validated according to standard NF V03-110 and document No. SANTE/11312/2021, guidelines relating to the validation of quantitative analysis methods in food products. Method performance criteria (e.g. limit of quantification (LOQ), repeatability and reproducibility, trueness) were evaluated using the total error approach with e.noval® 4.1 software. Results of validation will be presented and discussed. This method was applied to quantify the 18 QACs in 108 dairy products samples and 93 meat and seafood products samples from the 3<sup>rd</sup> French TDS. Results obtained will be presented and discussed.

**Keywords:** biocide, French total diet study, food contaminants, occurrence, quaternary ammoniums

### 16

#### QUANTIFICATION OF PFAS IN RICE AND MAIZE: VALIDATION OF A UHPLC-HRMS/MS ISOTOPIC DILUTION APPROACH IN SUPPORT TO FOOD SAFETY

**Consolato Schiavone**<sup>\*(1)</sup>, **Francesco Romaniello**<sup>(1)</sup>, **Andrea Mario Rossi**<sup>(2)</sup>, **Chiara Portesi**<sup>(1)</sup>, **Rosa Avolio**<sup>(3)</sup>, **Maria Cesarina Abete**<sup>(3)</sup>

<sup>1)</sup> *Istituto Nazionale di Ricerca Metrologica, Italy*

<sup>2)</sup> *National Metrology Institute of Italy - INRiM, Italy*

<sup>3)</sup> *Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy*

*\*Corresponding author - E-mail: c.schiavone@inrim.it*

In the present work, an analytical method for the quantification of per and poly fluoroalkyl substances (PFAS) in rice and maize has been developed and then validated with a metrological approach. PFAS are a group of human-made chemicals used in a variety of industries and consumer products for their water- and grease-resistant properties. Studies have shown that PFAS can contaminate soil and water, and there is concern about their bioaccumulation in edible plants, fruits, and cereals. The presence of PFAS has been identified in rice and other food products, including maize, as indicated by studies and scientific literature. This is particularly alarming since some PFAS have been associated with adverse health effects and rice and maize account for over 20% of the annual food intake worldwide. Despite this evidence, the regulation currently in place is not covering cereal matrices and limits of quantification for matrices encompassed by the current legislation are defined for a small group of PFAS. In this study an UHPLC-HRMS/MS based method was validated, obtaining a LOQ (Limit of Quantification) ranging between 2 ng/kg and 32 ng/kg and robustness in line with EU guidelines and recommendation for PFAS in food. Additionally, a metrological approach was employed to estimate the uncertainty budget, utilizing modeling and experimental methods, and comparing the outcomes, aiming to characterize with high accuracy PFAS in rice and maize and support control bodies to assess contamination in suspected areas. A comparison of uncertainty of different approaches was conducted after applying the method to 30 real samples.

**Keywords:** PFAS, food safety, UHPLC-HRMS/MS, rice and maize, food contamination

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### 17

## SIMULTANEOUS DETERMINATION OF C10-17 POLYCHLORINATED ALKANES IN FOOD BY A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

**Laura Lazdiņa**\*<sup>(1)</sup>, **Ingus Perkons**<sup>(2)</sup>

<sup>1)</sup> Institute of Food Safety, Animal Health and Environment & BIOR, Latvia & Faculty of Medicine and Life Sciences, University of Latvia, Latvia

<sup>2)</sup> Institute of Food Safety, Animal Health and Environment & BIOR, Latvia

\*Corresponding author - E-mail: [laura.lazdina@bior.lv](mailto:laura.lazdina@bior.lv)

Polychlorinated n-alkanes (PCAs) are a complex group of persistent substances that make up most chlorinated paraffin (CP) mixtures. CP mixtures are widely produced and used as plasticizers and lubricants in packaging and appliances, causing environmental and food contamination concerns [1]. Reports say that long-term exposure to PCAs could be toxic or even lethal to living organisms, causing oxidative stress, disturbances in energy metabolism and even cancer [2]. Homologues of one group (PCAs-C<sub>10-13</sub>) are classified as persistent organic pollutants (POPs); however, due to similar properties, studies suggest that their replacements (i.e. PCAs-C<sub>14-30</sub>) could also potentially be toxic to living organisms. Therefore, the applications of PCAsC<sub>14-30</sub> could be restricted, and this would cause a need for practical, reliable and widely accessible analysis methods [3].

This study aimed to develop a liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) method for PCA analysis in food products. Various factors were taken into consideration during the development process. Initially, we evaluated five analytical columns and different gradient settings to identify the most suitable stationary phase and gradient parameters for achieving adequate separation between homologues with overlapping mass and charge ratios. Subsequently, we optimized the parameters and homologue fragmentation paths for tandem mass spectrometry. A comparative analysis was conducted to verify method performance. Reference materials such as fish tissue from the Joint Research Center were used to evaluate the method. In addition, various food samples from different matrices were analyzed to study the occurrence of PCAs in food. The results from the developed LC-ESI-MS/MS method were then compared with those obtained from the liquid chromatography-electrospray ionization-high resolution mass spectrometry (LC-ESI-HRMS) method.

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[3] van Mourik, L. M. et al. Chlorinated Paraffins in the Environment: A Review on Their Production, Fate, Levels and Trends between 2010 and 2015. *Chemosphere* 2016, 155, 415-428. <https://doi.org/10.1016/j.chemosphere.2016.04.037>.

**Keywords:** polychlorinated alkanes, LC-ESI-MS/MS, food contaminants

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### 18

#### HIGH METAL CONTENT IN MUSHROOMS OF THE GENUS MORCHELLA GROWN AFTER A FIRE

**Elena Ortega Caneda<sup>(1)</sup>, Estrella Espada Bellido<sup>\*(1)</sup>, Alejandro R. Lopez<sup>(1)</sup>, Damiano Monticelli<sup>(2)</sup>, José Gerardo Lopez Castillo<sup>(3)</sup>, Miguel Palma Lovillo<sup>(1)</sup>, Gerardo F. Barbero<sup>(1)</sup>**

<sup>1)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, 11510 Puerto Real, Cadiz, Spain, Spain

<sup>2)</sup> Department of Science and High Technology, University of Insubria, via Valleggio 11, Como 22100, Italy., Italy

<sup>3)</sup> Unidad de Protección de la Salud, Distrito Sanitario Granada-Metropolitano, Consejería de Salud y Familias, Junta de Andalucía, 18150 Gojar, Spain., Spain

\*Corresponding author - E-mail: [estrella.espada@uca.es](mailto:estrella.espada@uca.es)

Among the edible mushroom species, the genus *Morchella* is well known for its high gastronomic and commercial value, considered a luxury and very difficult to obtain. After the last fire in Sierra Bermeja (Malaga, Spain) in 2021, there was an explosive fructification of *Morchellas*, which were collected in large quantities and ended up being consumed and marketed without control or traceability. This specific area is characterised by a mineral called peridotite with a characteristic metal composition; however fire is capable of altering the physical and chemical properties of the soil, thus affecting the mineral composition of the mushrooms grown here. Therefore, the present study aims to determine the mineral content of Fe, Mg, K, Ca, Al, Cu, Zn, Cr and Ni, in twelve samples of mushrooms of the genus *Morchella*, and their respective fruiting soils, collected from burnt and non-burnt locations. FAAS, ICP-OES and ICP-MS were used for the determination of the metals after acid digestion. The results showed that the samples from the burnt area of Sierra Bermeja have considerably higher levels of Fe, Mg, Al, Cr and Ni than samples from other locations. No differences in Ca, K, Cu and Zn content were observed among the locations studied. The estimated daily intake of metals (EDIM) and the health risk index (HRI) conclude that the samples from the area affected by the Sierra Bermeja fire pose a risk to human health with respect to their Fe, Al, Cr and Ni content. Furthermore, in view of the above results, it is necessary to propose to the competent authorities a coordination between the Environment, Agriculture and Health sectors in order to achieve a harmonisation of their respective regulations on the different interrelated aspects of harvesting, primary production, direct sale and marketing of wild mushrooms.

**Keywords:** metals, mushrooms, morchella, fire, food safety

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19

### **OCCURRENCE AND DIETARY EXPOSURE OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN COMMONLY CONSUMED FOOD IN SINGAPORE**

**Ignatius Lim<sup>(1)</sup>, Ping Shen<sup>\*(2)</sup>, Wesley Yu<sup>(2)</sup>, Yee Soon Chin<sup>(2)</sup>, Raymond Shi<sup>(2)</sup>, Joanne Chan<sup>(2)</sup>**

<sup>1)</sup> National Centre for Food Science, Singapore Food Agency, Singapore

<sup>2)</sup> Singapore Food Agency, Singapore

\*Corresponding author - E-mail: shen\_ping@sfa.gov.sg

Per- and polyfluoroalkyl substances (PFAS), widely known as "forever chemicals," are a class of man-made chemicals with more than 2000 compounds used in a wide range of consumer and industrial products. Due to their persistent and bioaccumulative nature, scientific studies have shown that long-term exposure to high levels of PFAS may be associated with compromised immune responses, adverse effects on developmental systems, some cancers, and increased infertility. A study was conducted to determine the occurrence levels and dietary exposure of 4 PFAS (PFOA, PFOS, PFNA, and PFHxS) in commonly consumed food by the Singapore population. More than 500 food samples were prepared using different cooking methods commonly practiced in Singapore. A newly developed QuEChERS method was validated for the analysis of PFAS in these food samples, with a limit of quantification (LOQ) of 0.1 µg/kg. High concentrations of PFAS (maximum level of 2.1 µg/kg for PFOS, 2.8 µg/kg for PFOA, 0.6 µg/kg for PFHxS, and 0.4 µg/kg for PFNA) were detected in fish and seafood (clam, crab, and anchovy), some egg products (century eggs), and sauces containing seafood ingredients. Exposure assessment based on local consumption profiles implicated that intake of foods contaminated with high levels of PFAS, such as fish and seafood, may significantly increase dietary exposure to PFAS. This study is a preliminary attempt in Singapore adopting total diet approach to establish the occurrence profile of PFAS in the Singaporean diet. We will further improve the analytical sensitivity to address the issue of left-censored data from this study and to broaden the scope of monitoring to cover more PFAS congeners. The ultimate goal of the study is to establish a more precise estimate of dietary exposure to PFAS and formulate potential risk mitigation measures such as regulatory limit setting and consumer advisory to safeguard food safety and protect public health.

**Keywords:** PFAS, Singapore, exposure assessment, dietary exposure

I10

### COMPARISON OF ANALYTICAL TECHNIQUES FOR THE COMPREHENSIVE PFAS DETERMINATION: A MULTI-METHOD APPROACH

Veronika Svobodova<sup>(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Darina Dvorakova<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Denisa Parizkova<sup>(1)</sup>, Aristeidis Tsagkaris<sup>(1)</sup>, Jana Pulkrabova<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: pulkrabj@vscht.cz

Per- and polyfluoroalkyl substances (PFAS) are a widely used class of synthetic chemicals, including thousands of compounds used in various industrial applications and consumer products, including ski waxes. These substances have been recognised as significant pollutants. The entry into force of Commission Regulation 2020/784 on 4 July 2020 specifically regulates the presence of perfluorooctanoic acid (PFOA) in products sold in the EU, including ski waxes. Despite this regulation, there is still a lack of comprehensive studies looking at the full spectrum of PFAS in these products. Most studies focus primarily on targeted analyses and may underestimate the total concentrations of PFAS.

This study advances the field by developing and comparing several analytical methods for determining PFAS on the example of ski waxes. Four different approaches were tested: (i) ultra-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) using a triple quadrupole mass analyser for the targeted analysis of 46 PFAS compounds; (ii) UHPLC coupled with high-resolution mass spectrometry (HRMS) using an Orbitrap mass analyser for both targeted and non-targeted PFAS screening; (iii) mid-infrared spectroscopy and (iv) Raman spectroscopy using surface-enhanced Raman scattering (SERS) for the analysis of carbon-fluorine bonds.

The targeted analysis revealed significant concentrations of perfluorocarboxylic acids (PFCAs, C4-C16), with total concentrations in ski waxes ranging from 26 to 178 ng/g. In addition, non-targeted screening detected the presence of long-chain PFCAs (C17-C24) in several samples. Partial least squares (PLS) models correlating observed data from infrared and Raman spectroscopy with predicted data from UHPLC-MS/MS showed a higher proportion of unidentified substances with carbon-fluorine bonds in several wax samples. This study emphasises the importance of using a multi-method analytical approach combining targeted and non-targeted techniques for determining PFAS. By comparing the efficiency and effectiveness of these methods, including rapid, non-destructive spectrophotometric techniques, this research contributes to a more comprehensive understanding of the presence of PFAS in consumer products and provides valuable insights into the advantages and limitations of each analytical strategy.

**Keywords:** PFAS, environmental pollution, UHPLC-MS/MS, IR, RAMAN

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I11

### SPECIATION ANALYSIS BY IC-ICP-MS - ARSENIC SPECIES AND METHYL MERCURY - AN OVERVIEW

Klaudija Cavlovic<sup>(1)</sup>, Clara John<sup>(1)</sup>, Morhaf Ghadri<sup>(1)</sup>, Pascal Schreiter<sup>(1)</sup>, Lennart Schmiedeken<sup>(1)</sup>, Mareike Reichel<sup>(1)</sup>, Jan Sebastian Maenz<sup>(1)</sup>

<sup>1)</sup> Eurofins WEJ Contaminants GmbH, Germany

\*Corresponding author - E-mail: [klaudijacavlovic@eurofins.de](mailto:klaudijacavlovic@eurofins.de)

Since elements can be essential or toxic simply due to its oxidation state, elemental species analysis is of crucial importance for food safety monitoring.

The European Food Safety Authority (EFSA) established a relationship between inorganic arsenic (iAs) and certain types of cancer in 2009. In 2023, this was extended to other health problems that can be attributed to iAs. Since July 2024, an EFSA opinion on organic arsenic species in food was published, which recommends the detection of these and provides evidence that dimethylarsinic acid (DMA) triggers carcinogenesis.

Arsenic naturally occurs in the earth's crust but can also be released into environment and groundwater through human activity and contaminate food, e.g. rice or marine foods.

According to Regulation (EU) 2023/915, maximum levels for iAs (sum of As(III) and As(V)) in certain foods, e.g. rice or infant formula are set. EU Recommendation 2015/1381 requires to monitor arsenic and its species in various of foodstuffs. No maximum levels have been established for organic arsenic species, yet. Notably, the EFSA Opinion from July 2024 provides the European Commission (EC) a scientific basis for the possible establishment of maximum levels now.

In addition to arsenic species, methylmercury (MeHg), an organic mercury species, is also important to be monitored due to its high toxicity. The German Federal Institute for Risk Assessment (BfR) has specified a tolerable weekly intake (TWI) for MeHg. Despite its high toxicity, MeHg is currently not regulated by the European Union. Maximum levels have only been set for total mercury.

Especially regarding the high toxicity of some species (iAs, DMA, MeHg), speciation analysis is relevant and allows a detailed evaluation of the analytes present in a sample. Ion chromatography (IC) coupled with inductively coupled plasma mass spectrometry.

(ICP-MS) allows detailed analysis of several ions in one sample, as it is less affected by matrix influences and can be used to detect different oxidation states.

Therefore, two specific, robust methods with IC-ICP-MS were developed for species analysis. For the determination of four arsenic species, a method based on DIN EN 16802:2016 was developed and validated for a wide range of foods of plant, animal and marine origin. For MeHg, the detection is based on an FDA method and is validated for fish and seafood. Emphasis was placed on the use of suitable consumables and equipment e.g. a metal-free IC system. Another focus was on checking specificity, as the ICP-MS only measures specifically for the metal part.

**Keywords:** speciation, inorganic arsenic, dimethylarsinic acid (DMA), methyl mercury, IC-ICP-MS



I12

### STREAMLINED ANALYSIS OF SHORT-CHAIN PFAS USING DUAL-COLUMN INTEGRATION: A NOVEL APPROACH FOR HIGH-THROUGHPUT LABORATORIES

Victoria Erdely<sup>(1)</sup>, Torben Leifels<sup>(1)</sup>, Claudia Rathmann<sup>(2)</sup>, Linda Schlittenbauer<sup>(3)</sup>

<sup>1)</sup> Eurofins GfA Lab Service, Germany

<sup>2)</sup> Waters GmbH, Germany

<sup>3)</sup> Waters, Germany

\*Corresponding author - E-mail: VictoriaErdely@eurofins.de

As global attention increasingly focuses on the presence and impact of PFAS compounds in foodstuffs and environmental samples, the need to accurately analyze and confirm short-chain PFAS has become critical. According to EURL guidelines, the most precise method for quantifying and confirming short-chain PFAS, such as PFBA, involves using two different analytical columns with distinct stationary phases. While scientifically robust, this approach is impractical for high-throughput laboratories with demanding turnaround times. In collaboration with Waters, our laboratory has developed a solution to this challenge. Our method enables a single sample injection to be analyzed as a unified data file, with results derived from two separate columns for all relevant short-chain PFAS. This innovation eliminates delays associated with remeasuring and reanalyzing any presumed positive short-chain PFAS detected in the initial column.

**Keywords:** PFAS, short-Chain PFAS

I13

### MINERAL OIL ANALYSIS: A QUALITATIVE & QUANTITATIVE WORKFLOW USING GC×GC-TOFMS/FID

**Sebastiano Panto**<sup>\*(1)</sup>, **Dmitrii Rakov**<sup>(1)</sup>, **Aous Khalefa**<sup>(1)</sup>, **Lena Dubois**<sup>(1)</sup>, **Nick Jones**<sup>(1)</sup>

<sup>1)</sup> LECO EATC, Germany

\*Corresponding author - E-mail: [sebastiano\\_panto@leco.com](mailto:sebastiano_panto@leco.com)

Mineral oil hydrocarbons (MOHs) are complex mixtures deriving from crude oil. They consist of two main fractions: mineral oil saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH). The analysis of MOHs in food is a challenging task, due to the high complexity of the matrices and the high affinity of mineral oil towards the lipid fraction and other interfering components (e.g., carotenoids, terpenoids, olefins).

The method of election for the quantitative determination of MOHs is LC-GC-FID. The resulting chromatograms are typically containing "humps", also known as unresolved complex mixtures (UCM). The UCM is often very difficult to interpret as the FID is a non-selective detector, and additional confirmatory methods are needed. In this respect, GC×GC-ToFMS is a well-established solution to characterize the MOSH and MOAH fractions in detail revealing the presence of markers of contamination (e.g., hopanes, dibenzothiophenes, diisopropylnaphthalenes POSH, PAO, etc.) as well as natural interferences (e.g., terpenoids). In addition, the GC×GC-ToFMS contour plots provide highly detailed qualitative information on the characterization of MOSH and MOAH fractions answering, as an example, the request of the EFSA and the European Union to characterize more in detail the 3-7 ring MOAH fractions. Recently, a full solution, including all hardware and software tools, was developed and validated, enabling automatic processing of quantitative data (GC×GC-FID), thanks to the use of a novel software algorithm developed to integrate 2D humps. We can demonstrate full qualitative and quantitative results for a range of challenging food sample matrices, such as palm oil and coconut oil.

I14

### **AUTOMATED PFAS EXTRACTION FROM DIFFICULT FOOD AND FOOD PACKAGING MATRICES**

**Alicia Stell<sup>(1)</sup>, Benedict Liu<sup>(1)</sup>, Brittany Fessler<sup>(1)</sup>, Bobbie McManus<sup>\*(1)</sup>**

<sup>1)</sup> CEM Corporation, United States of America

\*Corresponding author - E-mail: [bobbie.mcmanus@cem.com](mailto:bobbie.mcmanus@cem.com)

There is increasing concern of Per- and Polyfluoroalkyl Substances (PFAS) in our environment as a whole, due to their persistent nature and noted widespread detection throughout a vast array of matrices. More and more regulations regarding PFAS are being implemented with action limits that continue to decrease. Having a harmonized method to accurately determine the PFAS content in food, as well as other matrices, is important to ensuring long-term detection and regulation. The solvent extraction of PFAS from these varied sample matrices can be challenging given the susceptibility to contamination and the low levels in which these compounds are present. Existing solvent extraction techniques are predominantly manual methods that are not rapid, simple, or efficient. In this study the EDGE PFAS™, an automated solvent extraction system, is explored. This system performs an efficient extraction of PFAS compounds from challenging food and food packaging matrices in one simple process. Upon completion, the final extract is filtered and ready for any minimal cleanup required, as well as analysis. Excellent recovery with tight reproducibility is presented. The EDGE PFAS method offers a rapid, simple, and efficient solvent extraction solution for PFAS testing.

**Keywords:** PFAS, solvent extraction, sample preparation

I15

### IN-HOUSE VALIDATION OF A SIMPLE RP-LC-ICPMS METHOD FOR MERCURY SPECIATION ANALYSIS: APPLICATION TO DANISH SEAFOOD SAMPLES

Doaa Abouelenein<sup>\* (1)</sup>, Yuka Omura Lund<sup>(1)</sup>, Maria Haar<sup>(2)</sup>, Katrin Loeschner<sup>(1)</sup>, Lene Duedahl-Olesen<sup>(1)</sup>, Jens J. Sloth<sup>(1)</sup>

<sup>1)</sup> Research Group for Analytical Food Chemistry, National Food Institute, Technical University of Denmark (DTU), DK-2800, Lyngby, Denmark, Denmark

<sup>2)</sup> Danish Veterinary and Food Administration (DVFA), Denmark

\*Corresponding author - E-mail: ddaab@dtu.dk

Given the severe health risks posed by mercury (Hg), especially the neurotoxic effects of methylmercury (MeHg) and recognizing the urgent demand for improved mercury analysis across food samples, especially seafood. We herein used the method described by Hight & Cheng (2006) [1], with some modifications. The method combines ultrasonication-assisted extraction with robust reversed phase chromatography-inductively coupled plasma mass spectrometry (RP-LC-ICP-MS). Through this technique, we achieved the separation of MeHg from inorganic Hg (iHg) in less than 3 minutes using a C-18 Kinetex 2.6  $\mu\text{m}$  C18 100 Å column (100 x 4.6 mm), following ultrasonic extraction using L-cysteine·HCl·H<sub>2</sub>O as the extractant agent. Total Hg content was calculated as the sum of MeHg and iHg.

Validation of our method included analysis of three certified reference materials (CRMs) and three marine-derived samples. Accuracy of MeHg values was consistent with the certified values at 95% confidence level and the recoveries ranged from 94- 100.8% for all samples. We ensured method reliability through triplicate measurements by two operators, yielding relative standard deviations under repeatability conditions (RSD<sub>r</sub>) between 1.1- 9.4% and in-house reproducibility conditions (RSD<sub>R</sub>) ranging from 1.7- 12.3%. The MeHg detection limits (LODs) were 0.09  $\mu\text{g}/\text{kg}$  and 0.34  $\mu\text{g}/\text{kg}$ , while the quantification limits (LOQs) were 0.29  $\mu\text{g}/\text{kg}$  and 1.14  $\mu\text{g}/\text{kg}$  in edible seafood (fresh, dilution factor 50) and in lyophilized reference materials (dried, dilution factor 200), respectively.

The developed method was proved to be highly sensitive and suitable for routine analysis, aligns with green analytical chemistry principles, featuring reduced toxic waste, safer HPLC mobile phases, and shorter separation times, thus cutting operational costs. Finally, the developed method was applied to 26 seafood samples of different species collected by the Danish Veterinary and Food Administration as part of their annual control program. The concentrations for total Hg and MeHg ranged from 0.002-0.186 and 0.001-0.175 mg/kg, respectively. The percentage of MeHg in relation to total Hg varied from 35 to 97 %, with the lowest proportion of MeHg found in mussel samples. This demonstrated that total Hg is not in all cases a good proxy for MeHg. This enhances our understanding of Hg distribution in various aquatic species, hence contributing to more reliable health risk evaluations.

[1] Hight, S. C., & Cheng, J. (2006). Determination of methylmercury and estimation of total mercury in seafood using high performance liquid chromatography (HPLC) and inductively coupled plasma-mass spectrometry (ICP-MS): Method development and validation. *Analytica Chimica Acta*, 567(2), 160-172. <https://doi.org/10.1016/j.aca.2006.03.048>.

**Keywords:** methylmercury, inorganic mercury, validation, RP-LC-ICP-MS

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## FOOD CONTAMINANTS (ENVIRONMENTAL)

I16

### DEVELOPMENT OF A CANARY BIOSENSOR FOR RAPID AND SENSITIVE DETECTION OF SHIGA TOXIN-PRODUCING E. COLI IN FOOD

Christina Tam<sup>(1)</sup>, Wen-Xian Du<sup>(1)</sup>, Xiaohua He<sup>\*(1)</sup>

<sup>1)</sup> USDA, ARS, WRRRC, United States of America

\*Corresponding author - E-mail: xiaohua.he@usda.gov

Shiga-toxin producing Escherichia coli (STEC) causes a wide spectrum of diseases including hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). Current testing methods for STEC normally include enrichment, cell plating, and sequencing, which is very time-consuming thus delaying diagnosis and treatment. In this study, a rapid, sensitive, and potentially portable assay that identifies STEC by detecting Shiga toxin 2 (Stx2) using the CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) B-cell based biosensor technology was developed. Studies demonstrated that the Stx2 biosensor was capable of detecting down to 0.4 cfu/g or mL of STEC present in ground beef, lettuce and milk after 8 to 16 hours incubation in an enrichment medium within 3 min. These results suggest this biosensor has the potential to provide detect-to-warn function, thus is valuable for surveillance programs that help with food product recalls.

**Keywords:** Shiga toxin-producing E. coli, CANARY biosensor, B-cell based assay, foodborne pathogens, food safety

**Acknowledgement:** This research was funded by the United States Department of Agriculture, Agricultural Research Service, National Program 108, Food Safety, CRIS Project 2030-42000-053-000-D.

I17

### DEVELOPMENT AND OPTIMIZATION OF AN SS AND NTS STRATEGY TO EXTEND CONSUMER EXPOSOME CHARACTERIZATION TO OVER 900 CHEMICAL RESIDUES AND CONTAMINANTS

Cassandre Jeannot<sup>(1)</sup>, Bruno Le Bizec<sup>(1)</sup>, Gaud Dervilly<sup>(1)</sup>, Julien Parinet<sup>\*(2)</sup>

<sup>1)</sup> ONIRIS, INRAE, LABERCA, France

<sup>2)</sup> ANSES, Laboratory of Food Safety, France

\*Corresponding author - E-mail: julien.parinet@anses.fr

Chronic exposure to chemical hazards through the environment and diet can contribute to the development of certain chronic diseases. From a public health perspective, it is essential to characterize as many as possible of the chemical substances to which humans are exposed throughout their lives, in order to implement effective health prevention strategies.

Food can be contaminated by numerous contaminants such as per- and polyfluoroalkyl substances (PFAS) and pesticides from industrial and agricultural activities. Despite their widespread use since the 1950s, PFAS are listed for a few of them in the Stockholm Convention as persistent organic pollutants (POPs) while four have recently been regulated in food. The European Food Safety Authority (EFSA) has estimated that exposure to certain PFAS is harmful to human health, resulting in a tolerable weekly intake (TWI) in certain foods. For the same purpose, a maximum residue level (MRLs) for individual pesticides residues in foodstuffs has also been established. However, hundreds of chemical contaminants from different families exist, which need to be monitored to provide a more complete picture of environmental contamination and human exposure.

Recent non-targeted profiling technologies are making it possible to assess the chemical exposome in an increasingly global way, offering access to knowledge of a wider spectrum of chemicals. Our first work, only focus on fluorinated substances, highlighted the ability to extract, detect and identify novel PFAS compounds. To develop multi-contaminants method, even more compounds were included in the optimization process, while following the same strategy. We have activated three levers to extend the number of identified contaminants: sample preparation (optimised QuEChERS), data acquisition (LC-HRMS) and data processing. As food, especially those from the aquatic environment, is one of the main routes of exposure to PFAS, pesticides, veterinary drugs and various mixtures of contaminants, our research focused on developing an alternative, suspect and non-targeted approach for identifying as many contaminants as possible in various foods, such as fish.

This work involves the development and optimization of a QuEChERS-type preparation method, together with a comparison of the most widely used chromatographic columns for non-targeted analysis of contaminants in foods associated with different gradients. This new non-specific approach proved conclusive, as extracted, detected and identified over 900 contaminants in various fish samples.

In the interests of better risk assessment, this methodology paves the way for broader characterisation of consumer exposure to these compounds of concern.

**Keywords:** non-targeted screening, pesticides, PFAS, food, QuEChERS

I18

### **DETERMINATION OF FURANIC COMPOUNDS IN SOUTH AFRICAN TRADITIONAL FOOD PRODUCTS USING HEADSPACE SOLID-PHASE MICROEXTRACTION WITH GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR**

Vusumzi Pakade<sup>(1)</sup>, Lawrence Madikizela<sup>\*(1)</sup>

<sup>1)</sup> University of South Africa, South Africa

\*Corresponding author - E-mail: madiklm@unisa.ac.za

A headspace-solid phase microextraction - gas chromatography-flame ionisation detector (HS-SPME-GC/FID) method was developed for the simultaneous determination of furan, 2-methylfuran and 2-furaldehyde in thermally processed Mopane worms, corn, and peanuts. The optimal HS-SPME conditions with polydimethylsiloxane/ carboxen/divinylbenzene (PDMS/CAR/DVB) fiber were 30 °C, 40 min, and 600 rpm stirring speed. The recoveries, detection, and quantification limits for the analytes in food samples were 67-106%, 0.54-3.5 µg/kg, and 1.8-12 µg/kg, respectively. These results showed that the developed method was accurate, reproducible, and sensitive for the determination of furan, 2-methylfuran, and 2-furaldehyde in complex food matrices with limited interference from other components. The optimised analytical method was applied for monitoring the presence of the furanic compounds in heat-processed South African foods. Although 2-furaldehyde was not detected in food samples, the maximum concentrations of 24 and 95 µg/kg were found for furan, and 2-methylfuran, respectively.

**Keywords:** furan, thermally processed food, HS-SPME, GC-FID, mopane worms

**Acknowledgement:** National Research Foundation of South Africa is acknowledged for financial support.

I19

### THE EMERGENCE OF PHARMACEUTICAL RESIDUES IN HUMAN FOOD CROPS AND GARDEN-SOIL CULTIVATED UNDER ACTUAL ENVIRONMENTAL CONDITIONS

Lawrence Madikizela<sup>\*(1)</sup>, Ronewa Ronewa<sup>(1)</sup>

<sup>1)</sup> University of South Africa, Institute for Nanotechnology and Water Sustainability, South Africa

\*Corresponding author - E-mail: madiklm@unisa.ac.za

The perseverance of pharmaceuticals in water has led to their occurrence in the agroecosystem potentially posing significant human health risks. This study examined the presence of efavirenz, ibuprofen, naproxen, sulfamethoxazole, and trimethoprim in home-grown cabbage, spinach, and garden soil. Ultrasound-assisted extraction incorporated with solid phase extraction techniques was used to prepare samples prior to LC-qTOF-MS analysis. The sample preparation method was validated through the computation of analyte recoveries after spiking the investigated matrices at two concentrations (5 and 15 ng/g). Analyte recoveries attained ranged from 80 - 111% in all the investigated matrices with the quantification limits between 0.28 to 25.3 ng/kg. The study further examined the effect of cooking vegetables on the presence of pharmaceuticals in the studied vegetables. Trimethoprim was the most prominent drug in vegetables with the concentration reaching 1501 ng/kg in cabbage samples. On the effect of cooking, the studied pharmaceuticals were detected at lower concentrations compared to the uncooked samples. Detected pharmaceutical concentrations in vegetables present negligible human risk. The study also tentatively identified 108 additional drugs in vegetables, highlighting the need for more monitoring of pharmaceuticals in human food crops.

**Keywords:** pharmaceuticals, human food-crops, ultrasound-assisted extraction, solid-phase extraction, health risk assessment

**Acknowledgement:** University South Africa, Institute for Nanotechnology and Water Sustainability and National Research Foundation of South Africa (Grant numbers: 138004 and PMDS22061020).



120

### DETERMINATION OF NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> AND N-NITROSAMINES IN VEGETABLES STORED AND PREPARED UNDER DIFFERENT CONDITIONS

**Daniel Clayton-Cuch**<sup>(1)</sup>, **Agnieszka Anna Niklas**<sup>\*(2)</sup>, **Heidi Amlund**<sup>(1)</sup>, **Leise Leise Kjelmann**<sup>(1)</sup>

<sup>1)</sup> Technical University of Denmark, Denmark

<sup>2)</sup> National Food Institute, Technical University of Denmark, Denmark

\*Corresponding author - E-mail: anik@food.dtu.dk

The objective of this work is to quantify NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and *N*-nitrosamines in various vegetables when stored or prepared under different conditions, as well as to better characterize the interplay of these compounds. Inappropriate storage of raw or cooked vegetables, especially of leafy green vegetables high in NO<sub>3</sub><sup>-</sup> can lead to the direct conversion of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup>, this may result in unexpected exposure to high concentrations of NO<sub>2</sub><sup>-</sup> and therefore an increased potential for methaemoglobinaemia. In addition, the conversion of NO<sub>3</sub><sup>-</sup> to NO<sub>2</sub><sup>-</sup> may create favorable conditions for the formation of carcinogenic *N*-nitrosamines while in the presence of amines.

This work showcases its novelty in that it explores the concentration of the 10 carcinogenic *N*-nitrosamines (which are included in the EFSA 2023 risk assessment, referred to as the TCNA's) within vegetables prepared and/or stored under conditions that mimic what is typical prior to consumption and/or analysis. Further, understanding the interplay between NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup> and *N*-nitrosamine formation under different conditions has not been extensively characterized prior to this work. A selection of diverse vegetable samples were analyzed, for which the quantification of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> were determined by ion chromatography (IC), on a system which integrates conductivity detection with UV detection. For the screening of *N*-nitrosamines, an Orbitrap Exploris 120 LC-MS instrument was used. The quantification of *N*-nitrosamines was performed on a LC-MS/MS using Bruker EVOQ ELITE instrumentation, through multiple reaction monitoring mode for higher sensitivity.

Preliminary results from this work indicate that a substantial proportion of vegetables contained varying concentrations of nitrogenous compounds. Certain vegetables had elevated levels, with leafy green vegetables being particularly notable for their high NO<sub>3</sub><sup>-</sup> concentrations. Different methods of storage or preparation did not seem to have a significant effect on the concentrations of NO<sub>3</sub><sup>-</sup>. Furthermore, some vegetables intended for raw consumption contained detectable levels of NO<sub>2</sub><sup>-</sup> when stored inappropriately, raising potential concerns through creating favorable conditions for *N*-nitrosamine formation. The elevated NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup> levels in vegetables intended for consumption further underscores the need for research into the potential formation of *N*-nitrosamines. As highlighted in the EFSA 2023 risk assessment on *N*-nitrosamines, a significant proportion of published detection methods are solely for meat product analysis which indicates the need for determination of TCNAs in different food matrices, including vegetables.

**Keywords:** nitrite, nitrate, *N*-nitrosamine, EFSA, vegetable

I21

### DEVELOPMENT OF METHODS TO IMPROVE AND VALIDATE MEASUREMENTS OF PFAS USING GAS CHROMATOGRAPHY - MASS SPECTROMETRY

Charlotte Hauschildt\*<sup>(1)</sup>, Christian Jung<sup>(1)</sup>, Roland Becker<sup>(1)</sup>

<sup>1)</sup> Department 1.7, Bundesanstalt für Materialforschung und -prüfung (BAM), Germany

\*Corresponding author - E-mail: charlotte.hauschildt@bam.de

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetically produced chemicals that includes an estimated 10.000 compounds. Known as "forever chemicals", the characteristic feature is the complete or partial fluorination of the carbon chain. Due to the persistence, toxicity and ubiquitous occurrence, research has focused on the qualification and quantification of the most important compounds as well as on the investigation of toxicity and possible routes of entry over the last 10 years [1,2].

Since 2022, four perfluorocarboxylic- and sulfonic acids have been regulated in food in the EU for the first time [3]. Liquid chromatography - mass spectrometry is the analytical standard to test for PFAS, as the spectrum of detectable compounds is significantly more comprehensive than it is currently the case with gas chromatography - mass spectrometry (GC-MS). However, to be able to test for PFAS contamination in a more process-independent manner and to make the analysis more widely accessible, methods based on GC-MS are currently being developed. Since GC-MS methods only cover a fraction of the compounds belonging to the group of PFAS so far, further development of the corresponding measurement methods is inevitable [2].

The here described project is part of the EU project „Metrology for food safety in the circular economy: targeted and screening methods for contaminants in food and recycled packaging" (23IND13 - ScreenFood) [4]. The aim is to develop sensitive analytical GC-MS methods that contribute to an improved qualification and quantification of various PFAS (both currently regulated and emerging PFAS) in selected food and food packaging matrices. Of particular interest are native and recycled polymers such as PET. Besides, various techniques, including solvent-free variants such as TD-GC-MS, will be tested for a quick and easy analysis. This poster will present the overall project objectives and first results.

[1] Schiavone, C., et al., Appl. Sci. 2023, 13, 6696. <https://doi.org/10.3390/app13116696>

[2] Shafique, U., et al., Analytica Chimica Acta 949 (2017) 8e22.

<https://doi.org/10.1016/j.aca.2016.10.026>.

[3] Europäische Kommission, Verordnung (EU) 2022/2388.

<http://data.europa.eu/eli/reg/2022/2388/oj>.

[4] 23IND13 ScreenFood, 2024. <https://www.euramet.org/research-innovation/search-research-projects/details/project/metrology-for-food-safety-in-the-circular-economy-targeted-and-screening-methods-for-contaminants-in-food-and-recycled-packaging>.

**Keywords:** PFAS, GC-MS, food, food contact material, EU project

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122

### CONTRIBUTION TO TOXICITY OF PCDD AND PCDF IN PUYE (GALAXIAS SPP) COLLECTED FROM FRESH WATER, IN SOUTH OF CHILE

**Rodrigo Loyola-Sepulveda**<sup>\*(1)</sup>, Marco Salamanca O.<sup>(2)</sup>, Claudia Figueroa Sn. M.<sup>(1)</sup>, Alex Mendoza<sup>(1)</sup>

<sup>1)</sup> Laboratorio de Oceanografía Química, Universidad de Concepción, Chile

<sup>2)</sup> Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Chile

\*Corresponding author - E-mail: rodrigoloyola@udec.cl

Polychlorinated dibenzo-p-dioxins (PCDD) and Polychlorinated dibenzo-p-furans (PCDF) are widely distributed in the environment. The sources of these compounds may have different sources and, once being released to environment are finally bioconcentrated in organisms, especially those with high content of fat. Puya (*Galaxias spp*) is a small fish that lives in fresh water of some rivers located in the south of Chile, which is widely used in local gastronomy for some preparations and considered by locals a gourmet dish. Concentrations of PCDD and PCDF were measured in a long-term monitoring program in Puya, collected in Rio Cruces, located in the Region de los Rios, close to the city of Valdivia, south of Chile. Samples were carried to the laboratory then freeze dried and homogenized. The extraction and purification of PCDD and PCDF in the samples were performed by Pressurized liquid extraction (PLE-FMS.Inc) and Power Prep System (FMS.Inc) using conventional acidic Silica, neutral alumina and carbon columns. Quantification was carried out by isotopic dilution using a HRGC-HRMS Thermo DFS, extraction and quantification procedures based on the method EPA-1613. PCDD and PCDF were found in all samples of Puya analyzed, including the congeners 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD. Concentration and toxicity ranged from 0,00108 to 0,01153 ng.g<sup>-1</sup> and 0,00001 to 0,00095 pg.Kg<sup>-1</sup>. PCDD and PCDF were found in all samples analyzed.

**Keywords:** PCDD/F, galaxias spp, toxicity, Chile

**Acknowledgement:** Authors acknowledge to the administration of the "Laboratorio de Oceanografía Química" of Universidad de Concepción, for their financial support to carry out this research. The staff of the laboratory are thanked to their contribution in the acquisition of chemicals supplies that allowed extraction, analysis and sampling.

123

### THE EFFECT OF VARIOUS WASHING METHODS ON PESTICIDE RESIDUES, TOXIC AND ESSENTIAL ELEMENTS REMOVAL IN RICE

Khulood Alnabati<sup>(1)</sup>, Dalal Alqahtani<sup>(1)</sup>, Rakan Alajami<sup>(1)</sup>, Tagreed Alsalem<sup>(1)</sup>, Sara Almanna<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: kknabati@sfd.gov.sa

This study examined the effects of various treatments on removing pesticide residues and toxic elements in rice. In parallel, nutritional elements, magnesium (Mg), potassium (K), and phosphorous (P), were measured to investigate the effect of these washing treatments on the nutritional value of rice. A naturally contaminated rice sample containing five widespread used pesticides (azoxystrobin, buprofezin, carbendazim, and propiconazole) and toxic elements, arsenic (As), cadmium (Cd), and essential elements, was washed using several washing agents, including boiling water, 5% sodium bicarbonate (baking soda), 5% acetic acid (vinegar), 5% citric acid, and 5% sodium chloride (salt). The washing method was chosen based on its availability and widespread usage; soaking for 10 min was assumed to be reasonable. Our results showed that using 5% acetic acid significantly reduced azoxystrobin by 63%, buprofezin by 70%, carbendazim by 75%, and propiconazole by 61%. However, As and Cd were significantly reduced in sodium chloride by 57% and 32%, respectively. Furthermore, a significant reduction in essential nutrient elements was found in Mg (42%), K (37%), and P (23%) when rice was treated with 5% citric acid. Overall, washing agents reduced analytes in the following manners pesticides, toxic elements, and essential elements when using acetic acid, sodium chloride, and citric acid separately.

**Acknowledgement:** My name is khulood khalid alnabati and I am affiliated with the Reference Laboratory for Food Chemistry (RFL) at the Saudi Food and Drug Authority (SFDA). I have been working as an expert researcher in the field of inorganic chemistry for the past 4 years. Our department's main focus is on the development, analysis, and validation of a novel method for detecting metals and metalloids in food products.

124

### CHEMICAL HAZARD PROFILING OF BLACK SOLDIER FLY LARVAE (HERMETIA ILLUCENS)

**Marie Papin**<sup>(1)</sup>, Erwan Engel<sup>(1)</sup>, Christelle Planche<sup>(1)</sup>, Patrick Borel<sup>(2)</sup>

<sup>1)</sup> INRAE, UR 370 Animal Products Quality Unit, MASS Group, France

<sup>2)</sup> Center for CardioVascular and Nutrition Research, C2VN, Aix Marseille Univ, INSERM, INRAE, France

\*Corresponding author - E-mail: marie.papin@inrae.fr

Black Soldier Fly larvae (BSFL), *Hermetia Illucens*, are increasingly used as a new feed source in the EU. BSFL was proven to be able to convert and then recycle biowaste into high-quality nutrients for the feed industry. However, biowaste usually contains a lot of chemical hazards. Although BSFL are fed with these biowaste, the fate of the contaminants remains largely unknown, despite being a threat for both the insect industry and the higher trophic levels. The aim of this study was to profile BSFL in pesticides, mycotoxins, dioxins, furans, PCBs and PFAs and to link it with the levels of contamination of these chemical hazards in the substrates on which they had been reared. Larvae were reared on six different substrates: four authorized in the EU including carrots, apricots, salad and wheat bran and two currently unauthorized including school canteen and supermarket wastes. Rearing substrates and larvae 14 days old were analysed by two accredited labs (COFRAC): Inovalys (Angers, France) and Laberca (Nantes, France). Eighteen mycotoxins including aflatoxins, trichothecenes, fumonisins, ochratoxin, zearalenone and ergot alkaloid were searched. More than 500 pesticides, 18 PCBs dioxin-like and non-dioxin like, 7 dioxins (PCDD), 10 furans (PCDF) and 25 PFAS were also screened. The presence and concentrations of these chemical hazards in both rearing substrate and larvae will be discussed.

**Keywords:** insect, biowaste recycling, persistent organic pollutants, pesticides, mycotoxins

125

### A NOVEL APPROACH IN ADDRESSING THE CHALLENGES OF MONITORING MULTI-CLASSES OF DIOXINS AND OTHER POP IN A SINGLE RUN BY GC-ION MOBILITY-HRMS

**Carsten Baessmann**<sup>(1)</sup>, **Javier Lopez**<sup>(2)</sup>, **Miguel Angel Perez**<sup>(2)</sup>, **Arnd Ingendoh**<sup>(1)</sup>, **Hugo Mueller**<sup>(3)</sup>, **Gauthier Eppe**<sup>(3)</sup>

<sup>1)</sup> Bruker Daltonics GmbH & Co KG, Germany

<sup>2)</sup> Bruker Espanola, S.A., Spain

<sup>3)</sup> Mass Spectrometry Laboratory, MolSys Research Unit, Chemistry Department, University of Liège, Belgium

\*Corresponding author - E-mail: carsten.baessmann@bruker.com

Polychlorinated Dioxins, Furans and PCBs were analyzed in real-life samples like rapeseed oil, milk fat, sludge extract and ash. Their analysis is complex due to low regulatory exposure limits and difficult sample matrices. They are persistent organic pollutants (POP), widely found in environmental samples and can have severe health consequences. 1  $\mu$ L samples were injected and separated by GC with a GC-APCI source coupled to a trapped-ion-mobility-QTOF which enables fast and sensitive quantitative analysis of the different classes of compounds in a single GC/HRMS run. Criteria for validation and quantification of compounds were high mass accuracy, retention time, isotope pattern matching, MS/MS qualifiers and collision cross sections (CCS) from ion mobility filtering.

A TargetScreener 4D method "ready-to-use" was developed for the simultaneous analysis of all of these classes in the very same GC-HRMS run. Criteria for the quantification were two ions associated to each analyte (principal ion  $[M]^+$  in full scan MS and product ion  $[M-CClO]^+$  in MS/MS) with an ion ratio tolerance of  $\pm 30\%$  for both ions and expected CCS tolerance of  $\pm 7\%$ . Due to the use of TIMS, real-life samples like the ash extract spiked with a mixture containing several congeners of dioxins and furans (specially congeners of TCDF and TCDD) could be diluted by 10x before injecting. That has the inherent advantages of an improved reproducibility by lowering the number of ions, cost saving by reducing expensive standard consumption, and lower matrix effect due to the dilution. The congeners can be tracked first by their relative retention time to avoid erroneous integration. Then, ion mobilograms are generated with a clear integration for an accurate quantitation. A novel algorithm allows to integrate even a small amount of the detected analyte and generate the suitable chromatogram. This is of special importance be able to deconvolute and integrate chromatographic or mobilographic peaks in case of interferences or noisy backgrounds, as well as for better reproducibility for integrated peaks and improved quantitation figures. The LOD of all PCBs was at 10 ppt, with the LOQ at around 20 ppt. For some other compounds like PCB 81 or PCB 157, the LOQ was even below 10 ppt. All data was compared with results from conventional sector field analysis.

**Keywords:** POP, dioxin, furan, PCB, GC-APCI-TIMS-QTOF

126

### **DETECTING PFAS BEYOND THE CURRENT REGULATIVE REQUEST: A COMPREHENSIVE OVERVIEW OF THE CONTAMINATION IN DUTCH SURFACE WATER AND EFFLUENTS BY UPHPLC-ION MOBILITY-HRMS**

**Carsten Baessmann**<sup>(1)</sup>, David Liwara<sup>(2)</sup>, Ilona Nordhorn<sup>(1)</sup>, Birgit Schneider<sup>(1)</sup>, Sicco Brandsma<sup>(2)</sup>, Pim Leonards<sup>(2)</sup>

<sup>1)</sup> Bruker Daltonics GmbH & Co KG, Germany

<sup>2)</sup> Vrije Universiteit Amsterdam, The Netherlands

\*Corresponding author - E-mail: carsten.baessmann@bruker.com

PFAS are known as “forever chemicals” due to their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms. Ca. 5000 PFAS are marketed worldwide, making their systematic environmental monitoring an extremely challenging task. On top, there is a lack of reference standards or spectral libraries, and there are plenty of isomers. Adding trapped ion mobility spectrometry (TIMS) to UHPLC-HRMS allows for comprehensive monitoring of organic micropollutants in environmental and food matrices such as water or food extracts. Presented here is a complete solution for PFAS characterization in food and environmental samples, combining ion mobility supported target analysis with wide scope targeted and non-target screening against the complete set of 5000 compounds. Surface water was sampled at 20 sites and effluents at 10 sites in The Netherlands and pre-concentrated by SPE. Data acquisition was performed on a timsTOF Pro2 (Bruker) equipped with ion mobility. Kendrick mass defect (KMD) analysis filtered potential PFAS from the matrix background, based on the fluorine content (repeating CF<sub>2</sub> units). Spectra were compared with the Norman network and NIST suspect lists of 5000 entries for non-targeted analysis. These contain information about the PFAS elemental composition and the InChI structure which were matched with the four criteria of exact mass, isotope pattern, MS/MS fragmentation and CCS value of the experimental data for an automated and untargeted identification of all PFAS present in the sample. The ion mobility feature of the system was utilized for several purposes. First, it could separate coeluting isobars and isomers. Second, the TIMS filter resulted in higher sensitivity and lower detection limits of the targeted PFAS as well as significantly higher quality of full-scan MS and bbCID MS/MS spectra. Finally, collisional cross sections (CCS) as additional identification criteria enhanced the identification confidence with was based on retention time, exact mass, diagnostic fragmentation ions and the isotope pattern fit. Starting from a total of 15,700 detected features, 1181 potential PFAS have been filtered by KMD (92% data reduction). In total, 88 PFAS were found in the water samples, including genuine and degradation products with distinct differences for the various sampling sites which point to individual environmental circumstances and origins for PFAS.

**I27**

### **ULTRA-SENSITIVE PFAS ANALYSIS ACCORDING TO EU REGULATIONS IN WATER AND FOOD**

**Diego Martin Ortiz<sup>(1)</sup>, Javier Lopez Flores<sup>(1)</sup>, Rafaela Martin<sup>\*(1)</sup>**

<sup>1)</sup> Bruker, Spain

<sup>\*</sup>Corresponding author - E-mail: [rafaela.martin@bruker.com](mailto:rafaela.martin@bruker.com)

PFAS are highly present in the public and pose a threat to mankind and nature. Manufactured since the 1940s as water and grease repellents in consumer products, they are considered "forever chemicals". With their persistent, bio-accumulative, toxic (PBT) properties and ubiquitous presence in the environment and organisms, there is mounting evidence that exposure to PFAS may generate adverse health effects. EPA and EU regulations exist for the routine monitoring of certain PFAS in water and food with legal limits in sub-ppt range for the individual compound. Reaching these limits in a routine and robust manner is a challenge even for triple quadrupole systems of highest performance. Presented is a method for the routine targeted analysis by a novel, fast, robust and highly sensitive triple quadrupole which covers and exceeds the current EPA regulations for reliable and confident testing of water and food samples.

The standard PFAS list for the EPA regulation 533 was used for the evaluation of the method performance. An Elute HT UHPLC was coupled to an EVOQ DART-TQ+ (both Bruker, Bremen, Germany). The total runtime of the method incl. equilibration was 13.2 min with a gradient of 2 mM ammonium acetate in water and methanol. The columns were an Intensity Solo 2.0 100 x 2.0 mm (Bruker) and a delay column 50 x 2.0 mm (Restek). The TQ was operated in both polarities with source parameters and MRM transitions optimized. The scan speed was automatically calculated per compound with a minimum number of 12 spectra per peak and a dynamic window of 0.6 min per analyte.

Mobile phase compositions and extraction procedure (for food matrices) were optimised, different gradients and flow rates tested, equilibration time at the start of injection to avoid accumulation. Typical LOQs achieved for all PFAS were in the range 10-100 times lower than those required by EU and EPA guidelines and recommendations for drinking water and food. Linearities had typical values of  $R^2 \geq 0.999$ . The system shows high robustness with significantly reduced contamination and crosstalk for multiple injections. The method provides a robust, highly sensitive and rapid analysis of PFAS that meets and exceeds current regulations.

**Keywords:** PFAS, high throughput, triple quadrupole, regulation, robust, drinking water, food



128

### PEROXIDASE MIMICKING AND SERS ACTIVITY OF $\gamma$ -CYCLODEXTRIN SYNTHESIZED GOLD AND SILVER NANOPARTICLES FOR THE DETECTION OF PESTICIDES

Natasha Logan<sup>(1)</sup>, Cuong Cao<sup>\*(2)</sup>, Xiaotong Liu<sup>(1)</sup>

<sup>1)</sup> National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, United Kingdom

<sup>2)</sup> National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast; Material and Advanced Technologies for Healthcare, Queen's University of Belfast, United Kingdom

\*Corresponding author - E-mail: c.cao@qub.ac.uk

Chlorpyrifos (CPF), a widely used organophosphate pesticide in agricultural production, poses significant health risks as residues persist in the environment and accumulate in agricultural products. The persistence of CPF in the food chain highlights the urgent need for ultrasensitive detection methods to ensure food safety. This study presents a novel detection method utilizing noble metal nanoprobe synthesized with gamma-cyclodextrin ( $\gamma$ -CD), which exhibit outstanding catalytic and plasmonic properties.  $\gamma$ -CD, is known for its unique host-guest structure, enhancing the interactions between CPF with gold (Au) and silver (Ag) nanoparticles. These  $\gamma$ -CD capped nanoparticles also demonstrate remarkable oxidase-like characteristics, facilitating the oxidation of colorless 3,3',5,5'-tetramethylbenzidine (TMB) to a blue oxide (oxTMB), while simultaneously enhancing the Surface-Enhanced Raman Spectroscopy (SERS) signal from oxTMB. The presence of CPF induces aggregation of the nanoparticles, inhibiting the oxidation of TMB, which leads to a noticeable reduction in colour and SERS signal. Consequently, the absorbance peak from oxTMB at 370 nm and the Raman peak at 1190  $\text{cm}^{-1}$  exhibit an inverse linear relationship with increasing CPF concentrations. This correlation enables the development of a dual-mode sensing platform by combining SERS and colorimetric detection. The approach provides a straightforward, rapid, cost-effective, and highly sensitive method for detecting pesticides residues and holds significant promise for food safety and environmental monitoring.

**Keywords:** surface-enhanced raman spectroscopy, pesticides, chlorpyrifos, gamma-cyclodextrin, nanoparticles

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129

### ACHIEVING EXCEPTIONAL ROBUSTNESS FOR PFAS ANALYSIS IN FOOD WITH THE NEXT-GENERATION SCIEX 7500+ SYSTEM

**Holly Lee**<sup>(1)</sup>, **Ian Moore**<sup>(1)</sup>, **Craig Butt**<sup>(1)</sup>, **Elliott Jones**<sup>(1)</sup>

<sup>1)</sup> SCIEX, Canada

\*Corresponding author - E-mail: [holly.lee@sciex.com](mailto:holly.lee@sciex.com)

Since the European Commission (EU) established maximum residue limits (MRLs) for per- and polyfluoroalkyl substances (PFAS) in food, there has been an increased demand for robust and accurate LC-MS methods for routine monitoring. Robust mass spectrometers are crucial to prolong the long-term assay viability and ensure project timelines are met with minimal instrument downtime. Residue analysis in food matrices is challenged by the presence of interfering co-extractables, which can result in instrument contamination and system downtime. The SCIEX 7500+ system features new Mass Guard technology designed to improve instrument robustness while prolonging optimal sensitivity performance. This includes new hardware that filters out contaminating ions to create a cleaner ion beam prior to entering further downstream in the instrument. Here, an extensive robustness study was conducted to evaluate the performance of the SCIEX 7500+ system for PFAS analysis in salmon, avocado, spice powder and pet food extracts. Samples were extracted by QuEChERS without further SPE clean-up for accelerated testing. Robustness was evaluated by consecutive injections of matrix extracts and solvent standards without using a diverter valve or any interim maintenance. Despite the aggressive experimental conditions, the majority of PFAS compounds maintained >70% of the initial sensitivity even after >6,400 injections of food matrix extracts on the SCIEX 7500+ system. Coupled with the renowned sensitivity of the SCIEX 7500 system, the exceptional data stability and robustness demonstrated here for the SCIEX 7500+ system deliver a powerful and efficient platform for PFAS analysis in food.

**Keywords:** PFAS, robustness, matrix effects, contamination, food

I30

### ADVANCED ULTRA-SHORT-CHAIN PFAS ANALYSIS USING - UNEXPECTEDLY - REVERSED PHASE CHROMATOGRAPHY

**Ruben Kause**<sup>(1)</sup>, **Bas Van Dijk**<sup>(1)</sup>, **Katja Van Dongen**<sup>(1)</sup>, **Elise Hoek**<sup>(1)</sup>, **Stefan Van Leeuwen**<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: ruben.kause@wur.nl

Per- and polyfluoroalkyl substances (PFAS) are an extensive class of synthetic chemicals known for their remarkable chemical and heat resistance and water- and grease-repellent properties. The phase-out of long-chain PFAS has resulted in their replacement by short-chain and ultra-short-chain fluorinated alternatives ((ultra-)short-chain PFAS). Unlike most long-chain homologs, short-chain PFAS exhibit high water solubility and low vapor pressures, making them highly mobile and challenging to eliminate from the environment and to contain their emissions. Analytical challenges further complicate our understanding of short-chain PFAS, as conventional reversed-phase columns provide inadequate retention for these substances.

This study introduces a novel reverse-phase approach using LC-MS/MS for analyzing ultra-short-chain PFAS with a chain length exceeding one carbon atom. Using a polar PFP column, chromatographic selectivity was significantly improved compared to traditional C18-reversed-phase separation, utilizing dipole-dipole interactions and hydrogen bonding facilitated by fluorine's high electronegativity.

While ion-exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), supercritical fluid chromatography, and gas chromatography are commonly used alternatives for analyzing short-chain PFAS, each method has its limitations. HILIC effectively retains ultra-short-chain PFAS like trifluoroacetic acid (TFA) but struggles to achieve adequate selectivity for mid- to long-chain PFAS. Ion-exchange columns excel in retaining anionic PFAS but often require pH gradients, impacting column durability due to free silanol group dissolution. Supercritical fluid chromatography shows great potential in detecting (ultra-)short-chain PFAS but its widespread adoption is hindered by the need for specialized equipment. In contrast, gas chromatography is widely adopted but requires water-free sample extracts, diverging from typical analytical practices for PFAS analysis and reducing its suitability for general workflows.

To optimize chromatography, we tested four columns, including one HILIC column and three reversed-phase columns, and optimized the eluent and gradient. The developed method effectively detects (ultra-)short-chain PFAS and mid- and long-chain PFAS. This study specifically focused on a selected group of 17 (ultra-)short-chain PFAS, including TFA, trifluoromethane sulfonic acid (TFMS), and GenX.

We applied the method in a field study involving 21 sludge samples used as fertilizer for crops, and validated it across five different water sources, demonstrating its suitability and effectiveness for analyzing and quantifying (ultra-)short-chain PFAS. Our findings highlight the potential of this method to greatly improve the detection and understanding of these emerging contaminants. Future work should demonstrate if the PFP column will also work for neutral, cationic, and zwitterionic compounds.

**Keywords:** ultra-short-chain PFAS, PFAS, PFP-column, reversed phase chromatography, LC-MS/MS

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131

### IS THE STRATEGIC APPROACH OF SENTINEL ANIMAL SPECIES REALISTIC FOR RAPIDLY IDENTIFYING HALOGENATED EMERGING CONTAMINANTS IN THE HUMAN FOOD CHAIN?

Antonin Padiou<sup>(1)</sup>, Ronan Cariou<sup>(1)</sup>, Bruno Le Bizec<sup>(1)</sup>, Gaud Dervilly<sup>\*(1)</sup>

<sup>1)</sup> ONIRIS, INRAE, LABERCA, France

\*Corresponding author - E-mail: [gaud.dervilly@inrae.fr](mailto:gaud.dervilly@inrae.fr)

Human dietary exposure to halogenated contaminants, including POPs, can be characterised by analysing human tissues or foodstuffs, particularly food from animal origin (FFAO). However, the detection of emerging contaminants (ECs) in such matrices is limited by sensitivity and/or ethical issues. Sentinel animal species might help to overcome these limitations. Liminal species, whose habitat and diet are similar to those of humans, seem relevant. This study aimed to explore the liminal sentinel animal model for characterising halogenated contaminants in the human food chain.

Samples from liminal animal species were collected in French urban areas in 2015, 2021 and 2022. Gull eggs (*Larus argentatus* and *Larus michahellis*) were sampled in colonies located along the French coasts. Pigeon eggs (*Columba livia*) were sampled in Paris, Tours and Montpellier in contraceptive pigeon lofts. Adipose tissues and livers were sampled from pigeons and rats (*Rattus norvegicus*) euthanized by the municipal departments of Tours and Montpellier, respectively. FFAO samples (n = 51) were selected on the basis of the French food consumption habits (70 % FFAO consumption coverage) and purchased in Nantes in 2022. After extraction and purification, samples were analysed using GC- and LC-HRMS combining ionisation modes for GC-HRMS (ECNI and EI) and data acquisition mode for LC-ESI-HRMS (full-scan and MS<sup>2</sup>). Data treatment was performed with HaloSeeker software on the most intense signals in sentinel samples. Then, halogenated signals revealed were sought and semi-quantified in FFAO samples.

Among the 126 polyhalogenated compounds identified in the sentinel samples, 71 were also detected in FFAO samples. POPs such as 23 PCBs, 7 PBDEs and 3 organochlorinated pesticides (HCB, heptachlor epoxyde and dieldrin) were detected at higher concentrations in the sentinel samples. Moreover, 38 potential ECs were tentatively identified in both sample sets. A dichlorocarbazole dimer (C<sub>24</sub>H<sub>6</sub>N<sub>2</sub>Cl<sub>4</sub>) was tentatively identified in all sentinel matrices and in 4 meat and meat products samples at lower concentrations. The dichlorocarbazole monomers are considered as persistent ECs with dioxin-like properties but a dimer was not identified yet, to the best of our knowledge. Polyhalogenated biindoles and methylbipyrolles congeners, natural products from marine origin, were detected in gull eggs and fish and seafood at the same concentration ranges in both sample sets. A compound, tentatively identified as trichloroxanthone, was detected at higher concentration in various FFAO samples and in 2 pigeon's adipose tissues samples. For other detected compounds, structural identification remains a challenge. While the sentinel approach seems relevant for rapidly identifying emerging halogenated contaminants, it remains to be demonstrated that they really belong to the human exposome, by opting for a targeted screening, considering either the human diet or biological human matrices (e.g. blood, breast milk).

**Keywords:** halogenated emerging contaminants, sentinel species, human diet, non-targeted analysis, high-resolution mass spectrometry

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132

### MICROBIOLOGICAL STATUS OF DONOR HUMAN MILK INTENDED FOR PREMATURE INFANTS

Elena Sinkiewicz-Darol\*<sup>(1)</sup>, Iwona Adamczyk<sup>(1)</sup>, Katarzyna Kaliszczak<sup>(2)</sup>, Krzysztof Skowron<sup>(3)</sup>, Katarzyna Grudlewska-Buda<sup>(3)</sup>, Magdalena Twaruzek<sup>(4)</sup>

<sup>1)</sup> Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30 St., 85-064 Bydgoszcz, Poland & Human Milk Bank, Ludwik Rydygier Provincial Polyclinical Hospital in Toruń, St. Josef 53-59 St, 87-100 Toruń, Poland

<sup>2)</sup> Human Milk Bank, Ludwik Rydygier Provincial Polyclinical Hospital in Toruń, St. Josef 53-59 St, 87-100 Toruń, Poland & Department of Microbiological Diagnostics, Ludwik Rydygier Provincial Polyclinical Hospital in Torun, St. Josef 53-59 St., 87-100 Toruń, Poland

<sup>3)</sup> Department of Microbiology, Ludwik Rydygier Collegium Medicum, Nicolaus Copernicus University in Toruń, M. Curie Skłodowskiej 9 St., 85-094 Bydgoszcz, Poland

<sup>4)</sup> Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30 St., 85-064 Bydgoszcz, Poland

\*Corresponding author - E-mail: elena.sinkiewicz-darol@ukw.edu.pl

Human milk is considered the most suitable source of nutrition for infants. Donor human milk from human milk banks (HMB) is recommended as the best alternative for infants whose mothers' own milk is unavailable. Microbiological screening of milk donated to HMB is important to ensure the quality and safety of the pasteurised human milk.

The aim of this study was to describe the microbiological status of human milk donated to the Regional Human Milk Bank in Toruń, Poland.

Statistical data regarding the microbiological analysis of milk from 292 potential donors were collected by the Regional Human Milk Bank in Torun, Poland in the years 2013-2022. Total of 538 milk samples were tested. Conventional methods and MALDI-TOF mass spectrometry for microbiological analysis of human milk were used.

Only in 6% of human milk samples the bacteria level was above the required standard and/or the milk had potentially pathogenic bacteria. The main core of donors' breastmilk bacteria represents the skin microbiota, and the composition of the microbiota is strictly related to the surrounding environment. The most abundant genera detected in milk samples were the *Staphylococcus* group. There was no growth of fungi. Prolonged hospitalisation of infants' mothers and/or offsprings is associated with potentially pathogenic bacteria colonization in human milk. The use of the modern identification method MALDI-TOF resulted in more accurate results compared to the biochemical methods.

Analysis indicated that most of the tested milk samples (94%), both expressing at home and in hospital environments, meet the criteria for admission to the human milk bank. Effective techniques for identifying microorganisms ensure that donor milk from human milk banks meets the guidelines set for these units.

**I33**

### **THE ANALYSIS OF PFAS IN MILK BY LC-MS/MS**

**Tina Brandscher**<sup>(1)</sup>, **Sandra Ruiz Perez**<sup>(1)</sup>, **Jamie York**<sup>\*(2)</sup>

<sup>1)</sup> Restek GmbH, Germany

<sup>2)</sup> Restek Corporation, United States of America

\*Corresponding author - E-mail: jamie.york@restek.com

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured organic compounds that are used for a wide array of applications and products. The environmental prevalence and bioaccumulation of these compounds can lead to contamination of produce and other commodities meant for human consumption. One potential complication of this analysis is the presence of co-extractables, such as bile acids, found in the final sample. Bile acids, taurochenodeoxycholic acid (TCDCA), tauroursodeoxycholic acid (TUDCA), and taurodeoxycholic acid (TDCA), are endogenous compounds that are formed in the liver. These compounds share the same mass transition with some of the target PFAS in this method and can be detected at high concentrations in samples of animal origin. In this application, a workflow was developed for the analysis of 28 PFAS compounds in milk, resolving the target PFAS from potential bile acid interferences. To prepare samples, a QuEChERS approach coupled with dSPE was implemented. This workflow returned exceptional results for the four PFAS compounds required in the Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed released by the European Union Reference Laboratory for Halogenated POPs in Feed and Food. Recoveries for the four main PFAS compounds ranged from 100.7 - 113.0% and %RSD of 2.56 - 16.6% with acceptable method accuracy and precision results achieved for the majority of other compounds also monitored. While detectable levels of bile acid interferences were not observed in tested milk samples, the chromatographic method developed herein is suitable to apply to other matrix samples of animal origin for the detection of PFAS compounds where high levels of bile acids may be present.

**Keywords:** PFAS, liquid chromatography, milk, QuEChERS

134

### CONCENTRATIONS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN EGGS: PRELIMINARY RESULTS IN THE REPUBLIC OF CROATIA

Ines Varga<sup>\*(1)</sup>, Jelena Kaurinovic<sup>(1)</sup>, Nina Bilandzic<sup>(1)</sup>, Maja Dokic<sup>(1)</sup>, Ivana Varenina<sup>(1)</sup>, Bozica Solomun Kolanovic<sup>(1)</sup>, Marija Sedak<sup>(1)</sup>, Bruno Calopek<sup>(1)</sup>, Luka Cvetnic<sup>(2)</sup>

<sup>1)</sup> Laboratory for Residue Control, Department of Veterinary Public Health, Croatian Veterinary Institute, Croatia

<sup>2)</sup> Laboratory for Mastitis and Raw Milk Quality, Department for Bacteriology and Parasites, Croatian Veterinary Institute, Croatia

\*Corresponding author - E-mail: varga@veinst.hr

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic organic compounds that have been manufactured for more than seven decades. Due to the strong covalent carbon-fluorine bond, these persistent organic pollutants (POPs) are very stable in the environment, bioaccumulative in biota, and resistant to biodegradation, photo-oxidation, and hydrolysis. These compounds have been globally detected in marine and terrestrial organisms, as well as in the human population. Food is considered the primary source of exposure to these pollutants, with eggs being one of the most significant contributors.

Currently, there are no data on the occurrence of PFAS in food of animal origin in the Republic of Croatia. Preliminary research of isomers PFOS, PFOA, PFNA and PFHxS, was conducted on egg samples from three different types of farming in the Republic of Croatia. A total of 49 egg samples (19 organic eggs, 13 eggs from aviary systems and 17 eggs from cage systems) were analysed. Egg samples were extracted with acetonitrile and purified by dispersive solid phase extraction (dSPE). Analysis was performed using a 1290 Infinity II LC coupled to a 6495C Triple Quadrupole LC/MS system with iFunnel and Jet Stream Technology from Agilent Technologies, USA. Chromatographic separation was performed on a ZORBAX RRHD Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm) from Agilent using 2 mM ammonium acetate in ultrapure water (mobile phase A) and 2 mM ammonium acetate in methanol (mobile phase B).

The limit of quantification (LOQ) for four PFAS isomers ranges from 0.025 to 0.075 µg/kg. PFAS isomers were detected in 73.7% of organic eggs, 23.1% of eggs from aviaries, and 11.8% of eggs from cage systems. PFOS was identified in all egg samples that contained quantified PFAS isomers, with the highest mean concentration of 0.36 µg/kg found in organic eggs. In one organic egg sample, the concentration of PFOS was measured at 1.45 µg/kg, and the total concentration of the four isomers was 1.71 µg/kg, both exceeding the maximum permitted levels established by Regulation (EU) 2023/915 (1 µg/kg and 1.7 µg/kg). PFOA was quantified in only three organic eggs, with a mean concentration of 0.045 µg/kg. The mean PFNA values were 0.0640 µg/kg for organic eggs and 0.0284 µg/kg for eggs from cages (PFNA was not detected in eggs from aviaries). Mean PFHxS concentrations ranged from 0.033 to 0.050 µg/kg. The measured values for the sum of all four PFAS isomers across the three farming types were as follows (µg/kg): organic 0.42, aviaries 0.135, and cages 0.0843. The results of this study suggest that a much larger number of egg samples should be used to determine the exposure to these compounds through the consumption of eggs.

**Keywords:** PFAS, eggs, UHPLC-MS/MS, Croatia

**Acknowledgement:** The research was funded by the European Union - NextGenerationEU, project PFASsFoodWildlife.

I35

### PRESENCE OF POLYCHLORINATED BIPHENYLS IN DIFFERENT FISH FROM THE ADRIATIC SEA

Nina Bilandzic<sup>(1)</sup>, Maja Dokic<sup>(1)</sup>, Tamara Nekic<sup>(1)</sup>, Marija Sedak<sup>(1)</sup>, Bruno Calopek<sup>(1)</sup>, Ivana Varenina<sup>(1)</sup>, Ines Varga<sup>(1)</sup>, Bozica Solomun Kolanovic<sup>(1)</sup>

<sup>1)</sup> Laboratory for Residue Control, Department of Veterinary Public Health, Croatian Veterinary Institute, Croatia

\*Corresponding author - E-mail: bilandzic@veinst.hr

The marine environment is a primary location for the accumulation of persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs). These substances bind to suspended matter and sediments, where they are gradually released and accumulate in aquatic organisms. Despite numerous measures implemented to ban and restrict their use in an effort to reduce pollution, PCBs continue to enter the marine environment. Consequently, the highest concentrations of PCBs have been detected in fish, followed by shellfish and crustaceans. Six selected non-dioxin-like PCB congeners PCB-28, PCB-52, PCB-101, PCB-138, and PCB-153, and PCB-118 (ICES-7) are among the most frequently detected PCBs in the environment and serve as indicators of pollution.

The aim of this study was to assess the contamination levels of ICES-7 in various wild fish species. Specimens of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), sardine (*Sardina pilchardus*), and other fish were collected from different fishing zones along the Croatian Adriatic Sea between 2019 and 2023. A total of 137 fish samples were analyzed for ICES-7 using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS).

The frequency of quantification of PCB congeners above the limit of detection (LOD) varied significantly among the fish species. The lowest detection frequency was observed for PCB-28 (0-5.4%), while the highest frequencies were recorded for PCB-118 and PCB-153, ranging from 6.9% to 35.7% and 10.3% to 57.1%, respectively. Sardines exhibited the highest detection frequencies for all seven congeners. The congener profiles of the total ICES-7 were dominated by PCB-153 (26.5% to 40.5%) and PCB-138 (21.0% to 30.7%), followed by PCB-180 and PCB-101. Overall, the concentrations did not exceed the limits set by European Commission Regulation. The highest mean concentrations across all tested fish species were found for PCB-153 (0.88 to 4.35 µg/kg) and PCB-138 (0.80 to 3.80 µg/kg), followed by PCB-118 (0.25 to 2.34 µg/kg) and PCB-101 (0.29 to 2.20 µg/kg). PCB-28 and PCB-52 exhibited the lowest mean concentrations, while PCB-180 was detected only in sardines. The highest concentrations of all congeners were observed in sardines. The sum of the seven congeners (ICES-7) ranged from a lowest of 1.55 µg/kg in European seabass to a highest of 7.41 µg/kg in sardines. Statistical analysis revealed significant differences in the mean values of sum of ICES-7 among the four fish groups. Obtained results confirm the presence of these POPs in the marine environment of the Adriatic Sea.

**Keywords:** polychlorinated biphenyls, fishes, GC-MS/MS, Adriatic Sea

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136

### PFAS AND ONE HEALTH APPROACH. IMPROVEMENTS IN THE ENVIRONMENTAL, FOOD, PACKAGING, COSMETIC AND PHARMA SECTORS. WHAT ELSE?

**Emiliano De Dominicis\***<sup>(1)</sup>, **Federica Cattapan**<sup>(1)</sup>, **Andrea Vittadello**<sup>(1)</sup>, **Paola Verza**<sup>(1)</sup>, **Barbara Scantamburlo**<sup>(1)</sup>, **Alessio Mattiazzo**<sup>(1)</sup>, **Marco Redaelli**<sup>(1)</sup>, **Michela Casarotto**<sup>(1)</sup>, **Giulia Valente**<sup>(1)</sup>, **Claudia Piazza**<sup>(1)</sup>, **Cinzia Pellicorio**<sup>(1)</sup>, **Francesca Faraon**<sup>(1)</sup>, **Valentina Abbondandolo**<sup>(1)</sup>, **Sylvain Dahiot**<sup>(1)</sup>, **Sophie Robert**<sup>(2)</sup>, **Natalia Serrano**<sup>(3)</sup>, **Pascal Gonzalez**<sup>(3)</sup>, **Giampaolo Perinello**<sup>(4)</sup>, **Thomas Behnke**<sup>(5)</sup>, **Jay Alappat**<sup>(6)</sup>, **Walter Brandl**<sup>(6)</sup>, **Juan Carrasco**<sup>(7)</sup>, **Michael Ndlovu**<sup>(8)</sup>, **Robert Deng**<sup>(9)</sup>, **Asish Chakraborty**<sup>(10)</sup>, **Paolo Matteini**<sup>(11)</sup>, **Giuseppe Sammarco**<sup>(11)</sup>, **Francesco Rosi**<sup>(11)</sup>, **Cecilia Socolsky**<sup>(11)</sup>

<sup>1)</sup> Mérieux NutriSciences Italy, Italy

<sup>2)</sup> Mérieux NutriSciences, Global, France, France

<sup>3)</sup> Mérieux NutriSciences Spain, Spain

<sup>4)</sup> Mérieux NutriSciences Ireland, Ireland

<sup>5)</sup> Mérieux NutriSciences Germany, Germany

<sup>6)</sup> Mérieux NutriSciences North America, United States of America

<sup>7)</sup> Mérieux NutriSciences South America, Chile

<sup>8)</sup> Mérieux NutriSciences South Africa, South Africa

<sup>9)</sup> Mérieux NutriSciences China, China

<sup>10)</sup> Mérieux NutriSciences India, India

<sup>11)</sup> Mérieux NutriSciences Global, Italy

\*Corresponding author - E-mail: [emiliano.de.dominicis@mxns.com](mailto:emiliano.de.dominicis@mxns.com)

It was demonstrated that PFAS exposure negatively impacts the richness and biodiversity of microbiomes in different habitats, with a crucial impact in global health. According to the One Health concept, human health is closely connected to the health of animals, plants, and environments, including soil - in particular in connection with agriculture, and consequently with food safety.

The bioaccumulation and the persistence of PFAS in the environment (water, air, and soil) and the resulting spread in the food chain exponentially increase the risk of exposure, which is why investigating PFAS contamination in food and adopting timely regulations to protect public health is fundamental.

Nowadays, what about end-of-life of packaging related to extreme mobility of PFAS thinking to future restrictions under the new Packaging and Packaging Waste European Regulation? Investigating the intentional use and unintentional contamination of PFAS in packaging, not only food contact one but also cosmetic and pharmaceutical contact one, is a key action for legislative compliance and related safety.

And tomorrow? Water for animals and plants, animal feed, fertilizers, and more... are already seen as future regulatory needs to be met. Analyzing the presence of these substances throughout the entire agri-food chain will be the foundation for monitoring and mitigating this threat.

With adequate foresight.

Not just targeted.

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**Keywords:** PFAS, food, packaging, cosmetics, mass spectrometry

137

### PRELIMINARY STUDY OF PCNS CONCENTRATION IN FARM SAMPLES

**Sebastian Maszewski**\*<sup>(1)</sup>, **Malgorzata Warenik-Bany**<sup>(1)</sup>

<sup>1)</sup> National Veterinary Research Institute, Poland

\*Corresponding author - E-mail: [sebastian.maszewski@piwet.pulawy.pl](mailto:sebastian.maszewski@piwet.pulawy.pl)

**Introduction:** Polychlorinated naphthalenes (PCN) are a group of aromatic hydrocarbons which have been synthesized, commercially produced and industrially applied from 1930 due to similar to PCBs properties. Structure of this contaminants consisting of two benzene rings, where at least one of the eight hydrogen atoms in the external positions is substituted by chlorine. PCNs were also formed as by-products during the production of technical PCB mixtures. On the other hand, combustion processes responsible for the formation of dioxins, such as the burning of coal, coke, waste, biomass, or illegal waste disposal sites, cause the release of PCNs into the environment in quantities even higher than dioxins. Some of PCNs have toxic properties similar to dioxins with documented toxicity for living organisms ranging from 0.00005 to 0.004 (REP - relative potency) of toxicity of 2,3,7,8-TCDD. Structurally dioxin-like PCN flat molecules are persistent, lipophilic, stable in the environment and have the ability to accumulate in the food chain. The main toxic properties are induced by the Ah receptor include embryotoxicity, fetotoxicity, teratogenicity, immunotoxicity, and carcinogenicity. Due to the similar properties PCNs could be included to routine determination together with dioxins and PCBs with the same laboratory instrumentation.

**Material and methods:** The aim of the study was to determine of 9 PCN congeners containing from 5 to 8 chlorine atoms in the molecule (PCN-52/60, PCN-53, PCN-66/67, PCN-64/68, PCN-69, PCN-72/71, PCN-73, PCN-74, PCN-75) in farm samples. The tested materials consist of food (7 eggs, 4 cow milks), feed (15 fish meal, 2 compound feed, 4 plant origin feed) and 7 soil samples collected from individual small farms. The analysis was performed with IDMS method using the accelerated solvent extraction (ASE) and purification using chromatography on acidic silica gel, Florisil and activated carbon. Instrumental analysis was performed on HRGC-HRMS sector field mass spectrometer (DFS, Thermo Scientific). Recoveries of <sup>13</sup>C labelled standards were in the range of 37-123% and the limit of quantification for individual congeners was 0.025 pg/g of fat. The results are expressed on fat basis for food, 12% moisture for feed, and dry mass for soil as upper, medium and lower bound concentration.

**Result:** PCN was found in all samples tested. The range of determined concentrations in soil varies from 0.77 to almost 180 pg/g of dry soil. Soil average concentration was 64 pg/g dry soil. The feed concentration was lower and ranged from 0.63 to 1.62 ng/kg of feed with 12% of moisture. In the milk samples, an average total content of about 10 pg/g of fat was found, with the range of results from 4.5 to 18.75 pg/g of fat. PCN contents in the egg samples were significantly higher than milk. Average concentration was 360.63 pg/g fat, and the range was 45-867 pg/g fat. Due to contamination of the food chain, it indicates that research should be continued.

**Keywords:** PCNs, soil, feed, food

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I38

### QUANTIFICATION OF 60 PLASTICIZERS AND OTHER SELECTED ADDITIVES BY GC- AND LC-MS/MS IN GERMANY'S FIRST TOTAL DIET STUDY "BFR MEAL"

**Torsten Bruhn**<sup>(1)</sup>, **Andriy Kuklya**<sup>(1)</sup>, **Elisabeth Koch**<sup>(1)</sup>, **Ezgi Eyluel Bankoglu**<sup>(1)</sup>, **Irmela Sarvan**<sup>(1)</sup>, **Sebastian Zellmer**<sup>(1)</sup>, **Oliver Kappenstein**<sup>(1)</sup>, **Andreas Luch**<sup>(1)</sup>, **Thomas Tietz**<sup>(1)</sup>, **Alexander Roloff**<sup>(1)</sup>

<sup>1)</sup> Federal Institute for Risk Assessment, Germany

\*Corresponding author - E-mail: torsten.bruhn@bfr.bund.de

Plasticizers are used as additives in many synthetic polymers. Contamination of foods with these additives can occur along the entire food chain. Phthalates are currently the most widely used plasticizers. Some phthalates are classified as toxic to reproduction (category 1B) according to regulation (EC) No. 1272/2008 and identified as endocrine disruptors with respect to human health. Due to regulatory requirements and a growing environmental awareness, phthalate-based plasticizers are increasingly being replaced by non-phthalate alternatives.

In a recent publication, we presented an accurate multi-technique approach for the quantification of 60 plasticizers and other selected additives using GC- and LC-MS/MS at low concentrations and its application for beverages in the BfR MEAL Study [1]. The substances analyzed included but were not limited to phthalates, citrates, sebacates, adipates, succinates, azelates, benzoates, cyclohexane dicarboxylates, glycerol acetylates, and phosphates.

Here we present an extension of this approach for the analysis of various types of food matrices, which we divided into seven different commodity groups including - among others - foods with high water content, dairy products, meat and seafood, as well as foods with high fat content. The method involves matrix-dependent sample preparation and purification steps followed by GC- and LC-MS/MS analysis with limits of quantifications (LOQs) in the low  $\mu\text{g}\cdot\text{kg}^{-1}$  food range. The majority of analytes (50 out of 60) can be determined by GC-MS/MS. Additional LC-MS/MS analysis enables the determination of fatty acid amides and isomeric technical mixtures (e.g., diisononyl and diisodecyl phthalates) and allows cross-validation of results. The methods are characterized by high recoveries (80-120%) and precisions (<10%) for most investigated analytes.

This multi-technique approach was used to analyze 226 pooled samples from the BfR MEAL Study, Germany's first full-scale total diet study (TDS). Regulated plasticizers were found at very low levels. In most samples, the contents of *ortho*-phthalates were below the limit of detection (e.g., LOD for di(2-ethylhexyl) phthalate (DEHP):  $5 \mu\text{g}\cdot\text{kg}^{-1}$  food). DEHP was quantifiable in only 15% of the samples in a concentration range from 17 to  $720 \mu\text{g}\cdot\text{kg}^{-1}$  food and a median of  $45 \mu\text{g}\cdot\text{kg}^{-1}$  food. The range is comparable to the results from a UK TDS from 2007 and a Canadian TDS from 2013. However, DEHP was found in 55% of the 20 samples of the UK study and in 70% of the 159 samples of the Canadian study. Of the alternative plasticizers, acetyl tributyl citrate was found in ~46% of the samples with concentrations ranging from 0.01 to  $20 \text{mg}\cdot\text{kg}^{-1}$  while others like acetyl triethyl citrate were not detected in any food group. Erucamide was found in several food groups, mainly at levels below  $1 \text{mg}\cdot\text{kg}^{-1}$ .

[1] Food Chem., 2024, 446, 138874.

**Keywords:** plasticizer, total diet study, food, additives, multianalyte method

139

### WHAT DO 3-MCPD AND 2-CHLOROETHANOL HAVE IN COMMON? STUDY ON THE SOURCE FOR FALSE POSITIVE FINDINGS FOR 2-CHLOROETHANOL IN FOOD SUPPLEMENTS

**Anna Romanotto**\*<sup>(1)</sup>, **Deborah Bertuola**<sup>(1)</sup>, **Bruno Ramm**<sup>(1)</sup>, **Maike Greunwald**<sup>(1)</sup>

<sup>1)</sup> PiCA Prüfinstitut Chemische Analytik GmbH, Germany

\*Corresponding author - E-mail: [anna.romanotto@pica-berlin.de](mailto:anna.romanotto@pica-berlin.de)

It is known, that 2-Chloroethanol (2-CE) results of HS-GC-MS vs. GC-MS/MS analyses, particularly in food supplements, often do not match or sometimes are contrary. Especially since the ethylene oxide scandal of 2020-2021, 2-CE is analyzed as a routine parameter in the most of labs.

With our research we could prove the clear relationship between the 2-CE findings and polyethylene glycol (PEG) or ethylene glycol (EG) presence especially in food supplement samples. With this we can now provide an explanation for 2-CE as a false positive or focus the differences in the analysis results when using different methods.

Our study examined different combinations in applications of EG and PEG in the presence and absence of chloride ions for the formation of 2-CE. Before we tested and proved the development of 2-CE from EG and PEG in the injector of GC-MSMS. In the next step freshly harvested dried and powdered dandelion root samples were differently treated with EG and PEG. Clear formation of 2-CE was observed, particularly after treating the samples with EG/PEG in the presence of chloride ions (HCl) but also without HCl and measuring with GC-MSMS.

The important sources of PEG for consumers include the following:

- E1521 as a food additive [1]
- Packages: Food contact materials, various esters of PEG mentioned in the regulation [2]
- Modified PEG substances in agricultural use
- Ingredient in cosmetics tooth cleaning tablets, bath additives, liquid soaps up to 1 %

For proving of this hypothesis 30 food supplement samples were tested using HR-LC-MS for 40 different PEG compounds including esters and derivatives. In products with high levels of 2-chloroethanol using GC-MSMS we could also detect high levels of various PEG compounds.

Summarized that, the PEG presence is clear the reason for the differences in results between HS-GC-MS and GC-MSMS analyses, whereby the GC-MSMS measurement can lead to false-positive results. This cannot happen when measuring with HS-GC-MS, as glycols are not volatile, and 2-CE cannot therefore be formed in absence of glycols. The postulated path of 2-CE formation from EG/PEG is comparable to that of 3-MCPD from diglyceride ester, which happen in injector [3]. HCl as a source of the chloride can at the same time be formed from organochlorine substances in injector [4].

[1] Refined exposure assessment of polyethylene glycol (E 1521) from its use as a food additive; <https://www.efsa.europa.eu/en/efsajournal/pub/5293>.

[2] COMMISSION REGULATION (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

[3] Yue Zhao et al., Formation of 3-MCPD Fatty Acid Esters from Monostearoyl Glycerol and the Thermal Stability of 3-MCPD Monoesters, Journal of Agricultural and Food Chemistry, Vol 64/Issue 46.

[4] Gorge W., RINCPLES OF THERMAL DEGRADATION, chapter 4.1.4 HYDROGEN CHLORIDE, 2015, Pages 79-165.

**Keywords:** 2-chloroethanol, food supplements, GC-MSMS, HS-GC-MS, PEG

I40

### TRACE ANALYSIS POLYCHLORINATED DIBENZO-P-DIOXINS/FURANS ANALYSIS USING GC-MS/MS IN ACCORDANCE WITH AMENDMENTS TO EU REGULATIONS 644/2017 AND 771/2017 FOR FOOD AND FEED

**Adam Ladak**<sup>(1)</sup>, **Nicholas Warner**<sup>(1)</sup>, **Daniel Kutscher**<sup>(1)</sup>, **Kerstin Krätschmer**<sup>(2)</sup>, **Alexander Schächtele**<sup>(3)</sup>

<sup>1)</sup> Thermo Fisher Scientific, United Kingdom

<sup>2)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>3)</sup> European Union Reference Laboratory (EURL) for Halogenated POPs in Feed and Food, Germany

\*Corresponding author - E-mail: Adam.ladak@thermofisher.com

Polychlorinated dibenzo-p-dioxins/furans (PCDD/F), commonly known as dioxins, are persistent organic pollutants (POPs) classified under the Stockholm Convention. Due to their chemical stability and high fat solubility accumulate in food chains, posing a risk to human health through the consumption of food items such as dairy, meat, and fish. To protect the population, regulations are in place to monitor the presence of dioxins in food and feed. Currently, the maximum allowable levels (ML) for PCDD/Fs in feed stuffs are in the  $\text{pg}\cdot\text{g}^{-1}$  concentration range due to their toxicity. However, a recent review process has concluded that a reduction of up to 40% in these levels will be implemented in 2024 for certain feed items, such as animal/milk/egg fats ( $1.0 \text{ pg}\cdot\text{g}^{-1}$ ), fish oil ( $3.5 \text{ pg}\cdot\text{g}^{-1}$ ), and fish feed ( $1.0 \text{ pg}\cdot\text{g}^{-1}$ ). As of 2014, a change by the European commission permitted the use of gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) for the analysis of food and feed as an alternative to the GC-high resolution mass spectrometry (GC-HRMS). However, with reduction in ML for certain food items, performance of instrumentation must be assessed to demonstrate the ability to meet regulatory thresholds.

In this poster the compliance with the newly proposed regulatory limits for PCDD/F in food and feed samples according to EU regulations will be demonstrated. The Thermo Fisher Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system equipped with the NeverVent™ Advanced Electron Ionization (AEI) source was used in this analysis. To assess the instrument's sensitivity for the analysis of food and feed samples, a mixed standard containing six different of Tetrachlorodibenzodioxin (TCDD) was used at concentrations ranging between 2 to  $100 \text{ fg}\cdot\mu\text{L}^{-1}$ . Samples were provided by the European Union Reference Laboratories (EURL) in Germany and Wageningen Food Safety Research in the Netherlands and contained known trace levels of dioxins previously confirmed at these institutes. The samples were analyzed to ensure that the results were consistent with the previously determined concentrations of PCDD/F in food and feed matrices and assessed against current compliance requirements of EU regulations.

**Keywords:** dioxins, food, regulations, sensitivity

I41

### A FAST AND NOVEL WORKFLOW FOR SCREENING SMOKE FROM FOREST FIRES AFFECTING FOOD QUALITY BY SPMESH-DART-MS/MS

Terry L. Bates<sup>(1)</sup>, Francois Espourteille<sup>(1)</sup>, Eva-Maria Niehaus<sup>(2)</sup>

<sup>1)</sup> Bruker Scientific LLC, United States of America

<sup>2)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: Terry.Bates@bruker.com

Bushfire smoke can significantly impact the quality of crops (e.g., grapes, nuts, grains, herbs) used for foods and beverages in regions near the fires. When bushfire events occur near vineyards, levels of volatile phenols (VP) are monitored as markers of smoke impact via chromatography-based analyses. Inherent throughput limitations such assays often result in analytical bottlenecks when data is required within a compressed timeframe. In this work, we report a rapid, chromatography-free screening method for routinely monitored VP using Solid Phase Mesh Enhanced Sorption from Headspace coupled to Direct Analysis in Real Time-Mass Spectrometry (SPMESH-DART-MS). This workflow provides enhanced data quality, faster results, and lower cost per sample as compared to conventional analytical methods.

The screening workflow includes the VP 4-ethylphenol, 4-ethylguaiacol, guaiacol, 4-methylguaiacol, and o-cresol. Sample preparation (~1.5 hours) was performed for 24 samples in parallel. Following sample preparation and extraction, the SPMESH sheet was transferred to the automated positioning stage of an EVOQ DART-TQ<sup>+</sup> triple quad mass spectrometer. MS/MS parameters were optimized (collision energies, collision cell pressure, scan speed). Matrix matched calibration QC were analyzed, using d3-guaiacol as IS for all compounds. Regression curves were analyzed at 6 calibration levels in quadruplicate including matrix blanks. Recovery was assessed using 2 QCs analyzed in quadruplicate.

The automated DART-MS/MS analysis of 24 samples takes just 12 minutes. Data processing with a standard MS quantitation software showed linear regressions of  $R^2 \geq 0.99$  and recoveries of 90 - 110% at 5 µg/L and 25 µg/L concentrations. The total workflow time for 24 samples was < 1.75 h. Full workflow simplicity, sample throughput, and data quality meet or exceed the accepted metrics of conventional approaches.

**Keywords:** food quality, DART, wine, phenols, SPME

I42

### ENHANCED COMPOUND IDENTIFICATION IN NON-TARGET ANALYSIS USING A NOVEL GC-HRMS WITH SIMULTANEOUS EI AND CI

Eliska Ceznerova<sup>\* (1)</sup>, Sonja Klee<sup>(1)</sup>, Steffen Bräkling<sup>(1)</sup>, Arnd Ingendoh<sup>(2)</sup>, Eva-Maria Niehaus<sup>(2)</sup>

<sup>1)</sup> TOFWERK AG, Switzerland

<sup>2)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: [Eliska.ceznerova@tofwerk.com](mailto:Eliska.ceznerova@tofwerk.com)

Non-target analyses in GC-HRMS using electron ionization (EI) as ionization method suffer from unambiguous or insufficient scores in NIST searches of EI data and are prone to false positives. This often is caused by missing molecular ion information in EI mass spectra. Therefore, often additional GC runs are performed with a soft ionization method as for example chemical ionization (CI) to add the molecular ion information for filtering the NIST search results, when appropriate reference standards for compounds are not available or compounds are not listed in the library at all. However, that requires mechanical ion source switching, separate subsequent GC runs which increases instrument down-time as well as failure rates and generates issues with peak alignment between EI and CI runs. Presented here is a novel GC-HRMS which simultaneously operates an EI and a CI source on one single mass analyzer. Structural as well as accurate mass molecular ion information are generated in a single GC run which highly improves the confidence for identifying unknown compounds. Various studies will be discussed to proof the potential for non-targeted and suspect screening approaches, including applications in fields such as environmental contaminants or material emissions.

An 8500 GC was coupled to the ecTOF (Bruker, Bremen, Germany), using a 70 eV EI source and the novel HRP CI source [1,2]. Various GC methods and sampling procedures were employed depending on the analytical need of the study, including liquid injection of extracted samples, headspace sampling including SPME and thermal desorption using Tenax tubes. To generate the ideal molecular ion information different reactant ions (e.g.,  $N_2H^+$ ,  $H_3O^+$  and  $NH_4^+$ ) were used for the chemical ionization process.

The performance of the EI/CI-TOF is feasible for standard procedures employed by routine laboratories, e.g., target analysis for material emissions or steroid screening. Whilst standard GC-MS methods mainly focus on target analysis, especially suspect screening and non-target analysis is enabled and improved by the EI/CI-TOF. Especially when EI library hits are only accounted as "fair" with low corresponding probability, the additional chromatographic and CI information can be used to increase compound identification confidence. False positives from an EI-only approaches can easily be identified and often correctly annotated using the additional information generated by CI. Furthermore, compounds not listed at all in libraries have a high uncertainty for identification using an EI-only approach. Using the accurate mass information on the molecular ion provided by CI, sum formula for these unknowns can be derived. Combining this molecular information with the structural information generated by EI, tentative structure elucidation becomes feasible in many cases.

[1] Bräkling, S. *Anal. Chem.* 2022., 94 (15): 6057-6064.

[2] Bräkling, S. et al. *J. Rapid Commun. Mass Spectrom.*, 2023. 37:e9461, 3, 499-509.

**Keywords:** GC-HRMS, non-targeted analysis, food quality, compound identification, contaminants

I43

### DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN VEGETABLE ORIGIN FOODSTUFFS BY QUECHERS EXTRACTION AND LC-MSMS DETECTION

Simone Pellegrini<sup>(1)</sup>, Gianluca Facchinetti<sup>(1)</sup>

<sup>1)</sup> *Water & Life Lab Srl - Groupe Carso, Italy*

*\*Corresponding author - E-mail: simone.pellegrini@waterlifelab.it*

Per- and polyfluoroalkyl substances (PFAS) are gaining relevant attention as contaminants in environmental, food and health field due to their persistence and ubiquitous presence, which makes them known as “forever chemicals”.

The European Commission recently established limit values in Regulation (EU) 2023/915 for four of the most toxic PFAS in several animal origin products. In August 2022 was also published the Recommendation (EU) 2022/1431 that aims to monitor the levels of PFAS in a wide range of foodstuffs suggesting warning levels and therefore appropriate limits of quantification (LOQs) for PFOS, PFOA, PFNA and PFHxS. In addition, the Recommendation lists several other relevant PFAS to be monitored to collect as much data as possible. For vegetable origin and infant foodstuffs, suggested LOQs are quite analytically challenging: 0.002 µg/kg for PFOS, 0.001 µg/kg for PFOA and PFNA, 0.004 µg/kg for PFHxS.

The method developed allows to analyze a list of 24 PFAS in high and low water content vegetable origin matrices with a modified QuEChERS extraction, followed by a high-performance LC/MSMS system determination. No SPE purification step is involved in the procedure, since the most common weak anion exchange SPE cartridges available during the preliminary tests showed some poor recovery rates for some of the tested analytes. Therefore, the increase in sensitivity and the achievement of low LOQs requires a 5-fold concentration of sample extracts prior to injection in the LC/MSMS system. The use of isotopically labelled PFAS analogues, co-injected with sample extracts, compensates the possible matrix effects in instrumental response.

This approach allows to quantify PFOS and PFHxS at the LOQ suggested by Recommendation (UE) 2022/1431 (0.002 µg/kg and 0.004 µg/kg respectively), while for PFOA and PFNA the validated LOQ is 0.002 µg/kg, which is higher than the suggested LOQ (0.001 µg/kg), but below the lowest indicative level proposed by the Recommendation (0.005 µg/kg). For many of the other 20 PFAS determined, the reached LOQ is 0.010 µg/kg.

For the validation, three matrices were tested: apple, rice and spinach, at three different concentration levels: 0.002 µg/kg (PFOS, PFOA, PFNA, PFHxS), 0.010 µg/kg and 0.020 µg/kg. Recovery rates are included in the range between 80% and 110% and repeatability over 10 replicates showed RSD values below 10%.

**Keywords:** PFAS, foodstuffs, LC/MS-MS, QuEChERS



**I44**

### **DETERMINING PFAS IN FRUITS, VEGETABLES AND BABY FOOD USING LC-MS/MS**

**Claudia Rathmann**<sup>(1)</sup>, Nicola Dreolin<sup>(2)</sup>, Kari Organtini<sup>(2)</sup>, Stuart Adams<sup>(2)</sup>, Oliver Burt<sup>(2)</sup>

<sup>1)</sup> Waters GmbH, Germany

<sup>2)</sup> Waters Corporation, United Kingdom

\*Corresponding author - E-mail: claudia\_rathmann@waters.com

Per- and Polyfluoroalkyl Substances (PFAS) are a class of synthetic compounds widely used in various industrial and commercial applications, known for their persistence and potential adverse health effects. Regulatory bodies have increasingly prioritized stringent monitoring and control of PFAS levels in food due to their presence in agricultural environments and food chains, posing potential health risks to consumers.

This study focuses on the development of an optimized, sensitive and reliable LC-MS/MS methodology for the detection and quantification of ultra-trace levels of the 28 European Union regulated and recommended PFAS in fruits, vegetables and baby food. The method demonstrates an exceptionally low limit of quantification, reaching 0.0005 µg/kg for some compounds in matrix, while accurately detecting and quantifying the PFAS compounds listed in the Commission Recommendation 2022/1431 while meeting the acceptance criteria for Commission Implementing Regulation 2022/1428 for the control of PFAS in certain foodstuffs. The combination of enhanced sensitivity of the Xevo™ TQ Absolute Tandem Quadrupole MS System, with the increased clean-up efficacy of a bilayer dual-phase GCB/WAX SPE cartridge gave excellent recoveries, between 87 and 116% for the mandatory PFAS, and between 65 and 131% for most of the recommended compounds along with repeatability (RSD<sub>r</sub>) ≤10%.

**Keywords:** PFAS, EU 2022/1431, food safety, food of plant origin, baby food

I45

### IDENTIFICATION OF SHORT CHAIN PER AND POLYFLUORINATED ALKYL SUBSTANCES (PFAS) USING ION RATIOS WITH LOW MASS PRODUCT IONS

Claudia Rathmann<sup>(1)</sup>, Stuart Adams<sup>(2)</sup>, David Gordon<sup>(2)</sup>, Kate Whyatt<sup>(2)</sup>

<sup>1)</sup> Waters GmbH, Germany

<sup>2)</sup> Waters Corporation, United Kingdom

\*Corresponding author - E-mail: claudia\_rathmann@waters.com

As the environmental impact and health hazards of legacy long chain ( $\geq C8$ ) PFAS emerged, the compounds started to be substituted for short (C4-C7) and ultrashort chain ( $C\leq 3$ ) PFAS in manufacturing. The belief was these would have similar performance but less of an impact. However, the initial evidence suggests that there are still concerns regarding their effects. The analysis of carboxylate PFAS with chain lengths C3-C4 is problematic in terms of positively identifying these analytes due to the lack of a second product ion with suitable intensity to use for ion ratio calculations to meet common identification criteria. The use of  $m/z$  19 is possible but has largely been ignored due to its low mass being outside the performance envelope of many modern mass spectrometers. This response can be significantly improved using a modified high performance tandem quadrupole mass spectrometer.

For several short and ultrashort chain PFAS, predominately carboxylates with a carbon chain length of 5 or less (PFPrA, PFBA and PFPeA), there has been no mass product ion ( $m/z \geq 50$ ) identified to use to create ion ratios. Initial work has been carried out on a high-performance negative ion tandem quadrupole mass spectrometer to show that the detection of a fluoride ion ( $m/z$  19) was possible using the standard configuration, but the ion ratios measured were between 0.001 to 0.002. By modifying the mass spectrometer, improved sensitivity for the fluoride ion was achieved and initial results show the increase in response was 2 orders of magnitude. Using this modified system, the ion ratios measured were between 0.054 and 0.074 making confirmation of analyte identity by ion ratio a realistic possibility with this low mass product ion.

**Keywords:** PFAS, low mass product ions, Compound confirmation, EURL POP Guidance Document for PFAS, TFA PFPrA PFBA PFPeA

146

### SIMPLE AND EFFICIENT SPE AUTOMATION AND UPLC-MS/MS ANALYSIS OF PFAS IN MILK

**Cecile Pinto**<sup>(1)</sup>, **Daniel Ng**\*<sup>(2)</sup>, **Marcus Devakishen**<sup>(3)</sup>, **Kai Xiang Kuah**<sup>(3)</sup>, **Stuart Adams**<sup>(4)</sup>, **Joanne Ho**<sup>(4)</sup>

<sup>1)</sup> WATERS SA, France

<sup>2)</sup> Waters Asia Pacific Analytical Professional Services, Waters Corporation, Singapore

<sup>3)</sup> Waters Asia Pacific Marketing, Waters Corporation, Singapore

<sup>4)</sup> Waters Corporation, United Kingdom

\*Corresponding author - E-mail: daniel\_ng@waters.com

Per- and Polyfluoroalkyl Substances (PFAS) are a group of man-made chemicals that have multiple fluorine atoms attached to an alkyl chain which is used in many applications, some of which include packaging, surfactants, and firefighting fluids. Due to their wide-spread use and persistence in the environment, PFAS has been found in routes into humans via water, food and air, resulting in PFAS being detected in humans. The European Food Safety Authority (EFSA) predicts food to be a predominant channel for PFAS exposure, and regulatory bodies around the world have been placing increasingly stringent regulations on the testing and monitoring of PFAS in food. Milk, being the staple food product for infants and young children, would affect them disproportionately compared to adults, as such there is a need to monitor PFAS levels in milk matrices. To achieve quantification at low levels of PFAS in food matrices, sample preparation is required which comprise of many pipetting steps and can be time-consuming and error prone. The aim of this study is to automate as much of the workflow as possible for the analysis of PFAS in milk through the use of pipetting robots, stacked dual-phase solid-phase extraction (SPE) cartridges, and high sensitivity LC-MS/MS to meet EU 2022/1431 levels for PFAS in milk

The automation workflow developed in this study includes creating calibration standard solutions, QC samples, and SPE with Oasis GCB/WAX bilayer cartridges using Andrew+ Pipetting Robot and the Extraction+ Connected Device. Approximately 350 steps are automated when analyzing 12 milk samples, saving approximately 3.5 hours of analyst time. Sample were analyzed with enhanced sensitivity Acquity™ Premier UPLC coupled with Xevo™ TQ Absolute MS System, and the method was validated at 0.005 µg/kg for milk. When considering the entire panel of native PFAS together, mean percentage recovery across all fortification levels was 102 ± 18% for milk (min = 40.6%, max = 123.6%). For the four mandatory compounds for monitoring, PFOS, PFOA, PFNA, and PFHxS, apparent recoveries were between 98 and 118%. The use of automation improves efficiency and reduces errors especially in laboratories with multiple operators. A within-laboratory matrix group validation was carried out with 2 operators, over 3 non-consecutive days, and with 5 different milk matrices. For the mandatory PFAS compound for monitoring, majority of apparent recovery values were within ± 20% of the expected values.

**Keywords:** PFAS, food contaminants, food safety

I47

### SIMPLE AND FAST METHOD FOR PAHS QUANTIFICATION IN OIL & OIL-RICH FOOD USING MOLECULARLY IMPRINTED POLYMERS EXTRACTION

Gisele Usanase<sup>(1)</sup>, Aurelie Cortejade<sup>(1)</sup>, Cecile Vincent<sup>(1)</sup>, Roxane Chene<sup>(1)</sup>, Sami Bayoudh<sup>(2)</sup>, Michel Arotçarena<sup>\*(2)</sup>, Kaynoush Naraghi<sup>(2)</sup>, Mickaël Nicolas<sup>(1)</sup>

<sup>1)</sup> CARSO-LSEHL, France

<sup>2)</sup> AFFINISEP, France

\*Corresponding author - E-mail: michel.arotcarena@affinisep.com

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of organic compounds with two or more fused aromatic rings and are known to be carcinogenic. Human beings are exposed to PAHs mainly by intake of food. As these compounds are highly soluble in lipophilic matrices, oils and oils rich food can be an important source of contamination by PAHs. U.S. Environmental Protection Agency (EPA) has designated 16 PAHs as priority pollutants due to their potential toxicity in humans and other organisms and their prevalence and persistence in the environment. In addition, in 2023, EU Commission Regulation 2023/915, amending Regulation 835/2011 has set maximum levels in edible oils to 2µg/Kg of benzo[a]pyrene individually, and 10 µg/Kg of benzo[a]pyrene, benzo[b]fluoranthene, chrysene and benzo[a]anthracene combined.

Analysis of PAHs in certain matrices remains challenging, necessitating meticulous sample preparation to preserve the integrity of analytical devices and to reach lower limit of quantification (LOQ).

In this study, up to 25 PAHs compounds were analyzed by GC-MS/MS. Two extraction methods of PAHs in oil and oil rich food were compared: QuEChERS extraction, and Molecularly Imprinted Polymers (MIPs) applied to Solid Phase Extraction (SPE). Although, QuEChERS is a cost-effective solution, it does not always provide a thorough clean-up contrary to MIP extraction which allows a thorough sample cleaning. MIP sorbent also brings higher selectivity in comparison to some other extractions methods like classic polymeric or silica based SPE prior to analysis.

Better results were obtained with MIP extraction than with QuEChERS extraction. MIP cartridges were more selective, resulting in lower background interferences, facilitating the detection of lower concentrations of PAHs. Except for Naphthalene, after extraction, LOQs were 4 to 20 times lower with MIP compared to QuEChERS. The MIP extraction method was therefore chosen.

The developed method was validated in accordance with EU Commission Regulation 2023/915 of 25 April 2023 on established maximum levels for certain contaminants in food. 25 PAHs were validated in sunflower oil and 18 PAHs in avocado and walnut oil with limits of quantification (LOQ) at 0.5-1µg/Kg (except for naphthalene at 10µg/Kg). Excellent results were obtained with recovery yields ranging between 70% and 110%. Relative standard deviations were determined between 5% and 25%.

**Keywords:** polycyclic aromatic hydrocarbons (PAHs), oils, molecularly imprinted polymers (MIP), sample preparation, SPE

I48

### TRACE ANALYSIS OF 30 PERFLUORINATED COMPOUNDS IN SALMON TROUT AND TAP WATER USING SOLID PHASE EXTRACTION (SPE)

Sami Bayoudh<sup>(1)</sup>, Michel Arotçarena<sup>\* (1)</sup>, Kaynoush Naraghi<sup>(1)</sup>, Corentin Germain<sup>(1)</sup>, Juliette Gouley<sup>(1)</sup>, Louna Moles<sup>(1)</sup>

<sup>1)</sup> AFFINISEP, France

<sup>\*</sup>Corresponding author - E-mail: michel.arotcarena@affinisep.com

Perfluorinated compounds (PFAS) are a family of molecules composed of fluorocarbon chains of variable length and a functional group such as a carboxylic or sulphonic acid. They have been widely used since the 1950s in many products, such as fire-fighting foams, non-stick and hydrophobic coatings, and surfactants. Their composition makes them particularly resistant to chemicals, so they tend to accumulate in organisms and in the environment. Perfluorinated compounds have come under increasing scrutiny in recent years because of their alleged harmful effects on human health. Analysis of PFAS in certain matrices remain challenging, necessitating meticulous sample preparation to preserve the integrity of analytical devices and to reach lower limit of quantification (LOQ).

Polymer-based solid-phase extraction (SPE) has proven to be effective for the analysis of PFAS, as it can be used for concentration, purification and quantification in foodstuffs. The addition of a layer of Graphitized Carbon Black (GCB) allows a better cleaning for the most complex matrices.

In this work, a list of 30 historical and emerging PFAS were analyzed in salmon trout and in drinking water (tap water) using SPE to concentrate and purify the samples. 2g of salmon trout samples were spiked with the 30 PFAS at 0.2-100 µg/Kg and solvent extracted. Then, the extraction solutions were purified with a bi-layer SPE consisting of a polymeric phase and Graphitized Carbon Black (GCB).

For tap water polymeric SPE cartridges were used to treat 500mL samples spiked with the 30 PFAS at 0.004-0.4 µg/Kg. The analyses were carried out by LC-MSMS in addition with a delay column to remove the PFAS from LC solvent and tubing.

In salmon trout, the recoveries were higher than 81% for 28 compounds. PFHxDA and PFODA have shown lower but acceptable recoveries at 73% and 51% respectively. They all showed good repeatability with an average RSD value of 6% and a maximum of 18% for PFODA.

In tap water, the recoveries were higher than 90% for 29 compounds. MeFOSA has shown lower but acceptable recoveries at 66%. They all showed good repeatability in tap water with an average RSD value of 5% in tap water and a maximum of 12% for PFODA.

**Keywords:** perfluorinated compounds (PFAS), SPE, fish, water, sample preparation

149

### ULTRA-FAST MRM ACQUISITION AND QUANTITATION OF FOOD CONTAMINANTS IN MULTIPLE FOOD MATRICES

Jianru Stahl-Zeng<sup>(1)</sup>, Andre Schreiber<sup>(1)</sup>, Gitte Barknowitz<sup>(1)</sup>, Ian Moore<sup>(1)</sup>, Hassan El Khallabi<sup>(2)</sup>

<sup>1)</sup> SCIEX, Germany

<sup>2)</sup> Normec Groen Agro Control, The Netherlands

\*Corresponding author - E-mail: [jianru.stahl-zeng@sciex.com](mailto:jianru.stahl-zeng@sciex.com)

The intensive use of pesticides in agriculture has led to the need for rigorous and extensive use of analytical technologies to ensure that there is no impact on human populations. Mycotoxins are toxic fungal secondary metabolites that frequently contaminate food and feed worldwide, and hence represent a major hazard for food and feed. Maximum residue limits (MRL) are set for regulated residues that define the highest level of a pesticide or mycotoxin residue that is legally tolerated in food such that it is safe for consumers. Often these MRLs are set at low ppb level, to ensure highest safety, very sensitive, robust instrumentation and fast workflows are required to quantify these compounds down to their MRL.

Representative commodities were chosen according to guideline. Stock solutions were made that contained over 800 different pesticides and mycotoxins. Samples were then prepared using QuEChERS preparation protocol. Dilute and shoot approach is used after extraction without any further SPE preconcentration. The column used was reverse phase C18. 1 µL sample injected for each HPLC run is used. These experiments were performed using a triple quadrupole LC-MS/MS system. The system was operated in electrospray ionization (ESI) mode. To cover the full range of pesticide and mycotoxin, both positive and negative ionization mode were used, with rapid polarity switching, so all compounds could be quantified by only one injection. Scheduled MRM was used to analyze over 1400 MRM transitions.

When performing a method with such a vast number of compounds it is important to ensure that the quality of the data is not compromised and that every compound can be effectively quantified. Methods show very high sensitivity to meet different regulations and guidelines. For an assay with over 1500 MRM transitions to analyze, time scheduling of MRMs and instrument switching speed are keys to maintain high quantitative quality. Fast polarity switching time of the instrument ensures good data sampling rates are obtained across the LC peaks for different retention times. Accurate, robust fast quantitation results are presented align with SANTE guideline. Multiple MRMs per analyte also enabled ion ratio monitoring to ensure confident detection. Long batch of several days sample measurements with the representative matrices was run to demonstrate robustness of the method. Improved polarity switching speed allowed for improved quantitation quality for early eluting, polar pesticides. The ability to analyze such a broad panel of residues in a single injection saves significant time and resources in testing labs. Furthermore, a combined method for both pesticide and mycotoxin was developed. This combined method saves additional time for residue analysis in food testing labs.

**Keywords:** pesticides, mycotoxins, contaminants, rapid polarity switching, large multi-method

**Acknowledgement:** *The authors would like to thank Normec Groen Agro Control for their collaboration.*

## FOOD CONTAMINANTS (ENVIRONMENTAL)

I50

### QUANTITATION OF PFAS IN FOOD WITH PARTS PER TRILLION LEVELS OF SENSITIVITY

**Daniel McMillan**<sup>\*(1)</sup>, **Jianru Stahl-Zeng**<sup>(1)</sup>, **Michael Scherer**<sup>(1)</sup>, **Xanthippe Theurillat**<sup>(2)</sup>, **Ashley Grffin**<sup>(3)</sup>

<sup>1)</sup> *SCIEX, United Kingdom*

<sup>2)</sup> *Société des Produits Nestlé, Nestlé Research, Switzerland*

<sup>3)</sup> *Société des Produits Nestlé, Nestlé Quality Assurance Centre, United States of America*

*\*Corresponding author - E-mail: daniel.mcmillan@sciex.com*

This poster demonstrates a validated method for the analysis of 57 per- and polyfluoroalkyl substances (PFAS) compounds in a diverse range of food matrices. Using the SCIEX 7500 system and a simplified extraction method, the limit of quantitation (LOQ) levels met the Commission Regulation EU 2022/2388 for the 4 regulated PFAS compounds, including: PFOS, PFOA, PFNA and PFHxS. Specifically, LOQs were as low as 0.01 µg/kg for some PFAS in the food matrices (Table 1). The low LOQ levels were due to the sensitivity of the SCIEX 7500 system and the extensive contamination reduction steps that were implemented to reduce the PFAS background signal. The 7 food matrices tested included, baby food puree (beef), milk-based infant formula (as sold), full cream milk powder, fish, whole egg, soluble coffee and fish oil. The method showed good precision at the LOQ level for the 4 regulated compounds. The %CV was <20% for most matrices and <25% for all matrices. For more information refer to the publication on which this technical note is based.

Key features of PFAS analysis in multiple foods when using the SCIEX 7500 system:

- Broad PFAS coverage. A method was developed and validated for 57 PFAS compounds following the EURL POPs guidelines.
- Diverse food matrices. Multiple food matrices were tested including baby food puree (beef), milk-based infant formula (as sold), full cream milk powder, fish, whole egg, soluble coffee and fish oil.
- Parts-per-trillion (ppt, µg/kg) sensitivity. LOQs in food matrices analysed were as low as 0.01 µg/kg.

**Keywords:** PFAS, EU regulation, LC-MS/MS

I51

### UNVEILING THE COMPLEXITY OF ADVANCED GLYCATION END PRODUCTS (AGES): A NEW HILIC MS/MS METHOD FOR SIMULTANEOUS ANALYSIS OF 19 AGES AND THEIR COELUTING ISOMERS IN FOOD MODEL SYSTEMS

**Maria-Anna Kolovou<sup>(1)</sup>, Misha Vrolijk<sup>(2)</sup>, Dick Sijm<sup>(3)</sup>, Frederik-Jan Schooten<sup>(2)</sup>, Stefan Van Leeuwen<sup>\*(1)</sup>**

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>2)</sup> Department of Pharmacology and Toxicology, Faculty of Health, Medicine and Life Sciences, Maastricht University, The Netherlands

<sup>3)</sup> Netherlands Food and Consumer Product Safety Authority (NVWA), The Netherlands

\*Corresponding author - E-mail: stefan.vanleeuwen@wur.nl

Advanced Glycation End products (AGEs), a group of process-induced contaminants, have garnered significant attention in food safety research. They are formed through the Maillard reaction, a non-enzymatic process occurring in both food systems and the human body. This reaction involves the glycation of reducing sugars with amino groups and is induced by heat. AGEs play a dual role in food and health: while contributing to the flavor and quality of processed foods, excessive accumulation in the human body is potentially linked to adverse health effects. Earlier work from our laboratory has shown that dietary AGEs (dAGEs) can induce an inflammatory response in human macrophage-like cells. Therefore, the development of improved methods for the accurate quantification of AGEs in food and biological samples is crucial.

From an analytical perspective, the detection of AGEs presents significant challenges due to their high polarity, which complicates retention in chromatographic analysis. Moreover, some AGEs exist in various isomeric forms, and separating these isomers chromatographically has been an unmet challenge until now. While previous methods, such as reversed-phase liquid chromatography (RPLC), have provided high detection accuracy, they have not succeeded in separating AGE isomers or resolving issues related to retention without using mobile phase additives like ion-pairing agents and/or serious co-elution effects.

This study addresses these analytical difficulties by developing a novel hydrophilic interaction liquid chromatography (HILIC)-based method that successfully achieves both retention and isomer separation of AGEs. Specifically, the method enables the simultaneous detection of 19 AGEs in a single run. The method includes AGEs derived from arginine, lysine, and cysteine, as well as complex cross-links between lysine-lysine and lysine-arginine residues. To enhance chromatography, four different HILIC columns, the composition of the mobile phase, the gradient profile, run-time and temperature were assessed and optimized to achieve high resolution and symmetric peak shapes. Subsequently, the developed method was effectively applied to standard solutions and food model systems (i.e. a protein and reducing sugar mixed and heated to mimic food processing conditions). Looking ahead, these data suggest that, after method validation, this HILIC chromatography approach may be applied not only to food model systems, but also to real food and biological samples. This can substantially advance the database on AGEs occurrence in foods under processing conditions, allowing more accurate exposure assessments. To further advance the scientific quality of Maillard research, it would be beneficial to measure various AGEs in target organs or providing conclusive evidence that the compounds reach reaction sites, such as the kidneys.

**Keywords:** hydrophilic interaction liquid chromatography (HILIC), advanced glycation end products (AGEs), Maillard reaction, thermal processing contaminants, food safety

**Acknowledgement:** This research is supported by the Dutch Food and Consumer Safety Authority (NVWA). We furthermore acknowledge the analytical support of Henk Gerritsen and Stanislava Vonsovic for the analysis of AGEs at Wageningen Food Safety Research (WFSR).



I52

### **BENCHMARK OF NON-TARGETED LC-HRMS DATA PROCESSING SOFTWARE TO DEVELOP A STANDARDIZED WORKFLOW FOR ASSESSING CONTAMINATION OF DIFFERENT FOOD MATRICES**

**Deborah Martin<sup>(1)</sup>, Julien Parinet<sup>\*(2)</sup>**

<sup>1)</sup> ANSES, France

<sup>2)</sup> ANSES, Laboratory of Food Safety, France

\*Corresponding author - E-mail: julien.parinet@anses.fr

Many chemical contaminants are present in the environment because of human activity (agriculture) or unintentional environmental contamination. The fate of these compounds can be manifold: degradation, transformation into one or several metabolites and the migration. These compounds, including parent molecules and metabolites, can end up in food and cause health problems chronic exposure of populations. It is therefore important to have the necessary tools to quickly detect the presence of contaminants in order to take adequate health safety measures.

Today, analytical methods developed by laboratories are more and more efficient, making it possible to analyze a wide range of compounds and reach ever lower detection limits. In order to extend the scope to still unknown compounds or newly discovered compounds without available standards, non-targeted analyses can be carried out using high-resolution mass spectrometers (HRMS) coupled with liquid and gas chromatography (LC and GC). Several approaches can then be used to process data, such as the suspect screening approach, using online available mass spectra lists, or the non-targeted approach, using the various characteristics of the peak (isotopic profile, exact mass, MS<sup>2</sup> spectra) to propose a possible structure.

Several software for reprocessing LC and GC-HRMS data have therefore been developed. These different softwares have the same important steps of reprocessing, namely peak picking and deconvolution. An identification step then allows the annotation of peaks in comparison with lists of mass spectra. The aim of this project was the study of the different software parameters in order to find the right compromise for a single workflow to process different food matrices.

In this study, four software have been investigated: XCMS Online, MS-DIAL, MZmine and Compounds Discoverer. These softwares were chosen because they include open access and paid software, software developed by independent developers or HRMS manufacturers, or even online or downloadable software. To this end, QCs containing a reduced number of compounds were used for the evaluation of each software through testing different parameters of peak picking and deconvolution steps. The identification step was realized with mass spectra list imposed or to be implemented depending on the software. The evaluation was done on the number of matches between the list and the present compound for each software. To have a global view, other parameters such as accessibility, execution speed, file storage as well as the range of modifiable parameters were taken into account to choose the best software.

Once the best workflow had been determined, several QCs of different food matrices comprising a wider range of compounds were reprocessed with this workflow to assess its effectiveness. This enabled us to validate the standardization of this workflow to perform suspect screening analysis in different matrices studied in our laboratories.

**Keywords:** LC-HRMS, suspect screening, processing software, workflow, food contaminants

153

### FASTER ANALYSIS FOR FOREVER CHEMICALS: ACCELERATING PFAS ANALYSIS WITH UPLC-HRIM-MS

Alan McKenzie-Coe<sup>(1)</sup>, Jeremy Koelme<sup>(2)</sup>, Emma Rennie<sup>(3)</sup>, David Weil<sup>(3)</sup>, Paul Stelben<sup>(3)</sup>, Sarah Stow<sup>(3)</sup>, Krystal Pollitt<sup>(4)</sup>, David Godri<sup>(5)</sup>, Frederick Strathmann<sup>\*(1)</sup>, Daniel DeBord<sup>(1)</sup>

<sup>1)</sup> MOBILion Systems, United States of America

<sup>2)</sup> Innovative Omics, United States of America

<sup>3)</sup> Agilent Technologies, United States of America

<sup>4)</sup> Yale University, United States of America

<sup>5)</sup> 3rd Floor Solutions, Canada

\*Corresponding author - E-mail: frederick.strathmann@mobillionsystems.com

Per- and Polyfluoroalkyl Substances (PFAS) are chemicals that are resistant to degradation and are widely used in industrial and consumer products. The accurate detection and identification of these compounds is of growing importance to environmental characterization and regulation. The high chemical diversity of these chemical species, which includes numerous isomeric forms, makes comprehensive identification and quantitation difficult to impossible with existing techniques. This challenge is underscored by the lack of sufficient informatics tools for characterization. This work introduces a novel high-resolution ion mobility (HRIM) workflow that improves both the speed and depth of analysis for complex PFAS samples while also providing enhanced functionality for data interpretation.

The analytical performance of the workflow was assessed using a technical perfluorooctanoic acid standard (T-PFOA, Wellington Labs) and the NIST SRM 8690, which is a standardized Aqueous Film-Forming Foams (AFFF) Formulation that contains a wide variety of PFAS components. These standards were characterized with a UPLC-HRIM-MS workflow consisting of an Agilent 1290 RPLC column using gradient elution into a MOBIE<sup>®</sup> high-resolution ion mobility system (MOBILion Systems) coupled to an Agilent 6546 LC/Q-TOF mass spectrometer. Data were processed through Agilent IMMS Browser for visualization and feature extraction. Feature lists were imported and analyzed using novel ion mobility-enabled workflows implemented in FluoroMatch IM v1.2 (Innovative Omics) for feature identification, annotation, and visualization.

Using an optimized method from the T-PFOA experiments, we investigated an AFFF mixture which contains hundreds of highly isomeric and isobaric PFAS compounds. FluoroMatch IM provides interactive visualizations combining mass spectrometric evidence (CCS, drift time, retention time, and accurate mass) to discern PFAS homologous series and structures from non-targeted datasets. FluoroMatch IM performs CCS matching across 194+ PFAS contained in the CCS library. Leveraging FluoroMatch for feature annotation and visualization, we were able to identify novel species typically unresolved by LC alone. In particular, the patterned structure of various PFAS series generate reproducible relationships in  $m/z$  and mobility space. This behavior combined with Kendrick mass defect analysis allowed us to create trendlines that enabled interpolation and extrapolation to assist in the annotation of unknown PFAS without the need for purified individual standards.

**Keywords:** PFAS, 2D chromatography, HRMS, QTOF, isomer

**Acknowledgement:** Thomas Lubinsky

**I54**

### **SIMULTANEOUS ANALYSIS OF PESTICIDES IN WATER USING AI PEAK INTEGRATION**

**Waldemar Weber**\*<sup>(1)</sup>

<sup>1)</sup> Shimadzu Europa GmbH, Germany

\*Corresponding author - E-mail: [wwe@shimadzu.eu](mailto:wwe@shimadzu.eu)

The global problem of water pollution is jeopardizing the collective health. It occurs when harmful substances like chemicals or microorganisms contaminate oceans, lakes, streams, rivers or any other body of water, degrade water quality and render it toxic for humans, animals or the environment. In order to measure the multitude of pesticides and toxins in water, the multi-component simultaneous analysis of GC-MS is used. Among the tasks for chromatographers, peak integration is the one that has some difficulty to fit in. Most of the public methods or official analytical documents rarely define a strict rule for peak integration. Therefore, peak integration may be a process that has a risk for missing sufficient analytical objectivity. Peakintelligence for GCMS is an option software for LabSolutions Insight GCMS, which is trying to resemble the way of peak integration by professionals. This is totally independent of person, because there are no parameters for peak integration.

**Keywords:** peakintelligence, GCMS

I55

### VACUUM AND MINIATURIZED SOLID PHASE MICROEXTRACTION ANALYSIS OF PESTICIDES IN GRAPES

Mereke Alimzhanova<sup>(1)</sup>, Yerkanat Syrgabek<sup>\*(1)</sup>

<sup>1)</sup> Al Farabi Kazakh National University, Center of Physical-Chemical Methods of Research and Analysis, Kazakhstan

<sup>\*</sup>Corresponding author - E-mail: serkanat96@gmail.com

The contamination of grapes and products made from grapes with pesticide residues poses a serious risk to human health [1]. With growing concerns about food safety, it is essential to develop reliable, sensitive, and efficient analytical methods for detecting and quantifying pesticide residues [2]. This study aims to compare two advanced extraction methods: vacuum headspace solid-phase microextraction (Vac-HS-SPME) and miniaturized solid-phase microextraction (mini-HS-SPME). Both methods are used in combination with gas chromatography-mass spectrometry (GC-MS) to analyze pesticide residues in grapes.

The Vac-HS-SPME method employs vacuum conditions to enhance the extraction process. This technique allows for the simultaneous detection of six pesticides: boscalid, quinalofop-p-methyl, oxyfluorfen, fluroxypyr, metribuzin, and epoxycenazole. Key parameters such as extraction temperature (30-70°C), extraction time (1-40 min), fiber coating, desorption time (1-5 min), and vacuum time (60-120 s) were optimized. The results showed that Vac-HS-SPME offers superior selectivity and detection limits ranging from 0.11 to 0.61 µg mL<sup>-1</sup>. Additionally, this method significantly reduces sample preparation time due to the efficiency of the vacuum-assisted process. This makes it a powerful tool for analyzing pesticide residues in grapes, especially when dealing with complex mixtures.

The mini-HS-SPME method, designed to be compact and environmentally friendly, has also shown great effectiveness in detecting a wide range of pesticides. These include atrazine, simazine, prometryn, metribuzin, cyanazine, tebucanazole, and quinalofop-p-methyl. To make the mini-HS-SPME technique as effective as Vac-HS-SPME, we optimized crucial extraction parameters such as temperature (30-110°C), extraction time (1-120 min), sample volume (100-300 µL), pH, and salt addition. The results showed that the detection limits were between 0.03 and 0.31 µg mL<sup>-1</sup>.

The GAPI (Green Analytical Procedure Index) and AGREE tools highlighted the wider applicability of this method to various classes of pesticides. They also showed that mini-HS-SPME has a lower environmental impact compared to Vac-HS-SPME, making it an attractive choice for routine pesticide monitoring in food safety testing [3,4,5].

In conclusion, both Vac-HS-SPME and mini-HS-SPME offer significant improvements over traditional extraction methods. However, their application depends on the specific requirements of the analysis. Vac-HS-SPME is better suited for highly selective, vacuum-assisted extractions. It focuses on reducing detection limits for specific pesticides. On the other hand, mini-HS-SPME excels in providing a more sustainable and versatile approach for comprehensive analysis of a wider range of pesticide residues. Both methods contribute to improving food safety by offering reliable, cost-effective, and environmentally friendly solutions for analyzing pesticide residues in grapes.

**Keywords:** miniaturized solid-phase microextraction, grapes, pesticides, gas chromatography mass spectrometry, vacuum assisted solid phase microextraction

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156

### ANALYSIS OF GROUNDWATER CONTAMINATION BY PETROLEUM PRODUCTS USING MINI-SPME WITH GC-MS

Mereke Alimzhanova<sup>(1)</sup>, Bauyrzhan Abdykarimov<sup>\*(1)</sup>

<sup>1)</sup> *Al Farabi Kazakh National University, Center of Physical-Chemical Methods of Research and Analysis, Kazakhstan*

*\*Corresponding author - E-mail: abdykarimov.baur@gmail.com*

The contamination of groundwater with petroleum products poses a significant threat to agriculture and food safety, particularly in regions like West Kazakhstan [1, 2]. Groundwater used for irrigation can introduce harmful pollutants into the soil, adversely affecting crop quality and ecosystem health. This study focuses on the analysis of groundwater contamination by petroleum products using Gas Chromatography Mass Spectrometry coupled with miniaturized Solid-Phase Microextraction (mini-SPME with GC-MS), a highly sensitive and efficient analytical method.

Several cases of petroleum spills and leaks have been reported in West Kazakhstan, where the petroleum industry is prominent. The contamination of groundwater due to these incidents has raised concerns about the potential long-term effects on agricultural productivity and public health. Traditional water monitoring methods are often insufficient to detect low concentrations of volatile petroleum compounds in groundwater. mini-SPME with GC-MS, however, offers superior detection capabilities for trace contaminants [3].

In this study, we optimized key parameters such as extraction temperature (50-80°C), extraction time (10-1800 seconds), and fiber type to maximize detection efficiency. The results demonstrated that mini-SPME with GC-MS could accurately identify and quantify hydrocarbons in water samples with extraction temperature 80°C, extraction time 600s [4]. This method significantly reduces preparation time while offering high precision, making it a suitable solution for routine monitoring of groundwater contamination in petroleum-rich regions.

Mini-SPME with GC-MS offers considerable economic advantages due to its minimal solvent usage, reduced sample preparation time, and high-throughput capabilities. In comparison with traditional methods, mini-SPME with GC-MS requires fewer consumables and generates less waste, making it a cost-effective solution for large-scale environmental monitoring. Moreover, its ability to provide early warnings of contamination can help prevent the costly impacts of polluted irrigation water on agriculture, such as crop loss and soil degradation.

By employing mini-SPME with GC-MS, agricultural stakeholders in West Kazakhstan can gain early warnings about water quality issues, allowing for timely interventions to prevent crop contamination and ensure food safety. The study highlights the importance of adopting advanced monitoring techniques like mini-SPME with GC-MS in regions affected by industrial pollution. The method not only provides accurate and timely data but also presents a more sustainable and economically efficient approach to monitoring groundwater contamination.

**Keywords:** petroleum, GC-MS, mini-SPME, environmental monitoring, groundwater

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I57

### CONTAMINANTS IN FOOD STUFF: SOURCES AND STRATEGIES FOR DETECTION AND RESOLUTION BY LC/MS-MS

**Alexandra Paladi**<sup>(1)</sup>, **Alejandro Ladreyt**<sup>(1)</sup>

<sup>1)</sup> *Analytica Alimentaria GmbH, Germany*

*\*Corresponding author - E-mail: alexandra.paladi@aalimentaria.com*

Food contaminants are a significant public health concern, arising from a variety of sources including agricultural practices, processing methods, and environmental influences. The accurate identification and quantification of these contaminants in multi-residue pesticide analysis using liquid chromatography-tandem mass spectrometry (LC/MS-MS) require a comprehensive understanding of potential contamination sources. This work aims to investigate some of the most common sources of contamination encountered in a food safety laboratory and to propose effective practices for their identification and management. Identifying the sources of contamination in your laboratory is essential for maintaining food safety standards.

For instance, when dealing with residues of nicotine—an alkaloid found naturally in solanaceous plants such as tobacco—having an accurate quantification method is crucial for reliable results, considering that the maximum residue level (MRL) is 0.01 mg/kg for a variety of commodities. Contamination may occur from improper handling practices, such as exposure to nicotine from smoking or from the field where the commodity is grown. To ensure accurate quantification of nicotine, it is essential to develop stringent protocols for the preparation and storage of quality control samples used for quantification. The proper handling of analytical extracts is key for the correct identification and determination of the amount of nicotine present in the commodity.

Additionally, we examine the influence of packaging materials on contamination risk. Chemical residues from packaging, such as Triclosan—an antibacterial agent—can leach into food products. During our daily routine analysis, we identified that perfumed plastic bags used for transporting food items were a notable source of Triclosan contamination. Understanding these pathways is crucial for minimizing contamination during both processing and analysis.

Through this work, we aim to highlight the multifaceted nature of food contamination, emphasizing the need for rigorous laboratory practices. By increasing awareness of potential contamination sources and implementing effective strategies for their identification, we can enhance the reliability of analytical results and ultimately contribute to improved food safety standards. This research underscores the importance of a proactive approach in the laboratory to prevent contamination and ensure the integrity of food products.

**Keywords:** contaminants, LC/MS-MS, residues, maximum residue levels

I58

### A SURFACE ENHANCEMENT RAMAN SPECTROSCOPY (SERS) INVESTIGATION OF POTENTIAL RESIDUE CONTAMINATION OF MILK PRODUCTS FROM THE USE OF CHLORINE-FREE CLEANING PRODUCTS

Daniela Lopez<sup>\* (1)</sup>, Krishnakumar Chullipalliyalil<sup>(1)</sup>, Killian Barton<sup>(1)</sup>, Michael McAuliffe<sup>(1)</sup>, Ambrose Furey<sup>(2)</sup>

<sup>1)</sup> Centre for Advanced Photonics & Process Analysis, Munster Technological University, Ireland, Ireland

<sup>2)</sup> Mass Spectrometry group, Physical Science, Munster Technological University, Ireland, Ireland

\*Corresponding author - E-mail: daniela.lopez-henao@mycit.ie

This study investigation chlorine-free detergents as alternative to chlorine-based detergents in the dairy industry, focusing on potential residual by-products in milk after cleaning processes. The shift from chlorine-based to chlorine-free detergents has been driven by environmental and health concerns associated with chlorine's disinfection by-products (DBPs), such as trihalomethanes (THMs), which pose risks to human health. This research evaluates the interactions between chlorine-free detergents and milk components, particularly focusing on the by-products formed through reactions like saponification. The study employs Surface-Enhanced Raman Spectroscopy (SERS) and Nuclear Magnetic Resonance (NMR) techniques to detect and analyze these chlorine-free detergent residues.

Raman spectroscopy was used to establish spectral profiles of detergent by-products, leveraging silver nanoparticles to enhance detection sensitivity. Calibration curves were created for chlorine-free detergents and milk samples to quantify reaction by-products at various concentrations. NMR analysis provided complementary insights into elemental composition, detecting sodium and phosphorus as key indicators of detergent residues. Results highlight that chlorine-free detergents reduce hazardous by-products compared to chlorine-based alternatives, while maintaining effective cleaning properties. The study emphasizes the potential of SERS as a reliable, cost-effective tool for monitoring residues in dairy processing, contributing to safer food production practices and minimizing environmental impact.

**Keywords:** dairy, chlorine-free detergents, by-product residues, surface-enhanced Raman spectroscopy (SERS), nuclear magnetic resonance (NMR)

**Acknowledgement:** i) Teagasc Walsh Scholarships 2020 (Ref: 2020050) for funding Daniela Lopez MSc titled "Investigation of non-chlorine cleaning products and disinfectants as a source of alternative residues" and (ii) the 2019 awarded Dept. of Agriculture, Food and Marine FIRM project titled: "Investigation of chlorates in foods: using the dairy industry as a transferable knowledge model" Ref #2019R555.

I59

### CHEMICAL IONIZATION MASS SPECTROMETRY FOR REAL-TIME MONITORING OF PFAS

Manuel Hutterli\*<sup>(1)</sup>, Luca Cappellin<sup>(2)</sup>, Spiro Jorga<sup>(1)</sup>, Abigail Koss<sup>(1)</sup>, Omar El Hajj<sup>(1)</sup>, Veronika Pospisilova<sup>(1)</sup>

<sup>1)</sup> TOFWERK AG, Switzerland

<sup>2)</sup> TOFWERK, Switzerland & University of Padua, Italy

\*Corresponding author - E-mail: [hutterli@tofwerk.com](mailto:hutterli@tofwerk.com)

We demonstrate the application of chemical ionization mass spectrometry to detect and quantify PFAS at extremely low concentrations in gas-phase samples in real-time. This method has been applied in various scenarios, including emissions from food packaging materials.

Polyfluoroalkyl substances (PFAS) represent a large and diverse group of compounds widely used in industry to e.g. create fluoropolymer coatings for non-stick cookware, food packaging, pizza boxes and take-out containers, and many other products. Recently, these compounds have come under scrutiny from regulatory and health agencies due to their persistence in the environment, ability to travel and transfer between systems, and potential to cause serious health effects. Measuring these substances is increasingly critical because even extremely low concentrations (pptv level) can be significant for environmental and health concerns. Traditional instrumentation faces challenges in measuring such low concentrations. Another major challenge in PFAS research is the lack of efficient detection methods for direct, real-time measurement in the air.

Our recent advancements in chemical ionization mass spectrometry address these challenges by enabling the detection of PFAS at ultra-trace levels, down to parts-per-quadrillion (ppqv). We demonstrated the detection of PFAS mixing ratios in the air at parts-per-quadrillion levels, supported by calibration data for 11 perfluorinated carboxylic acids (PFCAs) and 2 fluorotelomer alcohols (FTOHs), and examples of PFAS detection in various sample headspaces.

We show that chemical ionization with iodide or nitrate reagent ion chemistry detects PFAS compounds with remarkably high sensitivity. The PFAS compounds are detected as the parent molecule M clustered with iodide reagent ions ( $[M+I]^-$ ) or with nitrate reagent ions ( $[M+NO_3]^-$ ). The molecular identity is based on the chemical formula determined from precise mass-to-charge ratio measurement and confirmed using isotope patterns. Due to their high halogen content, PFAS have a unique mass spectral signature.

As case studies, we report PFAS measurements in samples including ambient air samples from an industrial facility and headspace samples from cosmetics and food packaging. Remarkably, in all cases, our approach was able to detect PFAS in real-time. This real-time PFAS detection enables accurate emission tracking and potentially improved food safety and environmental protection measures.

**Keywords:** PFAS, food safety, chemical ionization mass spectrometry



160

### **AUTOMATED SAMPLE PREPARATION FOR TARGETED QUANTITATION AND NON-TARGETED ANALYSIS OF PFAS WITH HPLC-HRMS ANALYSIS BASED ON ORBITRAP TECHNOLOGY**

**Aristide Ganci\***<sup>(1)</sup>, **Valerie Thibert**<sup>(1)</sup>, **Jean-François Garnier**<sup>(1)</sup>, **Bénédicte Gauriat**<sup>(1)</sup>, **Richard Cochran**<sup>(1)</sup>, **Lee Ferguson**<sup>(2)</sup>

<sup>1)</sup> *Thermo Fisher Scientific, France*

<sup>2)</sup> *Department of Civil and Environmental Engineering and Nicholas School of the Environment, Duke University, United States of America*

\*Corresponding author - E-mail: [aristide.ganci@thermofisher.com](mailto:aristide.ganci@thermofisher.com)

Per- and Polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been in use in various industries due to their resistance to heat, water, and oil. However, their environmental persistence and potential adverse health effects has led to a growing concern on PFAS contamination monitoring in the environment. Therefore, the analysis of PFAS in different matrices has become increasingly important. PFAS include several chemical classes, which adds to the complexity of extracting and analyzing them in a single workflow. Further, regulations and different matrices are based on discrete lists of PFAS compounds.

Dispersive Liquid Liquid Microextraction (DLLME) is a highly effective sample preparation technique that has gained significant attention due to its simplicity, low cost, robustness, and high enrichment factors. DLLME is a miniaturized form of liquid-liquid extraction that uses minimal amounts of extraction solvent, making it a greener and environmentally friendly alternative. Furthermore, it can be easily automated, improving reproducibility and reducing potential for human error. Automation was conducted on a Thermo Scientific™ TriPlus™ RSH SMART liquid handling station.

Extracts of drinking water obtained by this fully automated novel extraction technique were measured by targeted quantitative methods to meet very low regulatory limits, while screening methods utilizing the high mass resolution and mass accuracy of the Thermo Scientific Orbitrap Exploris™ mass spectrometer explored potential unknown PFAS with confidence within a second injection of the same sample extract.

For targeted analysis, quantitation was performed for 56 target PFAS, with full scan (60k) acquisition. Results showed good accuracies at low (1 ng/L) and high (75 ng/L) spiking level, as well as reproducibility (<30%, n=7) over several days. The stability and cross-contamination of the extraction procedure were evaluated, and excellent results were obtained for all the tested compounds (bias <30%).

The non-targeted analysis workflow was performed with Thermo Scientific™ Compound Discoverer™ software. This provides a comprehensive package enabling confident annotation of unknown PFAS compounds as well as understanding differences in PFAS composition across samples. Annotation confidence was maximized by combining a multitude of the best resources available within the PFAS community, including EPA and NIST PFAS chemical databases, the FluoroMatch™ PFAS fragmentation database from Innovative Omics (<https://innovativeomics.com/software/fluoromatch-flow-covers-entire-pfas-workflow/>), the Duke University in-silico generated PFAS spectral library, and both the 2023 NIST HRMS MS/MS and mzCloud™ spectral libraries.

**Keywords:** DLLME, PFAS, automation, HRMS, versatility

161

### PFAS ANALYSIS STRATEGY STORY - DIRECT INJECTION, DLLME, LC-MS/MS, LC-ORBITRAP / GC-ORBITRAP

**Benedicte Gauriat<sup>(1)</sup>, Valerie Thibert<sup>(1)</sup>, Aristide Ganci<sup>(1)</sup>, Jean-François Garnier<sup>(1)</sup>**

<sup>1)</sup> Thermo Fisher Scientific, France

\*Corresponding author - E-mail: benedicte.gauriat@thermofisher.com

Per- and Polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that have been in use in various industries due to their resistance to heat, water, and oil. However, their environmental persistence and potential adverse health effects has led to a growing concern on PFAS contamination monitoring in the environment. Therefore, the analysis of PFAS in different matrices has become increasingly important. PFAS include several chemical classes, which adds to the complexity of extracting and analyzing them in a single workflow. Further, regulations and different matrices are based on discrete lists of PFAS.

This work aims to summarize the key components of PFAS analysis:

The choice between direct injection and the need for (automated) sample preparation is an important consideration in PFAS analysis. This largely depends on the instrument's dynamic range and detection limits compared to the one required by regulation, as well as the complexity of the analyzed matrix. Direct injection conducted on a high sensitivity Thermo Scientific™ TSQ Altis™ Plus allows for rapid analysis to meet very low regulatory limits for drinking water.

More complex matrixes, such as waste/industrial water, soil, food, and biological samples require specific sample preparation techniques to overcome matrix effects and achieve accurate quantification. Sample preparation, such as Dispersive Liquid Liquid Microextraction (DLLME) can drastically improve the concentration factor and reduce matrix interference, enhancing the overall method performance. DLLME is a miniaturized form of liquid-liquid extraction that uses minimal amounts of extraction solvent, making it a greener and environmentally friendly alternative. Furthermore, it can be easily automated, improving reproducibility and reducing potential for human error. This automation was conducted on a Thermo Scientific TriPlus™ RSH SMART liquid handling station.

Extracts of water samples obtained by this fully automated novel extraction technique were measured by both LC- and GC-high resolution accurate mass (HRAM) mass spectrometers. The Orbitrap™ HRAM technology allows further to not only conduct targeted quantitation but run non-targeted analysis to screen for potentially unknown PFAS with confidence within the same sample extract solution. This further permit to easily adapt to possible upcoming regulatory changes.

LC is commonly used separation techniques in PFAS analysis, but GC can offer an alternative for selected compounds and matrices. The selection of LC or GC for PFAS analysis depends on the chemical properties of the target compounds and the chromatographic conditions that can effectively separate and detect them.

**Keywords:** PFAS, automation, LC-MS/MS, GC-Orbitrap, LC-Orbitrap

162

### ROUTINE PFAS TESTING IN WASTEWATER USING TOP ASSAY AND ACQUITY™ QDA™ II MASS DETECTOR

Henry Foddy<sup>(1)</sup>, Cristian Cojocariu<sup>(1)</sup>, Vladimir Nikiforov<sup>(2)</sup>

<sup>1)</sup> Waters Corporation, United Kingdom

<sup>2)</sup> NILU, Norway

\*Corresponding author - E-mail: henry\_foddy@waters.com

Per- and polyfluoroalkyl substances (PFAS) have increasingly become a major environmental and public health concern due to their toxic properties and tendency to bioaccumulate in living organisms, such as crops and wildlife. Global widespread use of PFAS over many decades has meant that these compounds have become common and persistent environmental pollutants. Contamination of rivers, groundwater, and drinking water sources with PFAS has been reported globally. Concern in the United Kingdom has been amplified recently by increasing reports of wastewater and sewage leaks into rivers and other water bodies; this discharge of untreated or partially treated wastewater into rivers can exacerbate trophic transfer and persistence throughout vital ecosystems and food chains. The resistance of PFAS to traditional environmental remediation techniques makes addressing their impacts particularly challenging.

Traditional testing methods often fail to capture the full extent of PFAS pollution, primarily detecting legacy PFAS compounds and overlooking the myriads of precursor compounds and non-legacy PFAS compounds. The total oxidizable precursors (TOP) assay approach offers a means of bridging this gap; by oxidizing unknown PFAS precursors and intermediates and converting them into a limited set of well-characterized stable perfluorocarboxylic and perfluorosulfonic acids, it allows for a more complete insight into complex environmental samples. By utilising an LC-MS approach with the ACQUITY QDa II Mass Detector, this method provides a cost-effective means of risk assessment for environmental samples. It complements traditional testing methodologies by enhancing the identification and assessment of both legacy PFAS compounds and perfluoroalkyl acid (PFAA) precursors in each sample.

In this work, eight wastewater samples were collected downstream from wastewater treatment plants across the northwest of England. By comparing perfluoroalkyl carboxylic acids (PFCA) concentrations before and after oxidation, this method allows for routine assessment of total PFAA precursor content in a given sample. All wastewater samples tested showed a significant increase in total PFCA content post-amendment, with in-sample concentrations ranging from 63 to 2711 ng/L (ppt) - indicating a high degree of precursor contamination that traditional analytical methods may have failed to detect.

The LC-MS method, utilising the ACQUITY QDa II Mass Detector, offers an efficient and reliable alternative for mitigating risks associated with PFAS contamination. This high-throughput solution is well-suited for routine environmental screening of PFAS precursors, alleviating the need for more advanced and resource-intensive instrumentation. By enhancing the ability to detect and quantify PFAS precursors, this approach significantly improves overall environmental monitoring efficiency across local ecosystems.

**Keywords:** PFAS, routine testing, total oxidisable precursors assay, wastewater, LC-MS

163

### ELEVATING SPECIFICITY AND IDENTIFICATION CONFIDENCE FOR PFAS LIQUID CHROMATOGRAPHY AND CYCLIC ION MOBILITY MASS SPECTROMETRY

Susan Slade<sup>(1)</sup>, Michael McCullagh\*<sup>(1)</sup>, Georgios Theodoridis<sup>(2)</sup>, Robert Plumb<sup>(1)</sup>, Stuart Adams<sup>(1)</sup>, Sarah Dowd<sup>(1)</sup>, Artemis Lioupi<sup>(2)</sup>

<sup>1)</sup> Waters Corporation, United Kingdom

<sup>2)</sup> University of Thessaloniki, Greece

\*Corresponding author - E-mail: mike\_mccullagh@waters.com

Polyfluorinated alkyl substances (PFAS) exposure is a potential contributor to increased cancer occurrence in the human population. The bio-accumulative nature of PFAS enables monitoring of levels in human biofluids to help gain understanding into exposure levels and pathways. However, PFAS isomeric compounds can be challenging to efficiently separate using liquid chromatography (LC).

Cyclic Ion Mobility (cIM) provides an added dimension of separation and Collision Cross Section (CCS) values, which serve as a complementary identification descriptor. Using LC-cIM-MS, branched and linear PFAS isomers have been fully resolved.

Reproducibility of CCS values were investigated using a quadrupole-cIM-time-of-flight mass spectrometer to perform an inter-site/intra-site comparison and compared to published drift tube (DT) ion mobility PFAS CCS values.

With enhanced peak capacity of LC-cIM-MS, an increase in analysis efficiency of 75 % was achieved using a 5.5-minute gradient. At low concentrations where fragment information may be absent, CCS and arrival time distribution (ATD) provide an additional identification point alongside retention time (t<sub>r</sub>).

Human serum samples were analysed using LC-cIM-MS. Conformer profiles of perfluoroalkyl carboxylic acids were identified and their corresponding CCS identification fingerprints determined, thus providing newfound identification specificity for PFAS non-targeted screening assays allowing detection of individual PFAS isomers. Importantly chromatographic separation was achieved for PFOS and bile acid biomarkers. Using ion mobility resolution (R~145), we report ion mobility-enhanced specificity and resolution for biomarker isomers taurodeoxycholic, taurochenodeoxycholic and tauroursodeoxycholic acid.

Coeluting branched and linear PFAS isomers identified in anonymised human serum samples have been fully resolved and single component calculated concentrations determined. We illustrate the potential to correlate individual PFAS isomer structure, toxicity, cancer risk and environmental exposure.

**Keywords:** PFAS, Ion Mobility, non-targeted screening, LC-IM-MS

**164**

### **DETERMINATION OF VARIOUS PFAS IN EGG MATRIX USING STACKED INJECTION ON-LINE SPE COUPLED TO LC-MS/MS**

**Uwe Oppermann**<sup>(1)</sup>, **Anja Grüning**<sup>(1)</sup>, **Stephane Moreau**<sup>(1)</sup>

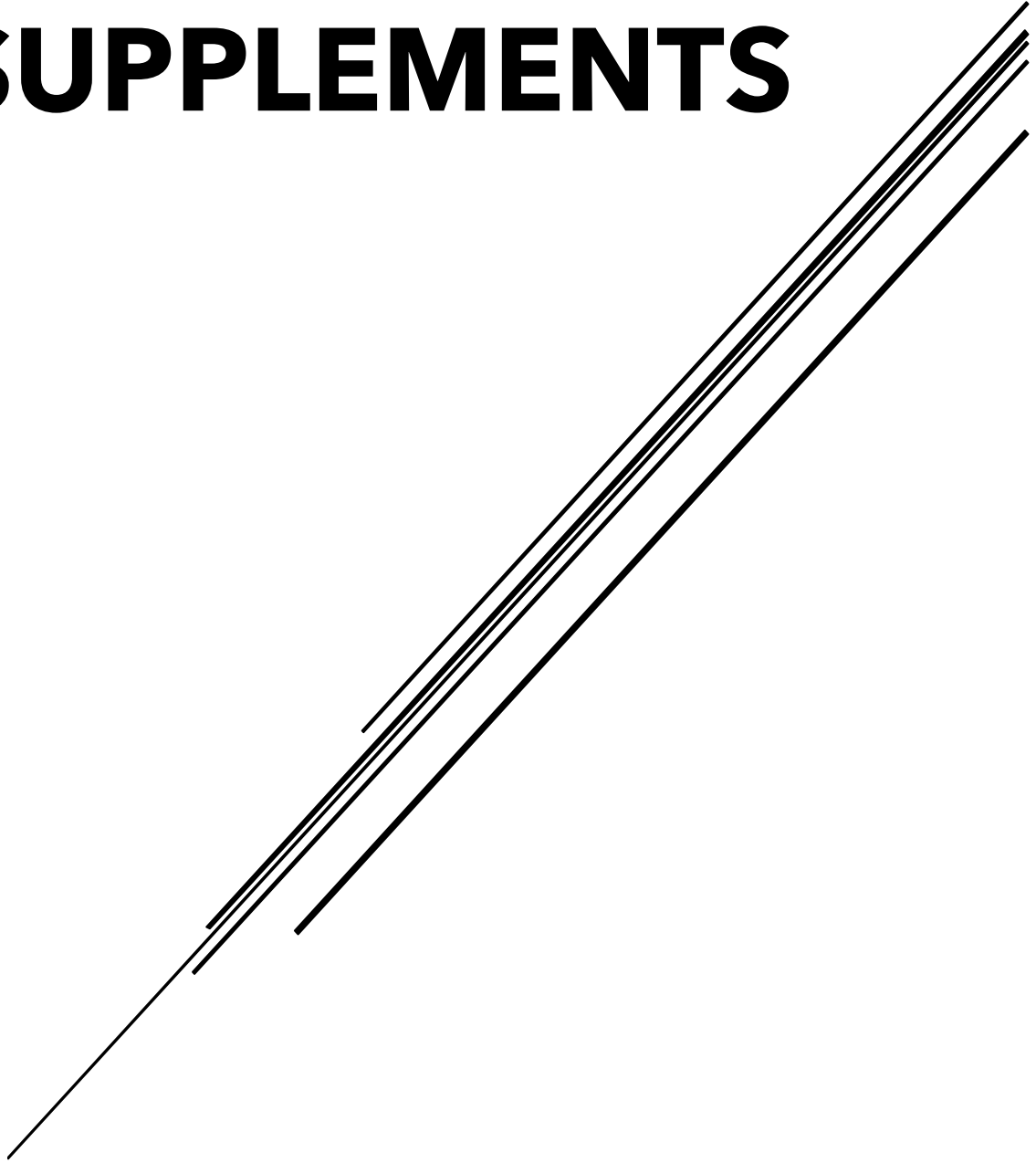
<sup>1)</sup> Shimadzu Europa GmbH, Germany

\*Corresponding author - E-mail: [uo@shimadzu.eu](mailto:uo@shimadzu.eu)

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) refer to a class of more than 4,000 individual chemicals that have been widely used since the 1950s, e.g. as fire retardants, food packaging materials or non-stick coatings. These compounds offer heat-resistant and oil-and-water-repellant properties as well as chemical and thermal stability, resistance to UV light and weathering. Due to their anthropogenic origin, PFAS cannot be degraded, and hence they accumulate and can now be detected ubiquitously in the environment. Due to this, PFAS also found their way into the food chain and accordingly into our food. Concerns about human exposure through diet, have led to studies on the status of food contamination being conducted in various countries. In December 2022 the EU commission released regulation 2022/2388 regarding maximum levels of perfluoroalkyl substances in certain foodstuffs [1]. In this poster, we describe the determination of various PFAS in egg matrix in a relevant concentration range. The analysis is based on a simple QuEChERS extraction coupled to an on-line SPE approach. This omits additional sample - preparation steps like dSPE. For the analytical setup it is important to have fast, sensitive and robust LC-MS/MS systems, which are providing the highest performance in terms of sensitivity and sample throughput in food testing laboratories. For the described application, the Shimadzu LCMS-8060NX triple- quadrupole mass spectrometer coupled with a Nexera X3 UHPLC system was used in order to demonstrate a sensitive method for determination of 27 PFAS in egg matrix with minimal sample preparation steps.

**Keywords:** PFAS, LCMS, food safety

# **FOOD SUPPLEMENTS**



### J1

#### PIGMENTS, NITRATES AND STANDARDIZATION: PROFILING RED BEETROOT (BETA VULGARIS L.) FOOD INGREDIENTS AND SUPPLEMENTS

**Marco Gozzi**<sup>(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Martina Cirlini<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>, Renato Bruni<sup>(1)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Italy

\*Corresponding author - E-mail: marco.gozzi@unipr.it

The interest for substances able to increase sports performance is steadily increasing, attracting the attention of a growing number of ordinary consumers, besides professional athletes. Both amateur and lifestyle users, interested in well-being and in improving endurance and strength during workout sessions, encouraged an increased commercial availability of ergogenic food supplements. At the same time, consumers shifted their attention to plant-based products, perceived as being more natural and beneficial, in the form of juices, extracts, powders, food supplements as well as food ingredients, the latter often used for supplementation purposes. In this complex scenario, several studies highlighted several critical issues involving adulteration, sophistication and, finally, a lack of standardization that gives rise to an extremely variable content of bioactive compounds in products available on the market. Within this context, red beetroot (*Beta vulgaris* L.) represents an emblematic example of a transition from a low-cost vegetable to a major ingredient with specific applications in sports nutrition. In fact, an extensive evidence-based background reported positive impacts in the regulation of muscle contraction, cellular respiration, and glucose uptake as well as a general modulation of cardiovascular function by affecting the nitric oxide bioavailability, which is the most investigated mechanism at the basis of the reported effects. Along with its high nitrate content, red beetroot is known to provide a rich phytochemical profile which includes compounds with antioxidant activity such as polyphenols along with pigments including betacyanins and betaxanthins. As commonplace in plants, the phytochemical profile is strongly affected by several factors, such as genetic background, agronomic practices, climatic conditions, and post-harvest management. A few reports have noticed extremely variable nitrate content from commercial beetroot products, suggesting a scenario in which bioactivity, efficacy, and ultimately safety may be inconsistent. At the same time some investigations suggested an involvement of beetroot phytochemicals in a synergistic modulation of the effects of nitrates leading to differences between the use of this botanical and the supplementation of pure nitrates. Contrarily to previous investigations, nitrate, polyphenols, antioxidants, betacyanin content, and profiling of thirteen beetroot-based food supplements and ingredients were investigated using spectrophotometry and LC-MS/MS techniques. Thirty-three different betacyanins were tentatively identified, showing a broad quantity and quality variability between samples regarding not only nitrates, but affecting also polyphenols, antioxidant activity and total betacyanin content as well.

**Keywords:** red beetroot, betacyanins, nitrates, food supplements

**Acknowledgement:** This work has been carried out in the frame of the ALIFAR project, funded by the Italian Ministry of University through the program 'Dipartimenti di Eccellenza 2023-2027'.

### J2

## OPTIMIZING HEMICELLULOSE HYDROTHERMAL EXTRACTION FROM LIGNOCELLULOSIC BIOMASSES USING AN ENZYMATIC APPROACH: EFFECT OF TEMPERATURE, ENZYMES, AND MATRIX

Pio Viscusi<sup>\*(1)</sup>, Veronica Lollì<sup>(1)</sup>, Augusta Caligiani<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

\*Corresponding author - E-mail: pio.viscusi@unipr.it

Conventional methods for hemicellulose extraction involve harsh conditions, i.e., high temperatures, high pressures, and the use of strong acids or bases. These conditions lead to the formation of degradation products and necessitate multiple purification steps. In a previous study, hazelnut shells were subjected to hydrothermal treatment at various temperatures (125, 150, 175, and 200°C for 1 hour), ranging from typical hemicellulose extraction temperatures to lower ones, highlighting that the lowest temperature tested (125°C) was ineffective for hemicellulose extraction.

The objective of this research was to recover hemicellulose and other fiber fractions from agro-food by-products under mild conditions, by promoting the breakdown of the lignocellulose matrix through a biotechnological pre-treatment.

Three different enzymatic pre-treatments (24 hours) with various lignolytic enzymes, Laccase from *Aspergillus sp.* (L), Laccase from *Trametes Versicolor* (T), and Manganese Peroxidase (M), were applied to hazelnut shells. Simultaneously, the effect of temperature was evaluated by subjecting the enzymatically pre-treated hazelnut shells to different extraction temperatures. A one-pot extraction at 150°C was conducted, along with a novel temperature-based fractionation approach involving sequential extractions at increasing temperatures (130, 140, 150, and 160°C) on the same laccase pre-treated matrix. Monosaccharide composition and total sugar yields of the extracts were determined by GC-MS and <sup>1</sup>H NMR, respectively.

The results demonstrated that the three enzymes behaved significantly differently on the substrate. For instance, L enhanced total sugar extraction yields at 150°C, whereas T was effective already at 130°C. Sequential extraction up to 150°C substantially increased the total sugar yield compared to the one-pot extraction at 150°C. Moreover, this approach produced three different fractions, achieving further fiber fractionation and resulting in higher hemicellulose purity in the final extract.

Based on these initial findings and due to its commercial availability, L was selected for further tests aimed at evaluating the effect of different matrices on the extraction process. The same procedure was applied to L pre-treated samples from various agro-food by-products, selected based on their lignin/hemicellulose content ratio. These matrices included almond shells (another nut by-product), hops leaves, pineapple and melon processing waste, and orange and apple peels. The goal was to test the performance of the enzymatic pre-treatment on a wide variety of substrates with different lignocellulose content and composition. Preliminary results indicated that L pre-treatment has a significantly different effect on fiber extraction depending on the substrate used.

**Keywords:** hemicellulose, laccases, green extractions, lignocellulosic biomasses



### J3

## A COMPREHENSIVE COMPARISON OF VARIOUS TECHNIQUES FOR CHARACTERISATION AND AUTHENTICATION OF FISH OIL-BASED FOOD SUPPLEMENTS

**Chi Mai Nguyenova**\*<sup>(1)</sup>, **Vojtech Hrbek**<sup>(1)</sup>, **Marek Dolezal**<sup>(1)</sup>, **Jan Poustka**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: nguyenox@vscht.cz*

Fish oil supplements have for many years seen high consumer interest and a wider range of products from producers. Fish oil supplements are available as natural fish oil containing triacylglycerols (TAGs) or as fish oil concentrates containing ethyl esters (EEs). The dominant type of ester in supplements in terms of authenticity are usually not declared, therefore the determination of ester type in samples is required. For this approach, Fourier Transform Infrared Spectroscopy (FTIR), Direct Injection High-Resolution Mass Spectrometry (DI-HRMS), Ultra Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry (UPLC-HRMS) and Gas Chromatography with Flame-Ionization Detector (GC-FID) have been employed. The aims of this study are to compare these applied analytical methods and to demonstrate various applicability for the routine inspection. The potentials of the different methods will be compared, as well as the time required for the complete analysis and the advantages and disadvantages of each method.

**Keywords:** fish oil supplements, triacylglycerol, ethyl ester, authenticity

**Acknowledgement:** *The work used facilities provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064).*

### J4

#### QUANTIFICATION OF 19 CANNABINOIDS IN COMMERCIAL CBD OILS AND SAFETY IMPLICATIONS OF THEIR CBD AND $\Delta^9$ -THC CONTENTS

**Niklas Lindekamp<sup>(1)</sup>, Stefan Weigel\*<sup>(1)</sup>, Benjamin Sachse<sup>(2)</sup>, Bernd Schäfer<sup>(2)</sup>, Sascha Rohn<sup>(3)</sup>, Nadja Triesch<sup>(1)</sup>**

<sup>1)</sup> German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Germany

<sup>2)</sup> German Federal Institute for Risk Assessment, Department Food Safety, Germany

<sup>3)</sup> Department of Food Chemistry and Analysis, Institute of Food Technology and Food Chemistry, TU Berlin, Germany

\*Corresponding author - E-mail: stefan.weigel@bfr.bund.de

CBD oils are a group of products typically consisting of hemp extracts containing cannabinoids such as cannabidiol (CBD) or tetrahydrocannabinol ( $\Delta^9$ -THC). These extracts are mixed with edible oils such as hemp seed, sesame or olive oil. Common consumer expectations when using CBD oils are pain and stress relief, relaxation, or improved sleep. CBD oils are often marketed as food/food supplements or cosmetics, although consumer surveys clearly indicate the use as self-medication. While the substance CBD is approved as a prescription medicine (anticonvulsant Epidyolex®) in the EU and US, CBD oils are not, and there is no clear clinical evidence for the effects consumers expect. The European Food Safety Authority (EFSA) has suspended the evaluation of pending applications for CBD under the *Novel Foods Regulation* due to data gaps and uncertainties regarding potential adverse health effects. CBD oils marketed as food or food supplements violate EU food law as they are considered unauthorised novel foods.  $\Delta^9$ -THC is regulated with maximum levels in hemp seeds and processed hemp seed products, but CBD oils are not regulated in this respect.  $\Delta^9$ -THC is relevant, because of its psychoactive effects. In its toxicological evaluation, EFSA has derived an acute reference dose (ARfD) of 1  $\mu$ g/kg body weight (bw).

An HPLC-MS/MS method was used to quantify 19 cannabinoids in 22 CBD, 2 cannabigerol (CBG) and 2 cannabinal (CBN) oils ( $n = 26$ ). The CBD content of the samples ranged from 3.1 to 24.2%. While EFSA has not yet derived a health-based guidance value, the *UK Advisory Committee on Novel Foods and Processes* (ACNFP) has established a provisional acceptable daily intake (ADI) of 0.15 mg/kg bw. For 24 samples, 2-7 drops would be sufficient to exceed this provisional ADI for a 70 kg person. At higher consumption levels of 20 to 100 drops or more per day, therapeutic doses recommended for Epidyolex® (350 mg/d for a 70 kg person) could be reached.  $\Delta^9$ -THC levels ranged from 5 to 1,576 mg/kg. For 13 samples, 1-20 drops would be sufficient to exceed the ARfD for a 70 kg person. For 7 of these 13 samples, only 2 drops would suffice. The compound (*R*)-HHC, which can cause effects similar to  $\Delta^9$ -THC, though likely higher doses are necessary, was quantified in 2 CBN oils at levels of 5 and 110 mg/kg.

**Keywords:** HPLC-MS/MS, ARfD, CBN oil, CBG oil, HHC

**Acknowledgement:** The authors thank Sebastian Kemter for his support in the laboratory.

### J5

#### DATE SYRUP: HEALTHY AND SAFE SWEETENER

**Benedetta Sgro**<sup>(1)</sup>, **Qada Benameur**<sup>(2)</sup>, **Nadra Rechidi-Sidhoum**<sup>(3)</sup>, **Vincenzo Lo Turco**<sup>(4)</sup>, **Angela Giorgia Potorti**<sup>(4)</sup>, **Giuseppa Di Bella**<sup>(4)</sup>

<sup>1)</sup> Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM), University of Messina. Department BIOMORF, University of Messina., Italy

<sup>2)</sup> Département d'agronomie, Faculté des Sciences de la Nature et de la Vie, Université Abdelhamid Ibn Badis de Mostaganem, Algeria

<sup>3)</sup> Département des Sciences Alimentaires, Faculté des Sciences de la Nature et de la Vie, Université Abdelhamid Ibn Badis de Mostaganem, Algeria

<sup>4)</sup> Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy

\*Corresponding author - E-mail: benedetta.sgro@studenti.unime.it

Date syrup, a traditional product of the Saharan regions, is gaining attention for its nutritional benefits and potential applications in the food industry. This syrup is derived from the extraction and concentration of dates (*Phoenix dactylifera* L.), the most abundant fruits in these areas, to ensure a product rich in natural sugars, minerals, and antioxidants [1]. Date syrup is therefore a healthier alternative to conventional sweeteners. Traditional medicine has used date syrup for its potential health benefits, making it a valuable dietary supplement for conditions such as diabetes, cardiovascular diseases, digestive disorders and nutritional deficiencies [2]. To ensure these therapeutic properties, date syrup must meet certain quality standards and be free of contaminants which can occur due to environmental factors and agricultural practices. Rigorous quality control and adherence to international safety standards are crucial to mitigate these risks. In this view, seven commercial date syrups from Algeria were analyzed to check their quality and safety by generating new or updated physico-chemical (pH, acidity, conductivity, total soluble solids) and compositional data (moisture, total sugar, ash, protein, total polyphenols). In addition, to check the product's safety, analysis of mineral elements, including potentially toxic elements, was performed by inductively coupled plasma mass spectrometry (ICP-MS) and direct mercury analyzer (DMA-80). The results confirm the high content of polyphenols (mean value  $13.98 \pm 7.62$  g/L) and mineral elements, except for one sample whose composition differed from the others due to the addition of glucose, sugar, water and caramel colour. The most abundant mineral is K (from  $1002.01 \pm 93.80$  to  $2999.98 \pm 344.51$  mg/kg) followed by Ca and Mg. Fe and Zn concentrations are also remarkable (mean value  $25.63 \pm 12.59$  and  $9.57 \pm 4.87$  mg/kg, respectively). Very low concentrations of potentially toxic elements are found. Ni is found at concentrations lower than analytical LOQ in all samples. Al, As, Cd, Pb, Sn and Tl are found at concentrations lower than 0.10 mg/kg in all samples. As the maximum levels for inorganic contaminants are not available for this specific food, the results obtained are compared with the maximum levels indicated for honey, fruits, fruit juice concentrates and food supplements [3]. In no case the results exceed the stated limits. In order to assess the safety of date syrup as a substitute for traditional sugar, the estimated daily intake of all minerals is calculated by considering the amount of sugar consumed in Algeria and compared with their reference values [4]. The results show a high coverage of the reference value for Co (40-110%) and interesting results for the other essential elements. Moreover, there are no health risks for potentially toxic elements as their reference values are not exceeded in any of the samples, confirming the safety of date syrups.

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## FOOD SUPPLEMENTS

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**Keywords:** date syrup, minerals, potentially toxic elements, food safety

### J6

#### EFFECT OF IN VITRO DIGESTION ON TOTAL PHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF GINGER AND PROPOLIS EXTRACTS

Katie Nina Doran<sup>(1)</sup>, Liliana Ordóñez<sup>(1)</sup>, Marc Griffiths<sup>(1)</sup>, Alexandros Ch Stratakos<sup>(1)</sup>

<sup>1)</sup> University of the West of England, United Kingdom

\*Corresponding author - E-mail: [katie.doran@uwe.ac.uk](mailto:katie.doran@uwe.ac.uk)

Ginger and propolis are natural products, both high in polyphenolic content which defines their antioxidant, antimicrobial, and anti-inflammatory properties. There has been an increasing interest in using plant-derived natural products as alternatives to chemically produced drugs due to a range of health-beneficial properties that natural products possess, especially in relation to gut health. The plant-derived natural products also have a lower toxicity and fewer side effects in patients. However, the mechanism of action of ginger and propolis extracts have not been fully investigated. Furthermore, it remains unclear whether the polyphenolic content and antioxidant activity of ginger and propolis are affected during the digestion process. This study investigated the effect of *in vitro* digestion using a static gut model (INFOGEST) on the total phenolic content (TPC), and antioxidant properties of ginger and propolis extracts. The INFOGEST model was applied to simulate oral, gastric and intestinal phases of the digestion process. The ginger and propolis extracts were obtained after 24h, 48h and 72h extraction using an aqueous solution of  $\beta$ -cyclodextrin. Antioxidant properties were determined using CUPric reducing antioxidant capacity (CUPRAC), (2,2-diphenyl-1-picrylhydrazyl) (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The analyses were performed before and after passing the ginger and propolis extracts through a static gut model. At all the extraction time-points, propolis extracts always had significantly higher TPC, CUPRAC, DPPH and ABTS activities when compared to ginger extracts. This might be due to the different composition of the phenolic compounds in these two natural products, and the lower stability of some of ginger-specific polyphenols such as 6-, 8- and 10-gingerols. The optimum extraction time for both, ginger and propolis, was 48h where the highest TPC, CUPRAC, DPPH and ABTS activities were observed when compared to 24h and 72h. After subjecting the extracts to INFOGEST digestion, the TPC and the antioxidant activities were significantly reduced in ginger and propolis extracts by approximately 85%. This might be due to the interaction of phenols with enzymes present at different digestion stages, or changing the conformation or the content of phenols due to pH variations at oral, gastric and intestinal digestion stages. The results demonstrate the importance of taking into account the effect of digestion when evaluating antioxidant properties of ginger and propolis. The work is ongoing on effect of *in vitro* digests on Caco-2 cells.

**Keywords:** ginger, propolis, in vitro digestion, antioxidant activity, extract characterisation

### J7

#### **SMOOTH THE PATH FOR HIGH THROUGHPUT LABORATORIES - A SIMPLE QUECHERS BASED-EXTRACTION MULTI-COMPOUND LC-MS/MS METHOD FOR THE DETECTION OF THE VITAMINS A, E, D AND K IN FOOD**

**Nancy Frank**<sup>(1)</sup>, **Lucas Hemmerle**<sup>(1)</sup>, **Laure Poquet**<sup>(1)</sup>, **Philippe Eugster**<sup>(1)</sup>

<sup>1)</sup> Nestlé Research Lausanne, Switzerland

\*Corresponding author - E-mail: Nancy.Frank@rdls.nestle.com

To ensure compliance of food products, routine laboratories have a high demand on fat-soluble vitamin analysis. To date, to cover the four fat-soluble vitamins and their different forms (e.g. Vitamin A and E and their esters, Vitamin K including Menaquinone-MK4 and -MK7 and Vitamin D with the vitamers D2 and D3), several individual methods based on either spectroscopy or mass spectrometry are usually used. An efficient multi-compound method covering those vitamins using liquid-liquid extraction with isooctane and detection based on super critical fluid tandem mass spectrometry (SFC-MS/MS) was published.

Here, we report the development of a liquid chromatography tandem mass spectrometry (LC-MS/MS) method as a simple alternative to this SFC-MS/MS covering the same compounds, same matrices and level of sensitivity. To decrease the amount of matrix extracted together with the fat-soluble vitamins, an alternative to the extraction with nonpolar solvents had to be used. The new method is based on "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) extraction with acetonitrile and phase separation using the so-called QuEChERS salt mix. The extracts are concentrated and injected for detection of the fat-soluble vitamins on the LC-MS/MS. The method consists of separation on C18-reversed phase column, APCI ionization, analysis by triple quadrupole mass spectrometry and isotope dilution for quantification using respective labelled standards. A commodity screening of different matrix groups (e.g. infant formula, infant cereals, ready to feed food, pet food, etc.) with known composition demonstrated the fit-for-purpose of the developed method for a high throughput application.

### J8

#### QUANTIFICATION OF SEXUAL ENHANCERS IN DIETARY SUPPLEMENTS BY LC-MS/MS

Meritxell Castello<sup>\*(1)</sup>, Eva Munoz<sup>(1)</sup>, Josep Calderon<sup>(1)</sup>, Margalida Artigues<sup>(2)</sup>, Jordi Diaz<sup>(2)</sup>, Sonia Gotsens<sup>(2)</sup>, Josep Lluís Lliberia<sup>(3)</sup>

<sup>1)</sup> *Agència de Salut Pública de Barcelona, Spain*

<sup>2)</sup> *Analytical and Applied Chemistry Department at IQS School of Engineering, Universitat Ramon Llull, Spain*

<sup>3)</sup> *AB Sciex Spain SL, Spain*

*\*Corresponding author - E-mail: mcastell@aspb.cat*

Dietary supplements are in limbo regarding their regulation, since they are not considered drugs nor foods. For this reason, sometimes these products contain active compounds that are not declared on the packaging or the labelling. This fact has become a profound concern for the authorities responsible for the assurance of public health. This concern is particularly pronounced in the realm of natural libido enhancement supplements, marketed as aids to male sexual function. In recent times, reports of serious adverse effects associated with the use of such natural enhancers have surfaced, prompting health authorities in numerous nations to issue alerts and advisories cautioning against their consumption. Despite these warnings, many natural sexual enhancers continue to hide medicinal compounds without proper disclosure on their labels, largely due to lax regulatory oversight in various jurisdictions.

To assess the presence of adulterants in samples of natural sexual enhancers, liquid chromatography coupled with mass spectrometry (LC-MS) techniques are commonly employed. These methods are designed to quantify the presence of PDE5 inhibitor drugs and their analogues within these enhancer products.

In the current study, a multiple reaction monitoring (MRM) approach was adopted for the quantification of these compounds, utilizing a UHPLC-QQQ instrument. The developed quantification was applied to ascertain the concentrations of sildenafil, tadalafil, vardenafil, yohimbine, and desmethyl carbodenafil in various dietary supplements. The method demonstrated a linear range spanning from 50 to 1000 µg/kg for all five compounds under evaluation. Furthermore, the method boasts a theoretical detection limit below 35 µg/kg and a theoretical quantification limit below 40 µg/kg for each of the studied compounds. Extensive analysis of fundamental analytical parameters was conducted using commercially available aphrodisiac dietary supplement samples. The results obtained showcased satisfactory precision (RSD ≤ 15%), accuracy (recovery rates within the range of 80–120%), and robustness (RSD ≤ 15%). Subsequently, the method has been accredited by ISO17025 standards.

Following the implementation of this analytical methodology, a total of 17 samples were subjected to rigorous examination. Alarmingly, our findings revealed that four of these samples tested positive for adulteration with undisclosed medicinal compounds. After our intervention, these compromised products were promptly removed from the market, mitigating the potential risks posed to consumers. This proactive measure underscores the importance of vigilant regulatory oversight and robust analytical techniques in safeguarding public health and ensuring the integrity of dietary supplements.

**Keywords:** liquid chromatography, mass spectrometry, dietary supplements, sexual enhancers

### J9

## QUALITY ASSURANCE OF SAFFRON SUPPLEMENTS VIA SUSPECT METABOLOMIC PROFILING

**Hilva Gjoni**<sup>\*(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Marco Biagi<sup>(1)</sup>, Renato Bruni<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

\*Corresponding author - E-mail: hilva.gjoni@unipr.it

According to the World Health Organization (WHO), COVID-19 pandemic has caused the biggest global crisis in generations, with severe outcomes on health systems, economies, and society. Indeed, the National Institute of Health (NIH) confirmed a rise in mental health issues since the beginning of the pandemic. As an alternative to synthetic drugs, some of those affected might seek alternative remedies, perceived as more natural approaches, to address their concerns and avoid potential side effects. These circumstances have led companies to market these products in diverse formulations and presentations, usually as food supplements. For instance, *Crocus sativus* L. extracts in supplement form are currently being sold due to their neuroprotective supposed effects, even though claims regarding this cannot be displayed on the packaging due to saffron being a botanical on hold. Saffron's stigmas contain crocin, picrocrocin, and safranal, responsible for color, taste, and aroma, respectively. Under controlled circumstances, crocins have been shown to protect against free radical damage in brain vasculature, acting as an anti-depressant. On the other hand, its petals are rich in flavonoids, increasing its antioxidant potential. Therefore, given that saffron is the most expensive spice in the world and susceptible to adulteration, especially in its powdered form, the quality assurance of saffron extracts used for supplement production was herein performed.

In this study, several *Crocus sativus* L. samples, sourced from an Italian-based company, were analyzed to assess their quality and authenticity. These samples constitute the company's raw materials for manufacturing their saffron-based supplements. Hence, after being subjected to a solid-liquid extraction (SLE) using a methanol-water mixture, the samples were analyzed through liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS). A thorough metabolomic characterization of the saffron-based powders was carried out by suspect analysis, allowing the putative identification of 53 compounds. Polyphenols (*i.e.*, mainly kaempferol, isorhamnetin, and quercetin derivatives) and crocins were pointed out.

The results showed some variability among samples. In particular, one discerned amidst the rest due to a higher content of flavonoids and a lower content of crocins, resulting from the presence of saffron petals in addition to the stigma. In this specific case, the producer declared the presence of petals. However, while flavonoids have beneficial antioxidant properties, undeclared petals could be problematic as they are less valuable than stigmas. Moreover, the variability among samples underscores the challenges in standardizing food supplements under current European regulations. Future research will investigate the possibility of saffron supplements being adulterated with petals, highlighting the need for stricter quality control.

**Keywords:** food supplements, *Crocus sativus*, botanicals, adulteration, profiling

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### J10

## IMPROVING CHROMATOGRAPHIC RESOLUTION OF THE JECFA METHOD FOR THE ANALYSIS OF STEVIOL GLYCOSIDES

**Cecile Pinto**<sup>(1)</sup>, **Jinchuan Yang**<sup>\*(2)</sup>, **Paul Rainville**<sup>(2)</sup>, **Stephanie Harden**<sup>(3)</sup>

<sup>1)</sup> Waters SA, France

<sup>2)</sup> Waters Corporation, United States of America

<sup>3)</sup> Waters, Switzerland

\*Corresponding author - E-mail: [jinchuan\\_yang@waters.com](mailto:jinchuan_yang@waters.com)

Steviol glycosides are often used as natural non-caloric sweeteners in food and beverages. This group of compounds shares the same steviol aglycone structure, but with different numbers and types of glycoside units (e.g., glucose, rhamnose, or xylose). The FAO/WHO JECFA Monograph 26 (2021) [1] includes the most up to date international standard for steviol glycosides, in which reversed-phase liquid chromatography (RPLC) is recommended for the determination of major and minor steviol glycosides. However, the chromatographic resolution of steviol glycosides obtained under the JECFA recommended conditions is inadequate with resolution of about 1.0 for the critical pair.

In this work, we screened five C18 columns, optimized the gradient elution program using the design of experiment (DoE) approach, and investigated the benefits of using a hybrid surface technology (MaxPeak™ HPS Technology) [2] on the RPLC of steviol glycosides. We were able to improve the JECFA RPLC method to achieve resolution of  $\geq 1.5$  using 2.5  $\mu\text{m}$  particle-size columns and resolution of  $\geq 2.0$  using sub-2  $\mu\text{m}$  particle-size columns. The method development and the method performance will be presented and discussed.

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**Keywords:** food additives, steviol glycosides

### J11

## CHARACTERIZATION OF CANNABIS CULTIVARS FOR MEDICINAL PURPOSES

**Frantisek Benes<sup>(1)</sup>, Zuzana Binova<sup>(1)</sup>, Matej Maly<sup>(1)</sup>, Maria Filatova<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: benesfr@vscht.cz*

Medicinal cannabis finds multiple uses including the treatment chronic pain, epilepsy, or neuropathy. A common issue with medicinal cannabis usage is its insufficient characterization, as only the content of *trans*- $\Delta$ 9-tetrahydrocannabinolic acid/*trans*- $\Delta$ 9-tetrahydrocannabinol (*trans*- $\Delta$ 9-THCA/*trans*- $\Delta$ 9-THC) and cannabidiolic acid/cannabidiol (CBDA/CBD) is typically monitored. However, other important biologically active substances occurring in cannabis (terpenes, flavonoids, alkaloids, etc.) can modulate the overall effects associated with the intake of cannabis based preparations by patients. Under such conditions, the selection of a suitable cannabis cultivar should be based on its comprehensive characterization.

In this study, UHPLC-HRMS/MS method was developed for the simultaneous quantification of 50 phytocannabinoids and 11 major phenolic compounds in cannabis methanolic extracts. The critical issue in the accurate analysis of such a number of phytocannabinoids is the effective chromatographic separation of isomeric molecules with practically identical mass spectra. An ACQUITY UPLC<sup>®</sup> BEH C18 (2.1 x 100 mm; 1.7  $\mu$ m) column with acetonitrile-water mobile phase gradient (ammonium formate and formic acid as additives) was used for this purpose. Recoveries ranged from 90 % to 102 %, and the repeatability, expressed as relative standard deviation (RSD), ranged from 5 to 15 %. The limits of quantification (LOQs) were in the range of 0.1 - 1.0 mg/kg. The validated method was employed to characterize eight medicinal cannabis varieties (one chemotype III = "high" CBD strain and seven chemotype I = "high" THC strains). In addition to phytocannabinoids and phenolic substances, the content of 33 terpenes in the samples was also monitored using a GC-HRMS method. The total phytocannabinoid content ranged from 10 to 26% w/w, while flavonoids and terpenes ranged from 0.03 to 1.0% w/w. Interestingly, in the chemotype III varieties, *cis*- $\Delta$ 9-THC (0.11% w/w) was contained at two times higher concentration than that of *trans*- $\Delta$ 9-THC (0.070% w/w). Additionally, the chemotype I varieties had a significantly different profile of terpenes, with  $\alpha$ -bisabolol being the dominant terpene, while  $\beta$ -caryophyllene was dominant in chemotype III varieties. While phytocannabinoid profiles were similar in the chemotype I varieties, the terpene profiles differed markedly. For instance, the Jack Herrer and Alien Punch varieties had significantly higher proportions of  $\alpha$ -terpinolene compared to other chemotype I varieties. In the follow up research, the bioavailability of bioactive compounds will be investigated in model systems.

**Keywords:** medicinal cannabis, phytocannabinoids, bioactive compounds, ultra-high performance liquid chromatography, high resolution mass spectrometry

**Acknowledgement:** *The work used [data/tools/services/facilities] provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064).*

### J12

## FORMATION OF SULFORAPHANE DURING LACTIC ACID FERMENTATION OF NASTURTIUM (*TROPAEOLUM MAJUS L.*)

**Anna Romanotto**<sup>(1)</sup>, **Kerstin Hansen**<sup>\*(2)</sup>

<sup>1)</sup> PiCA Preufinstitute fuer Chemische Analytik GmbH, Germany

<sup>2)</sup> Louie & gruenepflaume, Germany

\*Corresponding author - E-mail: kerstin@gruenepflaume.de

The known source of the very important, health-relevant secondary plant metabolite, sulforaphane (SFN) developed from glucosinolates (GSL), is the plants of the *Brassicaceae* family. This is primarily broccoli, but includes also garden cress, cabbage, mustard etc. [1]. The known precursor for SFN in broccoli was glucoraphanin (GPN) [2].

Nowadays, fermentation is no longer only the procedure, to protect food against spoilage, but also has numerous health benefits [3].

Nasturtium (*Tropaeolum majus L.*) belongs to the *Tropaeolaceae* family and, like the members of the *Brassicaceae* family, also contains GSL, but not GPN. We tested whether SFN can be formed during lactic acid fermentation in Nasturtium. The measurement technique used was UHPLC-MSMS after extraction with methanol.

In our analysis, fermented and non-fermented leaves and flowers of nasturtium were examined for the presence of SFN and also GPN. Clear amounts of SFN were found in the fermented product. Traces were also found in unfermented flowers, but not in the leaves. GPN was not found at any time. We were thus able to prove that SFN can also be formed from the other GSL apart from GPN during lactic acid fermentation. The source for SFN is also not only plants of the family *Brassicaceae*. In addition, through anaerobic lactic acid fermentation, SFN which is very sensitive to oxygen, can generally be stabilized and naturally protect SFN.

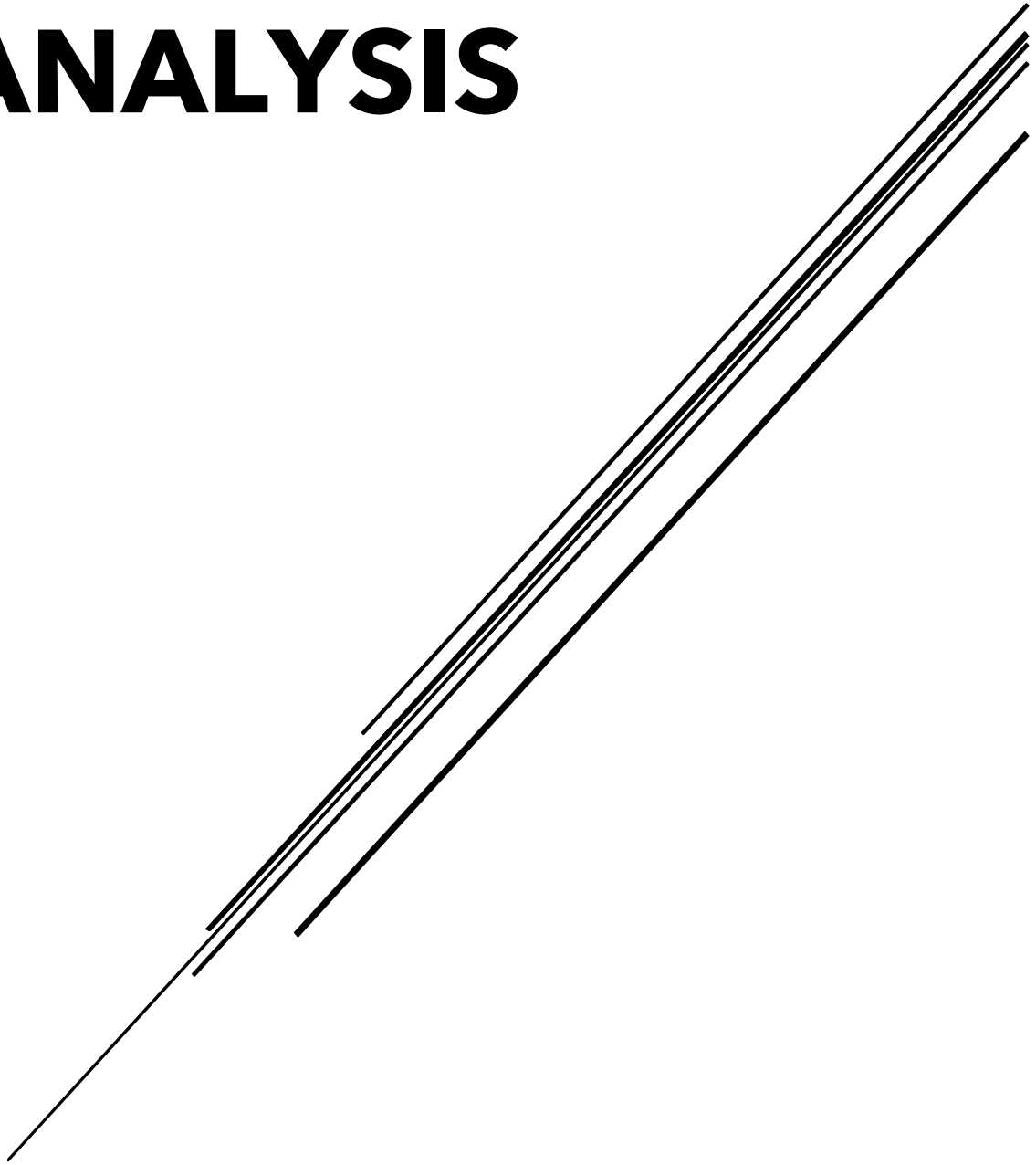
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**Keywords:** sulforaphane, glucosinolates, fermented vegetables, health benefits, secondary plant metabolite

# **GENERAL FOOD ANALYSIS**



## K1

### HIGH-THROUGHPUT, FULLY AUTOMATED SAMPLE PREPARATION FOR CONTAMINANT ANALYSIS

**Mareike Reichel**\*<sup>(1)</sup>, Clara John<sup>(1)</sup>, Jan Sebastian Mänz<sup>(1)</sup>

<sup>1)</sup> Eurofins WEJ Contaminants GmbH, Germany

\*Corresponding author - E-mail: mareikereichel@eurofins.de

Comprehensive manual sample preparation is a challenge when analysing contaminants in food and feed. Contract laboratories require trained personnel to perform the time-consuming steps of sample extraction, purification, evaporation, and dilution. Therefore, labour costs contribute the most to the cost of testing, and working hours limit the time in which a sample can be prepared.

In recent years, more and more automation solutions have been developed. Initially, semi-automated systems focussed on the handling of liquids or the integration of preparation steps into measuring systems. However, the trend is moving towards fully automated robotic systems. One of the biggest advantages of these systems is that samples can be processed 24 hours a day, 7 days a week, even without personnel.

A new robotic system has been set up at Eurofins in Hamburg for analysing contaminants in food and animal feed samples. The system consists of multiple different sample prep modules, three 6-axis ABB robots and is capable to process up to 400 samples per day. It was developed based on an earlier, simpler but also fully automated model that has been successfully used in our laboratory for more than 6 years for analysing mycotoxins and veterinary drugs. From spiking with standards, multiple extractions, salt dosing, centrifugation, phase separation, evaporation, and filtration to the preparation of dilutions and the filling of the final solution into a vial and its labelling, every step is fully automated. The only remaining manual steps are placing the samples on the input conveyor belt and removing the prepared vials for LC-MS/MS measurement. Comprehensive software makes it possible to run several different methods in parallel. The system covers various methods for mycotoxin (e.g. *Alternaria* toxins, patulin, fumonisins), process contaminants & adulterants (e.g. acrylamide, melamine, banned colourants) and veterinary drug residue analysis (e.g. amphenicols, sulfonamides, macrolides). Matrix-dependent individual sample preparation and thus new approaches for the development of robust and cost-efficient methods are also possible.

**Keywords:** robotic, sample preparation, mycotoxins, process contaminants, veterinary drug residues

**Acknowledgement:** *We would like to thank the manufacturer of the system, Elbatron GmbH, for the joint development, the trusting, intensive collaboration, the many years of cooperation and the realisation of our wishes in this system.*

## K2

### EFFECT-BASED ANALYSIS USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AS A TOOL TO EVALUATE COMPLEX MIXTURES

Paul Rogeboz<sup>(1)</sup>, Hélia Latado<sup>(1)</sup>, Claudine Cottet Fontannaz<sup>(1)</sup>, Blandine Descamps<sup>(1)</sup>, Amaury Patin<sup>(1)</sup>, Maricel Marin-Kuan<sup>(1)</sup>

<sup>1)</sup> Nestlé Research, Switzerland

\*Corresponding author - E-mail: Paul.Rogeboz@rd.nestle.com

Food is a complex mixture of chemicals. To understand the health significance of food chemicals is complex. Some chemicals may be associated with beneficial effects while others have been shown to be harmful, producing toxicity. Chemicals present in food mixtures and food-related materials need to be managed to ensure food safety. Establishing the safety of the numerous toxicologically uncharacterized chemicals is however very challenging. *In vitro* toxicology is a promising tool for hazard characterization and risk assessment. Effect-based analysis targeting key toxicological events brings mechanistic and relevant information for the human situation. However, classical liquid microwell plates are not adequate to evaluate food mixtures.

Indeed, despite several advantages as the standardization, the reproducibility, the recognition by the regulatory agencies (Rainer, Mayrhofer et al. 2019), some limitations can be highlighted like the compatibility of the solvent with the bioassays, the inadequate limit of detection with regulation (e.g. with AMES) (Rainer, Pinter et al. 2018, Schilter, Burnett et al. 2019), the matrix effect and a lack of full chemical identification. Therefore, an alternative effect-based analysis using high-performance thin layer chromatography (HPTLC) coupled to bioassays was identified as a promising approach to identify potential hazardous compounds (Debon, Rogeboz et al. 2022, Debon, Gentili et al. 2023). This new strategy allowed not only to solve several limitations related to classical multiwell systems but also to facilitate the chemical identification of the compounds responsible of the observed biological activity.

The current presentation will outline a framework using food mixtures as case studies including suitable extraction methods to avoid data misinterpretation, alternative effect-based analysis using HPTLC-based for endocrine activity and genotoxicity assessment. Despite some existing limitations, examples of successful identification of bioactive compounds will be highlighted providing a powerful contribution to the risk assessment.

**Keywords:** *in vitro* bioassay, genotoxicity, endocrine activity, HPTLC, safety of complex matrices

### K3

#### THE EVALUATION OF GROWTH-INHIBITORY EFFECT OF PLANT VOLATILE COMPOUNDS AGAINST FOOD PATHOGENIC MICROORGANISMS IN LIQUID AND VAPOR PHASE USING BROTH MICRODILUTION VOLATILIZATION METHOD

**Adrish Dutta**<sup>(1)</sup>, **Marketa Houdkova**<sup>(1)</sup>, **Ladislav Kokoska**<sup>(1)</sup>

<sup>1)</sup> *Department of Crop Sciences and Agroforestry, Faculty of Tropical AgriSciences, Czech University of Life Sciences Prague, Kamycka 129, Prague, 165 21, Czech Republic, Czech Republic*

*\*Corresponding author - E-mail: duttaa@ftz.czu.cz*

Foodborne pathogens are causing a huge number of diseases with significant effects on human health and economy [1]. It is of utmost need to find ways to protect food products by inhibiting the growth of food pathogens causing foodborne illness. Plant-derived volatile agents, which are relatively safe and environment friendly, seems to be prospective candidates for development of new generation of food preservatives [2]. For example, these agents can be used in the form of vapors which do not need to be applied directly to the food products but instead can be regularly distributed in the atmosphere protecting the food products from these pathogens. In this work, the growth-inhibitory effects of plant volatile compounds namely -thujaplicin, carvacrol, citral, menthol, thymol and thymoquinone were tested against various food pathogens such as *Aspergillus niger*, *Bacillus cereus*, *Clostridium perfringens*, *Enterococcus faecalis*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica Typhimurium*, *Shigella flexneri*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* using broth microdilution volatilization method [3]. As a result, thymoquinone exhibited a strong activity against all the tested pathogens with MICs in the range of 4 to 256 µg/mL and 8 to 128 µg/mL in liquid phase and vapor phase, respectively. The similar effect was produced by -thujaplicin which exhibited MICs ranging from 16 to 32 µg/mL in liquid phase and was >64 µg/mL in vapor phase. In addition, carvacrol and thymol showed moderate antibacterial effects in liquid (MICs 128-256 µg/mL) and vapor (128-512 µg/mL) phase. Among other compounds tested, citral exhibited weak growth-inhibitory effect with MICs ranging from 256 to 512 µg/mL and 512 to 1024 µg/mL for liquid and vapor phase, respectively. Based on above mentioned results, -thujaplicin and thymoquinone can be suggested for further research focused on development of volatile agents for antimicrobial atmosphere packaging of food. However, further experiments focused on the safety, organoleptic properties and efficacy in food models of these compounds will be necessary before their possible introduction to the food preservation practice.

**Keywords:** food pathogens, plant volatiles, food preservatives, broth microdilution volatilization, antimicrobials

**Acknowledgement:** *Czech University of Life Sciences Prague [project IGA 20243109].*

### K4

#### DEVELOPMENT OF AN ANTIOXIDANT ASSAY FOR ANTHOCYANINS IN RED CABBAGE USING LIQUID CHROMATOGRAPHY COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETRY

**Claudia Lombroni<sup>(1)</sup>, Ksenia Morozova<sup>\*(2)</sup>, Lucrezia Angeli<sup>(2)</sup>, Giovanna Ferrentino<sup>(2)</sup>, Matteo Scampicchio<sup>(2)</sup>**

<sup>1)</sup> University of Turin and Free University of Bozen-Bolzano, Italy

<sup>2)</sup> Free University of Bozen-Bolzano, Italy

\*Corresponding author - E-mail: Ksenia.Morozova@unibz.it

Red cabbage (*Brassica oleracea* var. *capitata* f. *Rubra*) is a food rich in bioactive compounds, particularly anthocyanins, which have significant potential as natural colorants and antioxidants. However, the antioxidant mechanisms and their links to molecular structures are not fully understood. Current methods like ORAC, DPPH, and FRAP assays are concentration-based and lack efficiency data on neutralizing radicals. Additionally, these assays provide overall reactivity data but overlook synergism and interactions, thereby failing to identify the most reactive anthocyanins.

This study aimed to address these limitations by developing an assay to evaluate the radical scavenging activity of anthocyanins in red cabbage using high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (HPLC-HRMS/MS) and diode array detection (DAD). Initially, the red cabbage extract was analyzed, identifying thirteen anthocyanins, with cyanidin 3-diglucoside 5-glucoside being the most predominant and cyanidin being added as internal standard. Next, the reaction between the anthocyanins in the extract and peroxy radicals (ROO•) released from the initiator 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was monitored under physiological conditions at 37°C and pH 7.4. During the reaction a different consumption rate among anthocyanins was observed. Overall, this approach allows a detailed analysis of individual anthocyanin reactivity, identifying the most reactive compounds and providing structure-activity insights.

The relative reduction in peak areas for each anthocyanin indicates specific efficiencies in neutralizing peroxy radicals, likely reflecting their unique molecular structures. Alatanin C, a cyanidin derivative with two glucose units and sinapic acid at the C3 position, exhibited the highest relative reduction and antioxidant efficiency. Interestingly, the reactivity of cyanidin was concentration dependent, shifting from first-order at 20-100 µM to zero-order at 100-200 µM. This suggests saturation of available radicals at higher anthocyanins concentration, implying that antioxidants should be tested only at low concentrations to avoid underestimation of their reactivity.

This approach effectively identifies anthocyanins with antioxidant properties, assesses their radical-scavenging activity, and relates their activity to chemical structures. These findings enhance understanding of anthocyanin reactivity, revealing their capacity to neutralize peroxy radicals and their potential as natural alternatives to synthetic additives.

**Keywords:** red cabbage, anthocyanins, AAPH, antioxidant activity, high-resolution mass spectrometry

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### K5

#### BAD AFTERTASTE IN TRUFFLE SOUP: IDENTIFYING THE CULPRITS IN A REAL-TIME NOSESPACE STUDY

Philipp Sulzer<sup>(1)</sup>, Andreas Mauracher<sup>(1)</sup>, Rene Gutmann<sup>(1)</sup>

<sup>1)</sup> IONICON Analytik, Austria

\*Corresponding author - E-mail: philipp.sulzer@ionicon.com

It is well-known that most "truffle flavored" products, such as oils and pastes, get their characteristic aroma from a more or less complex mixture of food chemicals rather than from real truffles, if only for cost reasons. While consuming food refined with artificial truffle aroma some report a rather pleasant first flavor sensation, which successively evolves into an increasingly disagreeable and pungent dining experience. In order to investigate the science behind this effect, we prepared "truffle soups" with eight different truffle products, as well as with genuine black (*tuber aestivum*) and white (*tuber magnatum pico*) truffles and analyzed the nosespace air of a test subject while tasting the soups in real-time. The experimental setup consisted of a latest generation Proton-Transfer-Reaction - Mass Spectrometry (PTR-MS) instrument with a 1s LoD of about 1 pptv and a mass resolution of about 15,000 m/Δm.

The infamous compound 2,4-dithiapentane, which only occurs in white truffles, was found in the headspace of all products, obviously except for the pure black truffle. Interestingly, its concentration was up to 30 times higher for products supposedly made from black truffles compared to pure white truffle. On the other hand, 2-acetyl-5-methylfuran, a compound also only occurring in white truffles, was not found in any product except for the pure white truffle, indicating that 2,4-dithiapentane had indeed been artificially added and did not originate from infusion by real white truffles.

Real-time nosespace analysis with PTR-MS is well-established. Disposable nosepieces are inserted into a test subject's nose and connected via a cold-spot-free transfer line to the PTR-MS instrument, which continuously draws several hundred sccm for chemical ionization under well-controlled conditions and subsequent detection in a TOF mass analyzer. Earlier nosespace studies during the consumption of soup were partly inconclusive, as the concentrations of several compounds known to be present in the soup did not correlate with soup consumption. One example is C<sub>5</sub>H<sub>10</sub>OS (protonated *m/z* 119, "garlic flavor"). With the current mass resolution of 15,000 m/Δm it was revealed that a total of five isobars can be distinguished at nominal *m/z* 119 with C<sub>5</sub>H<sub>10</sub>OS.H<sup>+</sup> having previously been completely masked by the four much higher concentrated isobars originating from human breath and inhaled air.

Ten "truffle soups" were prepared and repeatedly tasted during nosespace sampling. While after the first sip complex flavor mixtures could be detected, most compounds were not present anymore already after several seconds. However, for example, dimethyl sulfide ("cooking cabbage"), nonanal ("waxy and fatty"), and 2,4-dithiapentane were still quantified at considerably elevated levels in the nosespace even five minutes after a sip of truffle soup, which explains the transition from a pleasant to a quite disagreeable sensation while eating food infused with artificial truffle aroma.

**Keywords:** truffle, nosespace, real-time, PTR-MS, soup

### K6

#### IDENTIFICATION OF PROCESS-DEPENDENT MAILLARD ADDUCTS IN WHOLEMEAL DRY PASTA BY UNTARGETED HIGH RESOLUTION TANDEM MASS SPECTROMETRY: A COMPARATIVE ANALYSIS OF DIFFERENT DRYING CYCLES AND SEMOLINA COMPOSITION

Chiara Nitride<sup>(1)</sup>, Anna Luparelli<sup>(2)</sup>, Antonella Lamonaca<sup>(3)</sup>, Elisabetta De Angelis<sup>(2)</sup>, Linda Monaci<sup>(2)</sup>, **Rosa Pilolli**\*<sup>(2)</sup>

<sup>1)</sup> Department of Agricultural Sciences, University of Naples Federico II, Italy

<sup>2)</sup> Institute of Sciences of Food Production, National Research Council of Italy, Italy

<sup>3)</sup> Institute of Sciences of Food Production, ISPA- CNR, Italy

\*Corresponding author - E-mail: rosa.piolli@ispa.cnr.it

Durum wheat pasta is a common source of complex carbohydrates, which have been thoroughly investigated in terms of starch digestibility, but poorly analysed in terms of protein profile and digestibility.

In this contribution, the proteomic profile of wholemeal pasta prepared with different mixtures of semolina and using different drying cycles will be presented. Comparative investigation by high-resolution mass spectrometry of process-dependent formation of Maillard adducts (MAs) and chemical modifications (deamidation and oxidation) was accomplished and correlated to semolina composition and drying cycles. Spaghetti was selected as model pasta shape and produced in a pilot-scale, using two semolina with different protein content corresponding to medium and high quality, and two different drying cycles: (i) 21h at 50°C and 6h at 85°C. First, total protein extraction was performed under strong denaturant and reducing conditions for all sample types and the extracted proteins were quantified by colorimetric assay. Three independent samples were prepared for each sample and the differences in averaged contents resulted to be not statistically significant, confirming comparable and satisfactory extraction yields for all samples. Discovery high resolution MS/MS analysis and software-based identification were tailored to disclose the presence of MAs on lysine residue, as well as chemical modifications of methionine, glutamine, asparagine and arginine. More than four thousand peptide sequences have been identified for each sample with on average half of them presenting at least one modified amino acid. Most of the modifications were accounted by MAs on lysine (about 49%), and by deamidation of glutamine (about 29%) and asparagine (14%). Specifically, advanced glycation end product MGH1 ( $\Delta m = +96,0318$ ), carboxymethyl-lysine ( $\Delta m = +58,0049$ ) and carboxyethyl-lysine ( $\Delta m = +72,0206$ ) containing peptides resulted to be the most common process-dependent Maillard modifications. Detailed discussion about difference among the four types of pasta samples will be presented in the contribution in correlation with the semolina composition and the drying cycles.

**Keywords:** Maillard adducts, high resolution tandem mass spectrometry, pasta drying cycles, proteomic profile, protein heat damage

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### K7

#### DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE METHOD FOR VETERINARY DRUGS IN ANIMAL BASED-FOOD MATRICES USING LIQUID-CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY

Eloi Marilleau<sup>\*(1)</sup>, Yvan Gru<sup>(1)</sup>, Nicolas Cimetiere<sup>(2)</sup>, Alexandre Verdu<sup>(3)</sup>

<sup>1)</sup> INOVALYS, France

<sup>2)</sup> Univ Rennes, Ecole Nationale Supérieure de Chimie de Rennes, CNRS, ISCR (Rennes Institute of Chemical Sciences), UMR 6226, France

<sup>3)</sup> Bruker, France

\*Corresponding author - E-mail: [eloi.marilleau@inovalys.fr](mailto:eloi.marilleau@inovalys.fr)

Consumer health is at risk when veterinary drugs residues are found in animal-based food matrices like milk, eggs, and muscle. More and more multi-residue approaches using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) are being developed to check that residue levels do not exceed the legal limitations imposed by Europe for banned and unbanned substances [1]. High-resolution techniques have the advantage of producing more complete data thanks to Data Independent Analysis (DIA) method, which has high selectivity and sensitivity. Therefore, these methods have the capacity to carry out retrospective analysis for the research of new substances. This work has resulted in a rapid and sensitive method for screening and confirming about 200 veterinary drug residues in muscle. The method was based on a solid-liquid extraction (SLE) with water/acetonitrile followed by a second SLE with acetonitrile. Then a concentration of the supernatant organic phase by evaporation was made before injection in LC-Q-TOF. This method was validated according to the (EU) 2021/808 regulation for method performance in order to be further applied in routine analysis. Validation results showed that the method performed well for both qualitative and quantitative screening. Indeed, 85 % of the 257 substances were screened at 10 µg/kg. Approximately two-thirds could be quantified at this concentration. Moreover, for 50 % of the molecules, the screening level reached 1 µg/kg. The qualitative and quantitative confirmation results respectively showed that 69 % and 53 % of molecules reached the 10 µg/kg level. This method is currently being tested on interlaboratory samples to check whether it can be applied to real samples. Furthermore, these results will be compared to those produced by the ANSES laboratory to investigate the method applicability on other high-resolution mass spectrometry systems. Subsequently, further validations will be carried out on new matrices like milk, eggs, liver and kidney.

**Keywords:** high resolution mass spectrometry, veterinary drugs, multi-residue

### K8

#### **ADVANCING SAFETY ASSESSMENT - APPLICATION OF NON-TARGETED ANALYSIS FOR NEW FOODS AND FOOD PRODUCTION SYSTEMS**

Hui Yi Lim<sup>(1)</sup>, Bin Li<sup>(1)</sup>, Yai Foong Chew<sup>(1)</sup>, Lin Min Lee<sup>(1)</sup>, Wei Yong Desmond Teo<sup>(1)</sup>, Haiyan Li<sup>(1)</sup>, Joanna Khoo<sup>(1)</sup>, Sheot Harn Chan<sup>(1)</sup>, Dingyi Yu<sup>\*(1)</sup>

<sup>1)</sup> National Centre for Food Science, Singapore Food Agency, Singapore

\*Corresponding author - E-mail: Yu\_Dingyi@sfa.gov.sg

Driven by the need for sustainable food production to address global food security concerns, there has been a significant push towards new foods and food production systems in recent years. Innovations such as plant-based meat, cell-cultured meat, insect-based foods, and other alternative protein sources represent pivotal advancements in this endeavour. However, new technologies and unconventional agricultural inputs could introduce additional complexities and considerations to food safety. Rigorous food safety evaluation of these new foods is essential to uncover potential and unknown food safety risks.

To safeguard food safety and public health, it is crucial to establish comprehensive analytical strategies capable of swiftly identifying emerging food safety concerns. High resolution mass spectrometry (HRMS)-based non-targeted analysis (NTA) emerges as a powerful tool in this context, offering unparalleled insights into the diverse chemical composition of food products and production inputs. Nonetheless, NTA can be especially challenging for food analysis due to sample diversity and complexity. The sheer volume of data generated from the HRMS often results in a laborious data processing workflow.

This presentation outlines the workflow of an NTA approach and its application for the safety assessment of new foods and food production systems. Leveraging smart data processing methods, this approach swiftly identifies a diverse range of potential chemical hazards. Furthermore, this platform extends its capability to proteomics analysis, enabling detailed characterisation of protein/peptide profiles and the detection of potential foodborne allergens.

In brief, the established in-house NTA analytical system has demonstrated enhanced effectiveness for non-targeted analysis in foods, thus strengthening capabilities to safeguard food safety and public health.

**Keywords:** non-targeted analysis (NTA), food safety, chemical contaminants, proteomics, new foods and food production system

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### K9

#### SCREENING OF TOCOLS CONTENT IN DIFFERENT MAIZE KERNEL TYPE

**Jelena Vukadinovic**\*<sup>(1)</sup>, Jelena Srdic<sup>(1)</sup>, Violeta Andelkovic<sup>(1)</sup>, Nikola Grcic<sup>(1)</sup>, Milan Brankov<sup>(1)</sup>, Milomir Filipovic<sup>(1)</sup>, Snezana Mladenovic Drinic<sup>(1)</sup>

<sup>1)</sup> Maize Research Institute Zemun Polje, Serbia

\*Corresponding author - E-mail: jmesarovic@mrizp.rs

Maize (*Zea Mays* L.) is one of the main crops after rice and wheat commonly grown worldwide and one of the major food sources, especially for developing countries. Pigmented maize (yellow, black, purple, orange, red, and blue) and maize with specific traits contain secondary metabolites that have a positive role and protective impact on human health. Bioactive compounds naturally occurring in maize are tocopherols ( $\alpha$ -T,  $\beta$ -T,  $\gamma$ -T and  $\delta$ -T) and tocotrienols ( $\alpha$ -T3,  $\beta$ -T3,  $\gamma$ -T3 and  $\delta$ -T3), together called tocols or vitamin E. This study aimed to estimate the content of tocols in different maize kernel types and evaluate a possible linkage between them. In our study, the content of tocols in popcorn, white, yellow, red, orange, and sweet maize was determined using high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Obtained results revealed that the sweet maize has the highest content of  $\beta + \gamma$ -T (55.18 - 94.30  $\mu\text{g/g}$  dry weight (dw)) among all tested maize kernel types. Similarly, in yellow and orange maize kernel types was found the highest content of  $\alpha$ -T (9.44 - 20.54  $\mu\text{g/g}$  dw) in comparison to other maize kernel types. According to the Principal Component Analysis (PCA), samples of popcorn, sweet, and red maize were separated on loading plots indicating a unique linkage between kernel types and tocols content. The obtained results indicate that there is a need to improve the nutritional quality of maize kernel in terms of vitamin E content, especially for white and yellow, which are predominantly used in human nutrition in Europe.

**Keywords:** corn, HPLC, tocopherols, functional food, phytochemicals

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## GENERAL FOOD ANALYSIS

### K10

#### EXPLORATION OF NEW LOW-PRESSURE GC COLUMNS FOR FOOD AND ENVIRONMENT EMERGING CONTAMINANTS

Jana Hepner<sup>(1)</sup>, Whitney Dudek-Salisbury<sup>(1)</sup>, Chris English<sup>(1)</sup>

<sup>1)</sup> Restek Corporation, United States of America

\*Corresponding author - E-mail: jana.hepner@restek.com

The Low-Pressure GC (LPGC technique) has been successfully used in the past for pesticide residues' analysis. However, the technique is very versatile, and it allows for other applications, especially if different column phases are used. So far, the majority of the applications have been using the "5"-type phase (95% dimethylpolysiloxane, 5% diphenyl polymer). To expand on the previous applications, four additional column phases were selected (cyanopropylphenyl dimethylpolysiloxane; 50% dimethylsiloxane, 50% diphenyl; 65% dimethylsiloxane, 35% diphenyl; and trifluoropropylmethyl polysiloxane phases) to analyze various food and environmental contaminants, such as nitrosamines, alkylfurans, phthalates, arylamines and fluorotelomer alcohols. The LPGC techniques provided significant reduction in run times (up to 3.3x faster runs) and helium consumption reduction (up to 81% less helium used), while keeping an acceptable resolution.

**Keywords:** GC-MS, LPGC, pesticides, PFAS, contaminants

## K11

### MOROCCAN AND ITALIAN CAROBS (*CERATONIA SILIQUA L.*): CHEMICAL COMPOSITION, LIPID FRACTION, AND MINERAL PROFILE

Vincenzo Nava<sup>\*1)</sup>, Ambrogina Albergamo<sup>(1)</sup>, Angela Giorgia Potortì<sup>(1)</sup>, Vincenzo Lo Turco<sup>(1)</sup>, Giuseppa Di Bella<sup>(1)</sup>

<sup>1)</sup> Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy

\*Corresponding author - E-mail: vnava@unime.it

The carob (*Ceratonia siliqua L.*), a fruit typical of the Mediterranean but little known in the panorama of European crops, whose commercial value is increasing thanks to its multiple uses, is being revalued and considered ideal for contributing to the United Nations Sustainable Development Goals (SDGs) in the Mediterranean area [1]. Thanks to its nutritional value, carob is used as a health ingredient in many foods [2]. Its chemical composition can vary according to several factors such as genetic variability, environmental conditions, varieties, cultivation practices, geographical origin and harvesting period [3]. In this study, 90 Mediterranean carobs were characterized to determine their chemical composition, lipid fraction and mineral profile in pulp and seeds, and differentiating them according to their geographical origin (different provinces in Morocco and Italy). In addition, the potential toxicological risk for humans following carob consumption was assessed.

In general, the percentages of total lipids, protein and fibre were higher in the seeds than in the pulp. The ash content was more variable, while the percentage of sugars was higher in the pulp. The pulp had a higher percentage of palmitic and oleic acids than the seeds, which had a higher linoleic acid content. Among the sterols,  $\beta$ -sitosterol,  $\Delta$ -5-avenasterol and stigmasterol accounted for more than 70% of the total sterols in the pulp, while  $\beta$ -sitosterol alone accounted for more than 70% of the sterols in the seeds. Among the tocopherols,  $\gamma$ -tocopherol was the most abundant isomer in both pulp and seeds. Carob was also a good source of squalene. In general, all samples proved to be good sources of macro-mineral elements, of which potassium was the most abundant, followed by calcium, magnesium, and sodium. Most of the essential trace elements were present in higher concentrations in the seeds than in the pods. However, this varied according to the sample and the area of origin. About toxic and potentially toxic elements, and with reference to European Regulation 2023/915, carob has a maximum limit of 0.10 and 0.020 mg/kg for lead and cadmium respectively. Consequently, only some of the Moroccan carobs analyzed exceeded these limits. The PCA analysis showed that the lipid fraction, the chemical composition, and the mineral profile of carobs can help to distinguish samples according to their Moroccan or Italian origin. Finally, the evaluation of the potential toxicological risk showed that the consumption of one carob (10 g) per day results in a low intake of all the elements analyzed. The study can provide further insights into the chemical composition, lipid fraction and mineral profile of the Mediterranean carob, which is still poorly understood, and can contribute important information on the nutritional and functional value of this fruit. This is crucial to improving the market for carob and carob products by making consumers aware of its incredible properties.

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**Keywords:** *Ceratonia siliqua L.*, chemical composition, lipid fraction, mineral profile

### K12

#### QUANTITATION OF ALLYL ESTERS, LINALOOL, CITRAL, AND LINALYL ACETATE IN YOGURT AND SOFT DRINKS BY MEANS OF GC-MS

Anna Maria Gabler<sup>(1)</sup>, Michal Stupak<sup>(1)</sup>, Josephine Bönick<sup>(1)</sup>, Annemarie Richter<sup>(1)</sup>, Frederike Sommerfeld<sup>(1)</sup>, Susanne Esslinger<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: Anna-Maria.Gabler@bfr.bund.de

The pineapple and tropical fruit flavor of fruit yogurts and soft drinks is often supported by the addition of allyl esters - especially allyl hexanoate [1]. Allyl esters belong to the group of carboxylic acid esters and can be hydrolyzed to allyl alcohol. Allyl alcohol may be hepatotoxic because it is metabolized by alcohol dehydrogenase to acrolein [2]. Therefore, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has set a group ADI of 50 µg/kg body weight per day for allyl alcohol for three compounds (allyl hexanoate, allyl heptanoate and allyl isovalerate), which was confirmed for other allyl esters in subsequent studies [3]. In addition to allyl esters, the flavoring substances linalool, citral and linalyl acetate also contribute to the fruity and citrus-like aroma of foods and have a low group ADI value of 500 µg/kg body weight per day, expressed as citral [4]. Despite the toxicological assessment and potential health risks, there is still no validated method for the quantitation of allyl esters and the other flavorings. For this reason, the German National Reference Laboratory for Food Additives and Flavorings developed a GC-MS-based stable isotope dilution analysis for the quantitative determination of 22 allyl esters as well as linalool, citral (neral and geranial) and linalyl acetate.

First, suitable extraction methods were developed for the matrices "yogurt" and "soft drink". The chromatographic and mass spectrometric parameters were then optimized in order to study all compounds as selectively as possible. Validation parameters such as linearity, limit of detection and limit of quantification, repeatability and trueness were tested. Finally, the newly developed approach was applied to real samples. For instance, levels of 1-12 mg/kg (yogurt) and 1-24 mg/l (soft drinks) were determined for the compounds allyl hexanoate, linalool, allyl cyclohexane propionate and allyl phenoxy acetate.

For the first time, a GC-MS-based analytical method was developed for the quantitative determination of allyl esters in yogurt and soft drinks. This will provide a powerful tool for estimating dietary exposure to allyl esters and ensuring the monitoring of these compounds in certain food stuff.

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### K13

#### CHARACTERIZATION OF PLANT-BASED BEVERAGES BY 1D, 2D, AND QUANTITATIVE NMR

**Federico Brigante**<sup>(1)</sup>, **Pavel Solovyev**<sup>(1)</sup>, **Luana Bontempo**<sup>(1)</sup>

<sup>1)</sup> *Edmund Mach Foundation, Italy*

\*Corresponding author - E-mail: federico.brigante@fmach.it

Plant-based beverages (PBBs) are defined as emulsions obtained from different plant materials like legumes, cereals, pseudo-cereals, seeds, or nuts after the main steps of soaking, filtration, and thermal treatment. Naturally, they represent lactose-free options ideal for consumers with allergies or intolerance, and they possess environmental advantages compared to bovine milk, since the CO<sub>2</sub> fingerprint for their production is lower, their fiber content, and they favor animal welfare. They are also a staple food for those consumers that opt for new dietary habits such as veganism, vegetarianism, and flexitarianism [1,2]. In the last decade, efforts have been directed mostly towards their nutritional composition (in terms of macro and micronutrients), production processes, anti-nutritional factors (tannins, saponins, and enzyme inhibitors), their sensory acceptability, their protein availability, and the diversity of aroma-related compounds [1,3]. However, there is a large literature gap in the use of 1D, 2D NMR, and qNMR in the characterization of PBBs to gain deeper knowledge about their composition. The objective of this work was to characterize the polar extract of soy, oat, and almond PBBs by <sup>1</sup>H NMR, HSQC, and qNMR using sucrose as an external standard, with cow milk as a reference matrix. The analysis by <sup>1</sup>H NMR revealed that that the reference matrix was the one with the highest number of found compounds (68). The main chemical families were nitrogen compounds (excluding amino acids), carbohydrates, fatty acids, and organic acids. Then, soy PBB was the one closest to the reference in number of found and identified compounds, sharing fatty acids and carbohydrate signals but with distinct compounds like nucleosides, taurine, histidine, and histamine. Oat and almond PBBs showed around 30% less compounds (48 and 42, respectively) compared to the reference. Oat PBBs showed a richer carbohydrate profile with presence of mono, di, and oligosaccharides, especially maltose and raffinose. Almond PBB was the one with the lowest number of compounds among the 4 matrices, with pantothenate and myo-inositol as distinctive compounds. A total of 33 compounds were quantified, an analysis of variance with Tukey *post-hoc* test showed higher concentration of most organic acids and choline and its derivatives in cow milk. Soy PBB was enriched in compounds like stigmaterol and glucose-1-P, while oat PBB in beta-maltose, trigonelline, and valine. Finally, almond PBB showed significantly higher concentrations of tartrate, galacturonate, and malate. NMR and qNMR served as a fast and efficient tool for the characterization of plant-based beverages, bringing new knowledge about their composition to consumers and producers, and allowing the future proposal of authenticity and quality markers of these matrices after different production processes like incorporation into other matrices or fermentation.

**Keywords:** plant-based foods, food quality, nutrition, food composition, nuclear magnetic resonance

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# GENERAL FOOD ANALYSIS

## K14

### OPTIMAL SCHEME FOR STANDARD ADDITION

**Panagiotis Steliopoulos<sup>(1)</sup>, Franziska Scharmann<sup>(1)</sup>**

<sup>1)</sup> CVUA Karlsruhe, Germany

\*Corresponding author - E-mail: panagiotis.steliopoulos@cvuaka.bwl.de

The standard addition method is a well-known approach to quantification in analytical chemistry. It is used in order to circumvent systematic errors that are due to the sample preparation procedure or are caused by matrix effects. The standard addition technique involves adding varying amounts of the analyte to sample portions of fixed mass or fixed volume and submitting those portions to the sample preparation procedure. After measuring the final extract solutions, the observed signals are linearly regressed on the spiked amounts. The original unknown amount is estimated by the opposite of the abscissa intercept of the fitted straight line.

Despite a widespread belief, the optimal scheme for standard addition - optimal in that sense that it minimizes both the variance and the bias of the result - is not the popular scheme of equidistant additions. In this contribution, we proceed from the assumption that within the considered range the signal is related to the analyte concentration by a linear model with a normally distributed error term with constant variance and derive the optimal scheme for standard addition. Furthermore, we illustrate its performance, i.e., its influence on the bias and the variance of the result, in comparison to that of other schemes.

## K15

### EFFECT OF CLA-PRODUCING ADJUNCT CULTURES OF NUTRITIONAL VALUE OF SHEEP CHEESE

**Federica Litrenta**<sup>(1)</sup>, **Georgiana Bosco**<sup>(2)</sup>, **Annalisa Amato**<sup>(3)</sup>, **Carmelo Cavallo**<sup>(3)</sup>, **Marco Scalisi**<sup>(3)</sup>, **Cinzia Lucia Randazzo**<sup>(2)</sup>, **Vincenzo Nava**<sup>(3)</sup>, **Vincenzo Lopreiato**<sup>(3)</sup>, **Luigi Liotta**<sup>(3)</sup>

<sup>1)</sup> *Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy*

<sup>2)</sup> *University of Catania, Italy*

<sup>3)</sup> *University of Messina, Italy*

\*Corresponding author - E-mail: felitrenta@unime.it

Conjugated linoleic acid (CLA) production by microorganisms has attracted considerable attention due to its high biosynthetic capacity and potential application in the dairy industry. In particular, bacteria such as *Lactobacillus*, *Bifidobacterium* and *Propionibacterium* have shown the ability to synthesise CLA in an efficient way [1]. In this study two *Lactocaseibacillus rhamnosus* strains were used in a mixture and inoculated as starter culture in the traditional cheesemaking of a raw sheep's milk cheese. The chemical composition and fatty acids profile of cheeses (control cheese CT and experiment cheese LB) were evaluated after 24 hours (T0), and then at 15 (T15) and 30 (T30) days of ripening. Statistical analysis of the data showed that the chemical composition of the two groups CT and LB was similar. The lipid and protein contents were not significantly different between the two groups. Significant differences ( $p < 0.05$ ) were observed between the two groups for C16:0 iso, C16:0, C17:0 iso, C18:1n9(trans) and C18:3 n3 (9,12,15) cis fatty acids, which were higher in the LB group. Renes et. al. also observed that the use of a combination of CLA-producing strains generated C18:1n9 trans isomer increases in cheese [2]. No significant difference was observed between the two groups for C18:2 ci9, trans11(CLA). The CLA content ranged from 0.48 to 0.78 g/100 g in the CT group and from 0.61 to 0.76 g/100 g in the LB group. Regarding ripening time, significantly higher levels ( $p < 0.05$ ) of C16:0 and C18:3 n3(9,12,15) cis were found in the CT group at T30 compared to T0 and T15. In the LB group, significantly higher levels of C18:1n9(cis) were found at T15 and T30, while significantly higher levels of C18:0 were found at T0 compared to T15 and T30. Significantly lower levels of C 18:1 trans 11 were found in the LB group at T30. These results are in agreement with the authors Wu et. [3] Furthermore, a decrease in SFA and an increase in MUFA were observed in the LB group during ripening, probably due to the presence of both CLA-producing *Lactobacillus* strains, in agreement with the results obtained by Renes et al. [2] Although the cheeses produced did not differ significantly in terms of CLA content, the cheeses of the LB group presented a good nutritional profile. Overall, the use of these CLA-producing cultures could be a promising approach to improve the nutritional quality of cheese fat, with a focus on bioactive fatty acids, which would be of particular interest to the dairy industry to meet consumer demands.

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**Keywords:** conjugated linoleic acid, sheep cheese, fatty acids profile

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## K16

### MINERAL ELEMENTS IN COMMERCIAL BABY FOODS

Angela Giorgia Potorti<sup>(1)</sup>, Irene Maria Spanò<sup>\*(1)</sup>, Antonio Cambria<sup>(1)</sup>, Vincenzo Lo Turco<sup>(2)</sup>, Giuseppa Di Bella<sup>(1)</sup>

<sup>1)</sup> Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy., Italy

<sup>2)</sup> Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy

\*Corresponding author - E-mail: irene.spano@studenti.unime.it

Given the vulnerability of the organs and immune systems of infants and children, extreme care must be taken to ensure the safety and adequacy of the foods consumed. The World Health Organization (WHO) has stated that infants should be exclusively breastfed for the first six months of life and then introduced to foods that complement their growth and development. Foods designed to meet some or all the nutritional needs of children (age group: infants to three years) are often referred to as 'baby foods'<sup>1</sup>. However, these foods undergo processing and treatment (cooking, addition of additives to improve taste, etc.) that can introduce harmful xenobiotics into the food. Among these, toxic elements such as cadmium, arsenic, lead, and mercury can often be found in baby food due to their uptake by plants from soil and water or by migration from packaging materials [1]. In this context, the following study was aimed at monitoring the mineral content of 27 samples of baby foods (homogenized baby foods of different types and powdered milk) purchased in supermarkets in Messina (Italy) and at assessing the potential toxicological risk for children after consumption of these products. Potassium was the most abundant mineral in almost all the samples (range: 1179.67±22.72 - 6024.34±26.57 mg/kg), except in homogenized processed cheese, which had a higher calcium content (average: 3784.40±26.66 mg/kg). Among the essential trace elements, iron and zinc were the most abundant in all the samples analyzed. About toxic and potentially toxic elements, European Regulation 915/2023 sets maximum limits for As, Cd and Pb in some baby products. In general, the infant formula samples analyzed in this study exceeded the permitted levels of arsenic (0.020 mg/kg), cadmium (0.010 mg/kg) and lead (0.020 mg/kg). In addition, most of the infant formulae analyzed in this study had an arsenic content above the maximum permitted level. For the toxicological risk assessment, a range of consumption between 40 g/day and 250 g/day was considered as the most common amounts of tinned infant formulae, with reference to the EFSA guidelines, and an average weight of 9 kg for infants aged 7-11 months [2,3]. Consumption of these baby foods resulted in a complete intake (exceeding the recommended intakes) of Cr for homogenized veal, Mo for homogenized zucchini and potatoes, Na, Ca and Co for homogenized processed cheese and Ca, Co, and Se for powdered milk. However, consumption of most of the baby products analyzed resulted in intakes of toxic and potentially toxic elements (particularly As, Cd, Pb and Hg) exceeding the reference levels in almost all samples. In the light of the results obtained, it is therefore of paramount importance to continue to monitor the mineral content of these products to protect children's health.

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**Keywords:** commercial baby foods, mineral elements, toxicological risk assessment

### K17

#### **WP-CBR001 - A NEW MATRIX-CERTIFIED REFERENCE MATERIAL FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) AND TOXIC ELEMENTS IN WHEY PROTEIN POWDER**

Simon Lobsiger<sup>\*1)</sup>, Silvia Mallia<sup>1)</sup>, Lena Marki<sup>1)</sup>, Gisela Umbricht<sup>1)</sup>, Hanspeter Sprecher<sup>2)</sup>, Kathrin Breittruck<sup>2)</sup>, Markus Obkircher<sup>3)</sup>, Alexandra Jaus<sup>1)</sup>

<sup>1)</sup> Federal Institute of Metrology METAS, Switzerland

<sup>2)</sup> Sigma-Aldrich Production GmbH (a Subsidiary of Merck KGaA, Darmstadt, Germany), Switzerland

<sup>3)</sup> Sigma-Aldrich Chemicals Pvt Ltd (a Subsidiary of Merck KGaA, Darmstadt, Germany), India

\*Corresponding author - E-mail: [simon.lobsiger@metas.ch](mailto:simon.lobsiger@metas.ch)

Matrix-based certified reference materials (CRMs) play a key role in food contaminant analysis to ensure measurement accuracy and comparability, as they exhibit the same matrix effects during sample preparation and measurement as the food sample under investigation. However, the availability of such CRMs is still limited. Therefore, there is a great need for the supply of new CRMs, which ultimately contributes to food safety.

Here, we give an insight into the development of a new CRM (WP-CBR001), based on a whey protein powder, one of the most widely used food ingredients, for the determination of the four polycyclic aromatic hydrocarbons (PAHs) benz[a]anthracene (BaA), benzo[a]pyrene (BaP), benzo[b]fluoranthene (BbF) and chrysene (Chr) and the four toxic elements arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg). We will demonstrate the advantages of using the aforementioned matrix CRM. For example, we will illustrate the importance of choosing the most appropriate solvent for the extraction of PAHs in order to obtain correct results [1].

[1] S. Lobsiger et al., Anal. Bioanal. Chem., 2023, 415, 5819-5832 (10.1007/s00216-023-04863-9).

**Keywords:** certified reference material, PAHs, toxic elements, whey protein powder, food safety

**Acknowledgement:** We gratefully thank Hochdorf Swiss Nutrition AG (Sulgen) for providing the whey raw material and performing spray drying.

### K18

#### EVALUATION OF THE ANTIOXIDANT CAPACITY OF EXTRACTS OF MUSHROOMS WITH HIGH GASTRONOMIC VALUE

Elena Ortega Caneda<sup>(1)</sup>, Estrella Espada Bellido<sup>\*(1)</sup>, Rosa Maria Mateos Bernal<sup>(2)</sup>, Ceferino Carrera Fernandez<sup>(1)</sup>, Nuria Chinchilla Salcedo<sup>(3)</sup>, Miguel Palma Lovillo<sup>(1)</sup>, Gerardo Fernandez Barbero<sup>(1)</sup>

<sup>1)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, 11510 Puerto Real, Spain

<sup>2)</sup> Area of Biochemistry and Molecular Biology, department of Biomedicine, Biotechnology and Public Health, Faculty of Sciences, University of Cadiz, Spain

<sup>3)</sup> Allelopathy Group, Department of Organic Chemistry, Institute of Biomolecules (INBIO), Campus de Excelencia Internacional (ceiA3), School of Science, University of Cadiz, 11510 Puerto Real, Spain

\*Corresponding author - E-mail: [estrella.espada@uca.es](mailto:estrella.espada@uca.es)

Mushrooms are macrofungi that have been part of our diet for millions of years, valued not only for their flavour and versatility in cooking, but also for their nutritional and medicinal benefits. Among the edible species are the mushrooms of the genus *Morchella*, of high gastronomic and commercial value. These mushrooms are very difficult to obtain and have been used as traditional medicine in China for thousands of years. They have numerous therapeutic applications, being notable for their antibacterial, antioxidant, anti-inflammatory and anti-diabetic properties, among others. All these properties of mushrooms are due to their valuable nutritional profile and the presence of bioactive compounds such as tryptophan, which is characterised by its involvement in biological processes such as neurotransmission and its antioxidant capacity.

For this reason, the concentration of tryptophan in a total of twelve samples of mushrooms of the genus *Morchella* collected in different locations in Andalusia was determined. For this purpose, the extraction of the bioactive compound was optimised by ultrasound-assisted extraction (UAE) using a Box-Behnken design of experiment (BBD). The quantification of tryptophan concentration was performed by ultra-high performance liquid chromatography (UHPLC).

On the other hand, the antioxidant capacity of *Morchella* extracts was evaluated by DPPH and ABTS spectrophotometric methods, as well as two bioassays to determine the acetylcholinesterase (AChE) enzyme inhibitory activity and tyrosinase inhibitory activity. Good results were obtained for both antioxidant capacity and acetylcholinesterase and tyrosinase inhibitory activity, related to the concentration of tryptophan in the mushrooms.

On the other hand, the antioxidant activity "in vivo" of *Morchella* extract concentrated in tryptophan has been studied against stress caused by hyperglycaemia in a human fibroblast cell model, in order to verify the beneficial potential of these mushrooms at the cellular level.

**Keywords:** antioxidant power, tryptophan, morchella, mushroom, bioactive compounds

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### K19

#### PHYSICOCHEMICAL AND SENSORY PROPERTIES OF SOYMILK PREPARED FROM SOYBEANS SOAKED IN D-XYLOSE AND L-LEUCINE SOLUTIONS USING ULTRASONIC TREATMENT

Eunyeong Choi<sup>(1)</sup>, Kwang-Geun Lee<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Science and Biotechnology, Dongguk University-Seoul, South Korea

\*Corresponding author - E-mail: kwglee@dongguk.edu

This study investigated the physicochemical, sensory, and chemical properties of soymilk prepared from yellow and black soybeans soaked in D-xylose and L-leucine solutions, followed by ultrasonic treatment. The ultrasonic treatment was applied using a probe-type ultrasound device at an amplitude of 80% for durations of 5 and 10 minutes. The results revealed significant variations in the properties of soymilk depending on the type of soybean, soaking solution, and duration of ultrasonication. Color analysis indicated a reduction in the L\* value (lightness) in soymilk prepared from soybeans soaked in D-xylose (YSB\_XY30) accompanied by a significant increase in the browning index, suggesting enhanced Maillard reaction. Ultrasonic treatment effectively reduced particle size by up to 23.82%, particularly after 10 minutes (YSB\_XY30\_10min), leading to improved homogeneity and stability of the soymilk. The total phenolic content (TPC) and total flavonoid content (TFC) were highest in untreated soybeans, with a decrease observed after soaking in D-xylose, potentially due to phenolic-sugar interactions. The quantification of  $\alpha$ -dicarbonyl compounds, including glyoxal (GO), methyl glyoxal (MGO), diacetyl (DA) demonstrated higher levels in the D-xylose-soaked soymilk compared to the L-leucine-soaked samples. The total concentration of these compounds was highest in the control YSB samples. After 10 minutes of ultrasonic processing, total  $\alpha$ -dicarbonyl compounds dropped by 50.92% (from 41.55 to 20.39  $\mu\text{g/mL}$ ). These findings underscore the potential of combining D-xylose and L-leucine soaking with ultrasonic treatment to enhance the functional and sensory qualities of soymilk.

**Keywords:** soymilk, ultrasound treatment,  $\alpha$ -Dicarbonyl compounds, volatile compounds, antioxidant activity

**Acknowledgement:** This study was supported by the Basic Science Research Program through the National Research Foundation of Korea (grant number NRF, S2024-A049600153)

## K20

### TOTAL PHOSPHORUS AND PHOSPHATES CONTENTS IN MEAT PRODUCTS FROM THE BELGRADE MARKET, SERBIA

Danijela Vranic<sup>(1)</sup>, Radivoj Petronijevic<sup>(1)</sup>, Jasna Djinovic-Stojanovic<sup>(1)</sup>, Vesna Djordjevic<sup>(1)</sup>, Srdjan Stefanovic<sup>(1)</sup>, Vladimir Koricanac<sup>(1)</sup>, Dejana Trbovic<sup>(1)</sup>

<sup>1)</sup> Institute of Meat Hygiene and Technology, Serbia

\*Corresponding author - E-mail: danijela.vranic@inmes.rs

Phosphate additives have important role in manufacturing of meat products. They improve the safety and acceptability of meat products and extend shelf life. Inorganic phosphates used in meat industry are salts of phosphoric acid and their condensed forms (mono-, di-, tri- and polyphosphates). Current EU and national legislation on food additives authorized the most relevant group of phosphate additives as "phosphoric acid - phosphates - di-, tri- and polyphosphates" (E 338-452) in meat products up to 5000 mg/kg (0.5%), and the national regulation on meat products quality sets the permitted level of total phosphorus at less than 8 g/kg as P<sub>2</sub>O<sub>5</sub>. It is estimated that these additives are present in up to 65% of processed meat products. The specific measurement of additive-originating phosphates (although desirable) is currently not required by the legislation. The reason is difficulties of determination of phosphate origin due to their presence in biological tissues like meat, and limitations of analytical methodology. The most suitable method of ion chromatography (IC) cannot confirm origin of phosphates present in meat and, also, struggle to separate condensed phosphates higher than triphosphate. Calculation of added phosphates based on protein and total phosphorus determination are unusable if proteins of non-meat origin are present in product.

Total amount of 448 meat product were analysed. Total phosphorus, as P<sub>2</sub>O<sub>5</sub> (g/kg) and protein content were determined according to reference methods, and inorganic phosphates determination was performed by IC with conductometric detection. The highest average value of the total phosphorus content was found in smoked and dried meat products (6.31±1.11 g/kg), and the lowest in canned sausages (2.93±0.54 g/kg). Also, the highest content of inorganic phosphates, as P<sub>2</sub>O<sub>5</sub>, was found in smoked and dried meat products (3.37±1.10 g/kg) and, the lowest in the group of canned sausages (1.09±0.48 g/kg). In canned meat products and boiled sausages, average values for total phosphorus contents were 5.70 ±1.04 and 4.89±1.19 g/kg, respectively, while contents of inorganic phosphates were 3.14± 0.87 g/kg (canned meat products) and 2.39±0.94 g/kg (boiled sausages). Total phosphorus content was over the permitted value in two samples of boiled sausages (0.84%) and in one of smoked and dried meat products (1.58%) as well as inorganic phosphates content in the same product. In any case, it is important to monitor the use of phosphate content in meat products and ensure that levels are maintained below the limit, in terms of consumer health, but also in terms of quality and safety of the products.

**Keywords:** total phosphorus, phosphates, meat products, Serbian market

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## K21

### ASSESSMENT OF DIETARY INTAKE OF IRON IN SERBIAN ADULT BY CONSUMPTION OF DIFFERENT MEAT PRODUCTS

Jasna Djinovic-Stojanovic<sup>(1)</sup>, Ivana Brankovic Lazic<sup>(1)</sup>, Sasa Jankovic<sup>(1)</sup>, Danijela Vranic<sup>(1)</sup>, Munevera Begic<sup>(2)</sup>, Ognjen Krnjaja<sup>(1)</sup>, Vesna Djordjevic<sup>(1)</sup>

<sup>1)</sup> Institute of Meat Hygiene and Technology, Serbia

<sup>2)</sup> Faculty of Agriculture and Food Science, University of Sarajevo, Bosnia and Herzegovina

\*Corresponding author - E-mail: jasna.djinovic@inmes.rs

Meat products have played very important role of the Serbian diet. Traditional products from Serbia, such as different types of dry sausage, cooked and/or smoked sausages, bacon, canned meat etc. are widely available in all market chains in the Balkan region. These foods are widely consumed due to their acceptable price, desirable taste, high nutritional value and large variety of products. For most Serbian people foods of animal origin are primary sources of protein and nutrients such as vitamin B12, omega-3 fatty acids and bioavailable forms of essential elements. Iron is an essential element for almost all living organisms as it participates in a wide variety of metabolic processes. Inadequate iron intake and iron deficiency are recognized as a public health problem that affects around 2 billion people around the world.

This study provides original analytical data on the levels of iron (Fe) in different types of meat products which are commonly consumed in Serbia. On the other hand, this study was undertaken to estimate the contribution of different meat products consumption to the daily intake of iron. A total 137 meat products were collected in Serbian market during six months (January 2023 - June 2023). Samples were classified in 4 groups (dry fermented sausages, cooked sausages, bacon and canned meat). The levels of iron were determined by inductively coupled plasma mass spectrometry (ICP-MS). The following mean values were found (expressed as mean  $\pm$  standard deviation, ( $\mu\text{g/g}$ )): dry fermented sausages:  $22.7 \pm 11.5$ ; boiled sausages:  $19.3 \pm 9.4$ ; bacon:  $13.4 \pm 10.7$ ; canned meat:  $10.0 \pm 3.4$ . The levels of Fe in dry fermented sausage samples were statistically higher than the mean level measured in bacon and canned meat samples ( $p < 0.05$ ). Also, the levels in cooked sausages were statistically higher than the mean level measured in canned meat samples ( $p < 0.05$ ). The estimated daily dietary intake (EDI) of Fe was calculated using data of Fe levels obtained in this study as well as data of dietary intake of estimated meat products from the European Food Safety Authority (EFSA) database. The obtained results showed that boiled sausages have the highest contribution in EDI for iron ( $227.8 \mu\text{g/day}$ ) while bacon has the lowest ( $53.6 \mu\text{g/day}$ ). The results for EDI are expressed as % of the Recommended Dietary Allowance (RDA) for adults (male:  $8 \text{ mg/day}$ ; female:  $18 \text{ mg/day}$ ). Analyzed meat products from this study provide in total only 6.70% and 2.98% for men and women respectively of the RDA for Fe. It can be concluded that estimated meat products cannot be valuable source of Fe for Serbian adult populations.

**Keywords:** daily intake, iron, meat products, Serbian population

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## K22

### CALCIUM AND MAGNESIUM CONTENT IN BOILED SAUSAGES FROM THE SERBIAN MARKET

Jasna Djinic-Stojanovic<sup>\*(1)</sup>, Ivana Brankovic Lazic<sup>(1)</sup>, Sasa Jankovic<sup>(1)</sup>, Danijela Vranic<sup>(1)</sup>, Munevera Begic<sup>(2)</sup>, Srdjan Stefanovic<sup>(1)</sup>, Vesna Djordjevic<sup>(1)</sup>

<sup>1)</sup> Institute of Meat Hygiene and Technology, Serbia

<sup>2)</sup> Faculty of Agriculture and Food Science, University of Sarajevo, Bosnia and Herzegovina

\*Corresponding author - E-mail: jasna.djinovic@inmes.rs

Meat and meat products present a significant part in the human diet. In terms of their composition, they are rich in bioactive macro elements such as calcium (Ca), magnesium (Mg) and potassium (K), and the essential elements iron (Fe), copper (Cu), zinc (Zn), selenium (Se), and manganese (Mn). Macro- and micro elements are very important and necessary for adequate physiological functions of the human body and should be available through dietary intake. Mg is an essential nutrient for the human body, which is required for many physiological functions and has an important role as an enzymatic cofactor of more than 300 enzymatic reactions. Ca is also an essential macronutrient for humans which represents approximately 98% of the bones weight and 2% of body weight in an adult person. Calcium metabolism have role in in the control of some health issue (blood pressure, colon cancer, etc.).

Meat processing in Serbia have a long tradition. The most popular meat products are fresh or smoked pork and beef, as well as poultry. At the same time, different meat products such as different types of sausage are widely spread through all chains of supermarkets in the Balkan region. This study provides original analytical data on the levels of Ca and Mg in two types of boiled sausages (finely ground boiled sausages, FS, n=33 and coarsely ground boiled sausages, CS, n=30) which are commonly consumed in Serbia. Samples of boiled sausages were purchased from Serbian retail markets in the first half of 2023. The levels of Ca and Mg in meat products were determined by inductively coupled plasma mass spectrometry (ICP-MS). The mean levels of Ca in finely ground boiled sausages (607.6 mg/kg) in the current study were statistically higher than the mean levels measured in coarsely ground boiled sausages (318.4 mg/kg), ( $p < 0.05$ ). The mean Mg levels in two types of boiled sausages were not statistically different (163.8 mg/kg - FS, 181.9 mg/kg - CS). Significantly higher Ca levels in finely ground boiled sausages could be explained by use mechanically separated meat (MSM) for their production. Namely, MSM is a raw material which is widely used in the meat industry, obtained during the cutting and deboning of gutted poultry carcasses and their parts. Therefore, higher Ca levels in MSM and consequently in meat products may be due to the presence of residual bones in MSM. According to the Serbian regulation on the quality of minced meat, semi-produced meat and meat products, MSM may be used for production both finely and coarsely ground boiled sausages and it must be subject to declaration of the product. Producing practice in Serbia is such that MSM mostly used in higher amount in finely ground boiled sausages, which was confirmed by the declarations of the analyzed products.

**Keywords:** calcium, magnesium, boiled sausages, mechanically separated meat, Serbia

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## GENERAL FOOD ANALYSIS

### K23

#### **OCCURRENCE OF TOTAL AFLATOXINS (B1, B2, G1, G2) IN COMMERCIAL PEANUT AND PEANUT BUTTER FROM THE SAUDI MARKET IN THE PERIOD FROM 2015 TO 2020**

Yaser Almasoud\*<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: yfmasoud@sFDA.gov.sa

This study aimed to evaluate the occurrence of aflatoxins in peanut products from the Saudi market. A total of 472 samples of peanut and peanut butter imported from various countries were collected in the period from 2015 to 2020. Nuts products are susceptible to contamination with mycotoxin, especially aflatoxins, which results of mold growth during harvest or storage. All samples were analyzed by High-Performance Liquid Chromatography (HPLC). Total aflatoxins (B1, B2, G1, G2) were found to exceed the maximum level (ML) in 38 samples (8.1%) with a concentration range of 15.56-973.21 µg/kg, while 434 samples were below ML (91.9%). Specifically, in Peanut butter samples, 27 out of 333 were contaminated with Aflatoxins (8.8%), and 11 out of 139 peanut samples were contaminated with aflatoxins (8.6%). The results show that the percentage of Aflatoxins contamination was reduced in the period (2019 - 2020) compared to (2015 - 2018), due to the control of mycotoxins in Saudi Arabia which played an important role in reducing the contamination of AFs in peanut products.

**Keywords:** mycotoxin, Aflatoxins B1 B2 G1 G2, peanut, peanut butter

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## K24

### ACCURATE QUANTITATIVE PROFILING OF FATTY ACIDS IN PLANT-BASED FOOD USING BY ONE-STEP MICROWAVE-ASSISTED EXTRACTION AND DERIVATIZATION FOLLOWED BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH PARALLEL DETECTION BY FLAME IONIZATION DETECTOR AND MASS SPECTROMETRY

**Angelica Fina**<sup>(1)</sup>, **Andrea Caratti**<sup>(2)</sup>, **Donatella Ferrara**<sup>(3)</sup>, **Giorgia Botta**<sup>(1)</sup>, **Erica Liberto**<sup>(2)</sup>, **Simone Apolloni**<sup>(1)</sup>, **Marco Beccaria**<sup>(4)</sup>, **Diego Carnaroglio**<sup>(5)</sup>, **Giorgia Purcaro**<sup>(3)</sup>, **Chiara Cordero**<sup>\*(2)</sup>

<sup>1)</sup> *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Italy*

<sup>2)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>3)</sup> *Gembloux Agro-Bio Tech, University of Liège, Belgium*

<sup>4)</sup> *Department of Chemical, Pharmaceutical, and Agricultural Sciences (DOCPAS), University of Ferrara, Italy*

<sup>5)</sup> *Milestone srl, Italy*

*\*Corresponding author - E-mail: chiara.cordero@unito.it*

The quality assessment of dietary fats typically involves analyzing their fatty acid composition. In foods, fatty acids are primarily present as glycerol esters, in form of triglycerides. For high-resolution profiling using comprehensive two-dimensional gas chromatography (GC×GC), these fatty acids must be converted into their corresponding methyl ester form, known as fatty acid methyl esters (FAMES). Additionally, an initial separation of the lipid fraction from matrix interferences, such as proteins and fibers present in food samples, is essential. This study aimed to profile fatty acids in foods (available on the market) from two different dietary regimes: omnivorous and vegan/vegetarian. The analytical procedure utilized a one-step microwave-assisted extraction and derivatization (MAED) developed and validated vs. the Official Method Ce 2b-11 by the American Oil Chemical Society (AOCS) [1,2]. The extracted FAMES were then analyzed by GC×GC with reverse-inject differential-flow modulation and parallel detection by flame ionization detection (FID) and mass spectrometry (MS), to identify and quantify individual fatty acids. The GC×GC technique was chosen for its superior separation power compared to one-dimensional GC and the possibility to obtain structured retention patterns for homologs and isomers facilitating FAMES identification even without available reference standards. By external calibration and FID predicted relative response factors, FAMES were accurately quantified and their profiles adopted to evaluate several quality and nutritional indices. Of interest the  $\sum$ SFA,  $\sum$ MUFA,  $\sum$ PUFA, index of atherogenicity (IA) and thrombogenicity (IT), the hypocholesterolemic/hypercholesterolemic (HH) ratio, the health-promoting index (HPI), and the unsaturation index (UI) [2]. Moreover, for products with a short ingredient list, a consistency index corresponding to the percentage relative bias/error ( $\Delta\%$ ) between the experimental FAMES % in the product over the theoretical/expected one was calculated. Results on selected food samples exhibited deviations, with percentage values ranging from -80% to +140% compared to the declared or estimated values based on label information. Notably, processed products and plant-based alternatives to animal meat showed the most significant deviations. Nutritional indices supported a classification of products according to the fat quality while highlighting the importance for professionals in nutrition and dietetics to understand the actual impact of processing procedures on the fat quality and compositional consistency according to the ingredients list. The MAED procedure followed by GC×GC -MS/FID resulted highly flexible and informative supporting accurate definition of FAMES profiles in complex food products with minimal sample manipulation and analyst exposure to harmful solvents and reagents.

[1] doi:10.1016/j.sampre.2022.100039.

[2] doi:10.1016/j.jchromb.2024.124074.

**Keywords:** fatty acid methyl esters, microwave-assisted extraction and derivatization, comprehensive two-dimensional gas chromatography, reversed fill/flush flow modulation

## K25

### SEMI-AUTOMATED SAMPLING DURING TEA INFUSION: DETERMINATION OF THE TRANSFER OF SESQUITERPENE LACTONES FROM BOTANICAL PREPARATIONS (ARNICA MONTANA) INTO HOT TEA INFUSIONS

Franziska Kolrep\*<sup>(1)</sup>, Xenia Anabel Körner<sup>(1)</sup>, Oliver Kappenstein<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: [franziska.kolrep@bfr.bund.de](mailto:franziska.kolrep@bfr.bund.de)

A botanical preparation is any preparation obtained from plants by various processes. The extraction process of secondary plant constituents with an aqueous hot infusion is frequently practiced worldwide and is used for the preparation of different popular tea beverages. The infusion process itself can lead to a selective enrichment, depletion or formation of new substances not contained in the original plant material. Consequently, kinetic studies need to be conducted to answer the question of such different behavior of secondary plant substances originating from herbal preparations into ready-to-drink tea infusions. Such hot infusions are complex mixtures of secondary plant constituents that consist either of different plant parts or mixtures of different plant species. Besides the well-known beneficial effects of hot tea beverages, it is also important to consider some safety aspects related to the presence of contaminants, addictive, psychotropic or other substances of concern [1]. The EFSA database "Compendium of Botanicals" lists plant parts or preparations from plants, including their respective secondary plant constituents, which may be of concern for human health when ingested through foods [2]. This list also includes substances from the group of sesquiterpene lactones (SL), that are naturally occurring substances in plants with about 5000 structures showing a 15-carbon core structure which is derived from the assembling of three isoprene units and a gamma-lactone ring. Listed are the pseudogujanolide-type SLs (PSL) helenalin, dihydrohelenalin, arnifolin, chamissonolide and their esters present in arnica, helenium and ambrosia species or the eudesmanolide-type SL santonin produced by artemisia species for example [2]. Such plant species would therefore be considered a botanical source of contamination of tea. Hence, we targeted to develop and validate an HPLC-MS/MS method for the determination of these structurally very similar PSL in botanicals at the German Federal Institute for Risk Assessment (BfR). So far, this method comprises 44 SL including 19 PSL in addition to numerous flavonoids and caffeic acid ester. In order to improve the data basis for health risk assessment, we established a semi-automatic peristaltic pump sampling system to investigate the time-dependent transfer of SLs from the dry plant material into the hot infusion. Subsequently, *Arnica montana* was used as a model plant to establish kinetics of the transfer of SLs. The SL profiles in the hot infusion, in the infused plant material and in the dry starting material were compared. The results on the transfer into the hot tea infusion and the fate in the plant material of selected SLs are presented.

[1] Dusemund, B., et al., *Risk assessment of feed components of botanical origin - Approaches taken in the European Union*. Food and Chemical Toxicology, 2024. 188: p. 114654.

[2] EFSA Journal, 2012. 10(5): p. 2663.

**Keywords:** sesquiterpene lactones, semi-automated sampling, transfer, kinetics, arnica tea infusion

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### K26

#### FOLIAR APPLICATION OF SODIUM SELENATE: EFFECTS ON BIOACTIVE COMPOUNDS AND QUALITY OF RASPBERRY (*RUBUS IDAEUS* L.)

Manuel Martoccia<sup>\*1)</sup>, Helder Jose Couto Oliveira<sup>2)</sup>, Maurizio Aceto<sup>3)</sup>, Vincenzo Disca<sup>1)</sup>, Matteo Bordiga<sup>1)</sup>, Jean Daniel Coïsson<sup>1)</sup>

<sup>1)</sup> Department of Pharmaceutical Sciences, Università degli Studi del Piemonte Orientale "A. Avogadro", Italy

<sup>2)</sup> REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Portugal

<sup>3)</sup> Department for Sustainable Development and Ecological Transition, Università degli studi del Piemonte Orientale & A. Avogadro, Italy

\*Corresponding author - E-mail: manuel.martoccia@uniupo.it

Red raspberries are one of the most appreciated small fruits due to its flavor and high content of bioactive compounds and nutrients such as polyphenols, vitamins and minerals (Mazur et al., 2014). Selenium (Se) is an essential mineral for humans but not for plants. It is involved in the production of glutathione peroxidases (GPx), responsible for the reduction of systemic oxygen radical's levels, and is also involved in the modulation of thyroid hormones. Therefore, Se enrichment of plants may constitute an optimal strategy to increase the intake of this mineral and to prevent the deficit of selenium (Genchi et al., 2023). Nevertheless, the biofortification can modify the quality and the chemical composition of plants and fruits (Malagoli et al., 2015). In this context, a study on the variation of quality parameters and bioactive content after Se-enrichment was carried out on a red raspberry cultivar (cv). The *Diamond Jubilee*<sup>®</sup> variety was selected for this experiment and was cultivated using the traditional in-soil cultivation method. This re-blooming cv, first introduced in 2013 by US, exhibits a slightly lighter pigmentation and a longer shelf life of several days compared to other varieties. During the phenological phase of fruit swelling, sodium selenate solution (200 mg/L) was applied by spray-foliar application. Two samples were harvested in two different months (June and September) due to the re-blooming nature of the plants. Some plants were not enriched to have a control sample. After harvesting, moisture, titratable acidity, pH, solid soluble contents and maturation index were evaluated to define quality and shelf life of the berries. Se content was analyzed using ICP-MS technique and the total anthocyanins quantification was performed by UHPLC with double online detection (DAD and MS). If no marked differences were found in the proximate composition, significant differences in Se content were found between the two flowering periods, but not between Se enriched and control samples. The same trend was observed for the total anthocyanins content with differences between the two flowering periods but not between Se-enriched and control raspberries.

In conclusion, our results suggest that the foliar application of selenate does not seem to affect the total anthocyanin content, but differences in the content of the individual anthocyanins are noticeable. However, the Se content increased significantly during the second harvest.

**Keywords:** selenium, anthocyanins, antioxidant, biofortification

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## K27

### TRANSFER OF SELECTED PERSISTENT ORGANIC POLLUTANTS IN YELLOW MEALWORM LARVAE (*TENEBRIO MOLITOR*)

Corinna Kurbis<sup>(1)</sup>, Johanna Ziegler<sup>(1)</sup>, Lisa Klusmann<sup>(1)</sup>, Mariko Yamamoto<sup>(1)</sup>, Pia Sy<sup>(1)</sup>, Anne Jahnke<sup>(1)</sup>, Linda Uhr<sup>(1)</sup>, Anja Lüth<sup>(1)</sup>, David M. Schumacher<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Germany

\*Corresponding author - E-mail: corinna.kuerbis@bfr.bund.de

**Introduction:** The larvae of the yellow mealworm beetle (*Tenebrio molitor*) can be a dietary source for animals and humans. While consumed worldwide, in the EU it was authorised as feed only recently in aquaculture [1] and for feeding poultry and pigs [2]. Furthermore, dried *Tenebrio molitor* larvae were authorised in the EU in 2021 as novel food after the European Food Safety Authority (EFSA) assessed the larvae as safe for human consumption [3, 4]. Nevertheless, EFSA stated that only limited data are available on a transfer of chemical contaminants from contaminated substrate into larvae [4]. The aim of this study was to determine a possible transfer of different pollutants like dioxins, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) from contaminated oat-bran to yellow mealworm larvae.

**Materials and Methods:** Yellow mealworm larvae were exposed to oat-bran *ad libitum* for four weeks under controlled conditions for temperature and air humidity. The contaminated oat-bran was fortified with a mixture of dioxins (final concentrations of 0.1 µg/kg), PCB (0.1 µg/kg non-ortho-PCB, 0.5 µg/kg mono-ortho-PCB, 1 µg/kg ndl-PCB) and PBDE (up to 18 µg/kg). Weekly, larvae were taken and analyzed for concentrations and results compared to control larvae reared on blank oat-bran. At the same time, larvae were weighed, counted and feces was removed. Analysis of larvae was done after automated sample preparation (Miura) using a high-resolution gas-chromatography mass spectrometry (dioxins, PCB) and gas-chromatography tandem-mass spectrometry (PBDE).

**Results:** Yellow mealworm larvae kept on contaminated oat-bran developed similarly to control larvae reared on blank oat-bran regarding feed intake, weight and mortality. Furthermore, we observed a transfer of all compounds present in contaminated oat-bran into yellow mealworm larvae. After 3 weeks of exposure a steady-state was achieved with transfer rates of dioxins, PCBs and PBDEs ranging between 30 % and 100 %.

**Discussion and Conclusion:** We could demonstrate a transfer of dioxins, PCBs and PBDEs from feed into *Tenebrio molitor* larvae. A possibility to reduce the amounts might be fasting the larvae before processing into feed and food. Therefore, further investigations on the impact of fasting for 24 h and 48 h on contamination levels are on-going.

[1] Commission Regulation (EU) 2017/893, OJ L 138 pp. 92-116

[2] Commission Regulation (EU) 2021/1925, OJ L 393 pp. 4-8

[3] Commission Implementing Regulation (EU) 2021/882, OJ L 194 pp. 16-21

[4] EFSA Journal, 19(1):6343

**Keywords:** yellow mealworm, transfer, dioxins, PCB, PBDE

## K28

### QUANTIFICATION OF 30 PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN FRUITS AND VEGETABLES

**Anne Jahnke**<sup>(1)</sup>, **Corinna Kurbis**<sup>(1)</sup>, **Nicolas Mertin**<sup>(1)</sup>, **Kerstin Weidner**<sup>(1)</sup>, **Marvin Spieker**<sup>(1)</sup>, **Anja Lüth**<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: anne.jahnke@bfr.bund.de

**Introduction:** Per- and polyfluoroalkyl substances (PFASs) are persistent chemicals with widespread environmental distribution [1]. The European Food Safety Authority (EFSA) identifies fish, fruits and eggs as major sources of human exposure, with PFOA in fruits and vegetables being significant [2]. While no maximum levels for PFASs in fruits and vegetables have been established, Recommendation 2022/1431/EU suggests limits of quantification (LOQs) and indicative levels for four PFASs evaluated by EFSA (PFOS, PFOA, PFNA, PFHxS; EFSA- 4). For fruits and vegetables, the proposed LOQs are  $\leq 0.002 \mu\text{g}/\text{kg}$  for PFOS,  $\leq 0.001 \mu\text{g}/\text{kg}$  for PFOA and PFNA, and  $\leq 0.004 \mu\text{g}/\text{kg}$  for PFHxS [3]. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for 30 different PFASs. The possibility of grouping different fruits and vegetables matrices was evaluated and the feasibility of meeting the proposed LOQs and indicative levels was assessed.

**Materials and Methods:** Beetroot, apple, strawberry, lamb's lettuce, olive, and potato were analyzed. Homogenized samples (10 g) were extracted using water, acetonitrile and hydrochloric acid. The resulting extract was evaporated and reconstituted in ammonium acetate. The sample clean-up was performed by solid-phase extraction (SPE). Quantification was carried out on an LC-MS/MS instrument. Validation followed EURL guidelines [4], including the parameters LOQ, accuracy, reproducibility, and recovery assessment.

**Results:** Quantification of the EFSA-4 at proposed indicative levels was achieved for most samples, except PFHxS in beetroot and olive and PFOS in olive. The proposed LOQ was achievable for PFNA across all samples. Additionally, the proposed LOQ of  $0.002 \mu\text{g}/\text{kg}$  for PFOS was met in apples and lamb's lettuce. The LOQ of PFOA was met for the matrix beetroot and strawberry. Two matrix groups were established: group I for fruits and vegetables with high acid and/or water content, and group II for roots and tubers. The method could not be fully validated for all PFASs, primarily due to accuracy issues attributed to the absence of suitable internal standards.

**Discussion and Conclusion:** The analyzed fruits and vegetable samples demonstrates that the quantification at levels as low as  $0,001 \mu\text{g}/\text{kg}$  is feasible for certain matrices and PFASs using the developed method. Fruits and vegetables with high fat content may require further sample clean-up to achieve the LOQs proposed by Recommendation (EU) 2022/1431. Moreover, finding a blank matrix at the concentration range of the recommended LOQs for validation remains a significant challenge.

[1] Ackerman G., et al.; 2024, Nat. Geosci. 17, 340-346.

[2] Schrenk, D., et al.; 2020, EFSA Journal 18.

[3] Commission Recommendation (EU) 2022/1431, OJ L 221 pp. 105-109.

[4] EURL POPs; 2022, Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed.

**Keywords:** PFAS, method development, fruits and vegetables, LOQ



## K29

### **ANALYTICAL STRATEGY FOR THE DETERMINATION OF CAROTENOIDS AND THEIR FORMS IN FEED AND ANIMAL PRODUCTS**

**Aliaksandra Kharoshka**<sup>(1)</sup>, Kamila Mimrova<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>, Vera Schulzova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: kharosha@vscht.cz*

Carotenoids are tetraterpenoids well-known for their yellow, orange, and red colours. These compounds are classified into two main groups: carotenes (hydrocarbons) and xanthophylls (oxygen-containing carotenes). Several xanthophylls, particularly canthaxanthin and astaxanthin, are known for their use in animal feed, where they are added to enhance the colouration of an animal or its products, such as egg yolks. Although these compounds occur naturally in certain plants and algae, their synthetic forms are often added to feed to achieve a brighter colour. This practice is subject to regulation under EU law, which sets maximum levels for these pigments (either alone or in a mixture with other carotenoids) at 6-100 mg/kg feedstuff. Furthermore, the legislation states the commodities in which neither synthetic astaxanthin nor canthaxanthin may be added. As different formulations are used for feed preparation, it's crucial to understand what compounds and their forms to expect in a sample. In some cases, alkaline hydrolysis may be a mandatory step in sample preparation while some carotenoids may degrade during sample preparation. The main aim of this study was to design a fast and reliable analytical workflow for the determination of carotenoids and their forms (primarily canthaxanthin and astaxanthin) in feed and animal products.

In order to determine present carotenoids, different extraction strategies were tested, where the effect of saponification was assessed as well as the extraction solvent. The separation and determination were performed employing high-performance liquid chromatography (HPLC) with a reverse-phase C18 column combined with diode array detection (DAD). Identification was achieved by comparing the retention times and absorption spectra of the peaks in a sample with those of the available standards. As maximum levels were established, the method was validated including extraction efficiency tests. Finally, the method was applied for the monitoring of added carotenoids in animal feed and egg yolks to ensure both the safety and quality of food products. For identification of unknown forms as well as confirmation of carotenoid presence, mass spectrometry can be used.

**Keywords:** canthaxanthin, astaxanthin, feed, egg yolk

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### K30

#### **ANALYTICAL ASSESSMENT AND HEALTH CLAIMS STRENGTHENING IN GREEK OLIVE OIL: STUDYING THE INFLUENCE OF OLIVE PITTING ON QUALITY PARAMETERS OF THE PRODUCED OLIVE OIL**

**Panagiotis Loukas Gialouris<sup>(1)</sup>, Sofia Drakopoulou<sup>(1)</sup>, Eleni Nastou<sup>(1)</sup>, Anastasia Koupa<sup>(1)</sup>, Georgia Soutani<sup>(1)</sup>, Nikolaos Thomaidis<sup>\*(1)</sup>**

<sup>1)</sup> *Laboratory of Analytical Chemistry, Chemistry Department, National and Kapodistrian University of Athens, Greece*

*\*Corresponding author - E-mail: ntho@chem.uoa.gr*

Olive oil holds a prominent position in the global market, as a food product of high nutritional value. Extra virgin olive oil (EVOO) in particular, is widely recognized as an integral part of the Mediterranean diet and related to several health benefits. It is mainly composed of glycerides and fatty acids that represent more than 98% of the total oil weight. However, many of its distinctive properties are derived from the minor fraction of 2% w/w, including compounds of antioxidant capacity, such as pigments, tocopherols and polyphenols. International organizations, such as the European Union (EU) and the European Food Safety Authority (EFSA) have authorized several health claims acknowledging the health benefits associated to these compounds, under the legislative framework. Special attention has been paid on the phenolic content, as the consumption of olive oil that contains no less than 5 mg of hydroxytyrosol and its derivatives per 20 g of olive oil contributes to the protection of blood lipids from oxidative stress (EU 432/2012). Thus, the increased economic and nutritional value of olive oil makes the evaluation of its quality characteristics of utmost importance. Among the factors that affect its quality is the olive pitting, which is applied before extraction, and considered as one of the most crucial ones. In the present study, novel analytical techniques were implemented to investigate the quality attributes of olive oil, in terms of nutritional value and health claim strengthening. Olive oils from various regions and olive varieties of Greece were analyzed, prior and after destoning, to evaluate the influence of olive pitting on quality parameters of the produced olive oil. Specifically, phenolic compounds were identified using Ultra high-Performance Liquid Chromatography combined with Quadrupole Time-of-Flight Mass Spectrometry (UHPLC-QTOF-MS), while HPLC coupled to Diode Array Detector (HPLC-DAD) was applied to determine pigments, squalene, lutein, and tocopherols. A Gas Chromatography method coupled to Flame Ionization Detector (GC-FID) was also used for the determination of fatty acids. Following screening workflows, individual compounds belonging to different chemical classes were identified and quantified. The effect of olive pitting was also evaluated based on the concentration of hydroxytyrosol and its derivatives as well as the content of  $\alpha$ -tocopherol, monosaturated and polyunsaturated fatty acids, for which relevant health claims have been established. To the best of our knowledge, this is the first time that such a thorough investigation on olive pitting has been implemented, with UHPLC-QTOF-MS, HPLC-DAD and GC-FID methodologies being simultaneously employed, linking the presence of phenolic compounds, pigments and fatty acids to relevant health issue regulations.

**Keywords:** olive pitting, phenolic compounds, pigments, fatty acids, HRMS

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### K31

#### **COMPARISON OF VARIOUS ANALYTICAL METHODS FOR MONITORING OF CHANGES IN THE CHEMICAL COMPOSITION OF SELECTED PLANT OILS DURING LONG TERM STORAGE**

**Chi Mai Nguyenova**\*<sup>(1)</sup>, **Marek Dolezal**<sup>(1)</sup>, **Jan Poustka**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\**Corresponding author - E-mail: [nguyenox@vscht.cz](mailto:nguyenox@vscht.cz)*

The quality and shelf-life of plant oils play a very important role in the food industry. The most important quality factor of oil represents oxidative stability, i.e. the resistance of the oil to oxidation processes. This primarily depends on the chemical composition of oil, especially the content of monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). A higher degree of unsaturation increases the susceptibility to oxidative degradation. The presence of some naturally occurring antioxidants e.g. tocopherols, polyphenols and flavonoids, also positively affects the oxidative stability of oil. Several factors such as the presence of oxygen, light, a catalyst, and temperature can contribute to the degradation processes of oils. In this study different storage conditions of vegetable oils were tested. Various analytical methods, namely Fourier Transform Infrared Spectroscopy (FTIR), Direct Injection High-Resolution Mass Spectrometry (DI-HRMS) and Gas Chromatography with Flame-Ionization Detector (GC-FID), were applied aiming at monitoring of changes in chemical composition of oil and comparing their analytical abilities.

**Keywords:** oxidative stability, storage of oils, FTIR, DI-HRMS, GC-FID

**Acknowledgement:** *The work used facilities provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064).*

### K32

#### **VOLATILE COMPOUNDS, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF LACTIC ACID BACTERIA-BIOCONVERSIONED GRAPE BY-PRODUCT (VITIS LABRUSCA B.)**

**Geonhak Lee<sup>(1)</sup>, Kwang-Geun Lee<sup>\*(1)</sup>**

<sup>1)</sup> Department of Food Science and Biotechnology, Dongguk University, South Korea

\*Corresponding author - E-mail: kwglee@dongguk.edu

This study investigated antioxidant activity, anti-inflammatory activity, and volatile compounds of bioconversioned grape by-product (*Vitis labrusca* B.). Grape by-products were fermented by *Lactobacillus plantarum* NCDO 955, *Lactobacillus rhamnosus* GG, *Pediococcus acidilactici* HW01, and *Leuconostoc citreum* HW02. The results of viable counts and pH levels indicate that grape by-products are a suitable matrix for LAB fermentation. The total phenolic and flavonoid contents were increased by 33 % and 28 %, respectively. The antioxidant activity based on DPPH and ABTS radical scavenging activities was improved to 136% and 121%, respectively. To verify anti-inflammatory activity of bioconversion grape by-products, the mRNA expression levels of pro-inflammatory cytokines and chemokine, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1-beta (IL-1 $\beta$ ), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) in LPS-induced RAW 264.7 cells were analyzed by real-time polymerase chain reaction. Bioconversion grape by-products inhibited the mRNA expression of cytokines and chemokine by 40.9%, 60.2%, 52.1%, and 63.1%, respectively. Also, the inhibitory effects on TNF- $\alpha$ , IL-6, and MCP-1 were increased by 35.7%, 59.6%, and 49.8%, respectively, as measured by enzyme-linked immunosorbent assay. Total 34 volatile compounds including 5 acids, 13 alcohols, 4 aldehydes, 7 esters, 3 ketones, and one terpenes were analyzed by solid phase microextraction (SPME) using a gas chromatography-mass spectrometer detector (GC-MSD). Phenylethyl alcohol, methyl salicylate, butyl butyrate, and hexyl butyrate were the dominant volatile compounds of GBE. After fermentation, total acids, alcohols, and esters were increased by 486%, 174%, and 276 %, respectively. Ketones were increased by 20 times in GBE fermented by *Lactobacillus rhamnosus* GG. Aldehydes including 2-Hexanal and furfural were decreased after fermentation. The total volatile compounds were increased by 227% by *Lactobacillus rhamnosus* GG. These results showed that lactic acid bacteria could improve volatile compounds and enhance the antioxidant activity and anti-inflammatory activity of grape by-products.

**Keywords:** grape by-product, lactic acid bacteria, volatile compound, antioxidant activity, anti-inflammatory activity

**Acknowledgement:** This study was supported by the ministry of oceans and fisheries of Korea.

### K33

#### **INNOVATIVE AMINO ACID ANALYSIS: ACCURATE AND ROBUST UNDERIVATIZED AMINO ACIDS ANALYSIS IN FOOD AND FEED USING HYPERCARB COLUMN COMBINED WITH UNIQUE TANDEM LC CONFIGURATION FOR INCREASED THROUGHPUT**

**Valerie Thibert**<sup>(1)</sup>, **Maryline Carvalho**<sup>\*(1)</sup>, **Serdar Bilgesoy**<sup>(2)</sup>, **Andreas Becker**<sup>(3)</sup>

<sup>1)</sup> Thermo Fisher Scientific EMEA Customer Solution Centre, France

<sup>2)</sup> Thermo Fisher Scientific CCS Product Management, Turkey

<sup>3)</sup> Thermo Fisher Scientific, Germany

\*Corresponding author - E-mail: maryline.carvalho@thermofisher.Com

The analysis of amino acids is essential for various applications in the food, beverage, clinical, research, and biomedical industries since amino acids represent the building blocks for proteins. Amino acids content determination needs to be monitored in various industries for new product development, and quality control purposes to ensure safety and to prevent fraud.

Ion exchange chromatography followed by photometric detection after post-column reaction with ninhydrin, is considered as the gold standard for amino acids analysis. Many laboratories will commonly use this or other approaches employing pre- or post-column derivatization followed by fluorescence or UV detection. Although these methods are well understood and have been historically well received, they are often time-consuming and induce high operating costs.

An alternative to derivatization would be the use of mass spectrometry detection, which opens the possibility to work by direct injection. A reversed-phase chromatographic approach is not appropriate because of poor retention of the amino acids, and separation of some important isomers cannot be achieved. Alternative mechanisms can be used such as ion exchange or hydrophilic interaction chromatography (HILIC), but in both cases the pH of the mobile phase needs to be controlled rigorously. The pH related to the sample type and its preparation could also be critical for method robustness.

This work describes an approach without derivatization based on Thermo Scientific™ Hypercarb™ column separation combined with single quadrupole mass spectrometry detection. The Hypercarb column provides unique retention and separation of underivatized amino acids. This stationary phase is stereo-selective and can separate geometric isomers (i.e. isoleucine and leucine critical pair). 19 amino acids were targeted, all addressed in the ISO 13903:20051 global standard and the European Commission (EC) regulation 152/20092 for the determination of total amino acids content in feedstuff. Thermo Scientific™ Vanquish™ Duo LC HPLC system was used in tandem LC configuration. This allows efficient column cleaning and equilibration steps, boosting laboratory sample throughput. Also, risks of carry-over and cross-contamination are minimized, and the chromatographic method reliability is maximized. Tandem LC mode therefore enhances and maintains Hypercarb column performances for routine purposes. Detection is then performed with a Thermo Scientific™ ISQ™ EM mass spectrometer. Its selectivity allows for overcoming chromatographic coelution and increases confidence in results. The full profile of total amino acids was achieved within 20 minutes. Method accuracy and precision were confirmed with proficiency test based on feed samples. Robustness was verified over 1000 real samples' injections with stable retention times and signal.

**Keywords:** total amino acids, underivatized, feed, food, hypercarb porous graphitic carbon

## GENERAL FOOD ANALYSIS

### K34

#### PFAS ANALYSIS IN FOOD USING AN AUTOMATED SOLVENT EXTRACTION SYSTEM AND THE LATEST LC-TQ

**Day Powell**\*<sup>(1)</sup>

<sup>1)</sup> *Agilent Technologies, United Kingdom*

\**Corresponding author - E-mail: day.powell@agilent.com*

Poster to demonstrate low level PFAS analysis in various food samples using an automated solvent extraction system as opposed to the conventional QuEChERS extraction. This novel approach to the extraction of food samples allows for automation of the preparation not possible with QuEChERS extraction. The automated solvent extraction allows for equivalent extraction efficiency but with much lower solvent usage, reducing environmental impacts of high solvent usage and waste, also more reproducible extractions as the instrumentation is very reproducible.

Using the latest in LC-TQ (Liquid Chromatography - Triple Quadrupole Mass Spectrometry) PFAS can be detected in the complex food matrices to sub PPB levels with over 50 PFAS analysed. Using a feed injection allows for large volume injections of solvent, which using a conventional LC injection would cause poor peak shape, the new feed injection allows an infusion of high solvent sample with the low solvent mobile phase, maintaining peak shape. This allows for much lower levels of PFAS to be detected.

Comparison of automated extraction and QuEChERS detailed to show the differences and the future possibilities.

**Keywords:** PFAS, food, automation

### K35

#### 5-HMF QUANTIFICATION AND VOLATILE PROFILING IN HONEY BY VAC-HS-SPME-GC×GC-MS

Damien Eggermont<sup>(1)</sup>, Francesca Pardi<sup>(2)</sup>, **Giorgia Purcaro**\*<sup>(1)</sup>

<sup>1)</sup> Gembloux Agro-Bio Tech, University of Liège, Belgium

<sup>2)</sup> La Sapienza, University of Roma, Italy

\*Corresponding author - E-mail: gpurcaro@uliege.be

Over the last decades, the food industry and their actors have been faced with increasingly challenging requirements. Food fraud and adulteration, particularly in expensive or protected edible products, have become increasingly sophisticated and difficult to detect. Therefore, analytical methods must continuously evolve to meet these new necessities. Among these evolutions, we can notably cite the use of greener and more sustainable techniques.

In this context, solid-phase microextraction (SPME), introduced nearly 35 years ago, along with its headspace mode (HS-SPME), stands out for its simplicity and efficiency in capturing and concentrating a wide range of compounds without the mandatory use of solvents before analysis. However, HS-SPME faces challenges in extracting low-volatile compounds, where higher temperatures or extended extraction times might be needed. Unfortunately, these conditions can be incompatible with delicate matrices and analytes, while also prolonging the analysis process. Additionally, extended extraction times can worsen the displacement effect when it occurs.

To address this issue, several strategies have been developed over the past decades. One approach is to modify the equilibrium conditions by conducting extraction under vacuum (Vac-SPME). This technique reduces the gas-phase resistance to mass transfer, thereby accelerating the volatilization of low-volatile compounds in the headspace.

In this study, Vac-SPME has been optimized to quantify 5-hydroxymethylfurfural (5-HMF), a processing contaminant formed during thermal treatment and aging, in honey using GC×GC-MS, while also exploring the possibility of simultaneously analyzing the volatile profile. 5-HMF serves as a quality marker and is regulated by the EU (2001/110/EC) with a maximum allowable limit of 40 mg/kg. The proposed method was validated through matrix-matched calibration. The achieved LOD and LOQ were 1.6 and 4.7 mg/kg, respectively, with a recovery rate of 98% and an RSD of 21%. When compared to the official HPLC method using eight real-world samples, an average 6% bias was observed. The International Honey Commission's official method requires a direct injection into the HPLC within a very short time after dilution (IHC, 2009). Therefore, from a greenness viewpoint, the proposed method showed better performance, evaluated with AGREE metrics, as well as comparable practicality to the official one, evaluated with the BAGI metric.

### K36

#### **BIOACTIVE COMPOUNDS OF GREEK PROPOLIS: A QUALITATIVE AND QUANTITATIVE INVESTIGATION EXPLOITING UHPLC-VIP-HESI-TIMS-QTOF-MS**

**Eleni Nastou<sup>(1)</sup>, Dafni Preza-Mayo-Kataki<sup>(1)</sup>, Panagiotis Loukas Gialouris<sup>(1)</sup>, Nikolaos Thomaidis<sup>\*(1)</sup>**

<sup>1)</sup> *Laboratory of Analytical Chemistry, Chemistry Department, National and Kapodistrian University of Athens, Greece*

*\*Corresponding author - E-mail: ntho@chem.uoa.gr*

Over the last years, a growing scientific interest in the analysis of propolis has been developed, due to its health-promoting properties. Propolis is a wax-like mixture of resin and secretions from the salivary glands of the bees (*A. mellifera*). It consists of plant resins (50%), waxes (30%), aromatic and essential oils (10%), pollens (5%), and phenolic compounds (5%). Existing evidence relates its positive health impact to the high content of specific bioactive components, especially phenolic compounds. It is the main ingredient in a plethora of pharmaceutical products, such as food supplements, and cosmetics. Thus, their determination is of utmost importance.

In this study, a novel analytical methodology has been developed and validated, using Ultra High-Pressure Liquid Chromatography (UHPLC) coupled with Trapped Ion Mobility Spectrometry - Quadrupole Time-of-Flight Mass Spectrometry (TIMS-QTOF-MS) with Vacuum Insulated Probe Heated Electrospray Ionization (VIP-HESI) as the ion source. VIP-HESI presents significant analytical advantages, such as increased sensitivity, and robustness, while it reduces source contamination, and memory effects due to active exhaust. In addition, the existence of the moving vacuum-insulated probe decreases thermal degradation, resulting in zero loss or fragmentation of sensitive compounds. Moreover, the incorporation of TIMS in HRMS workflows provides an extra dimension of identification, introducing a new parameter, the Collision Cross-Section value (CCS value). This 4D metabolomics approach integrating *m/z*, Retention Time (RT), qualifier ions, and CCS values increases the identification confidence of the analytes.

The developed method was validated in terms of linearity, matrix effect, LODs, and LOQs calculation, as well as accuracy (trueness and precision). A number of 52 phenolic compounds commonly detected in propolis were included in the validation dataset, bringing satisfactory results. The developed methodology was applied to 36 propolis samples from different regions across Greece for the comprehensive characterization of their phenolic content. More than 40 bioactive compounds were determined through target screening. After a thorough literature review, information on bioactive compounds previously mentioned in bee products was gathered, and a suspect database of 190 compounds was created. Suspect screening results revealed the presence of an additional 140 compounds that were identified with high confidence. Thus, target and suspect screening methodologies, allowed the identification of a significant number of bioactive compounds, leading to an extended finger-printing of Greek propolis. To the best of our knowledge, such a wide chemical characterization of propolis based on its bioactive content has never been achieved.

**Keywords:** propolis, VIP-HESI, HRMS, target screening, suspect screening



## K37

### PHYSICAL CHARACTERIZATION OF NOVEL OLIVE POWDER

**Melis Cetin Karasu<sup>(1)</sup>, Mecit Halil Oztop<sup>\* (1)</sup>, Behic Mert<sup>(1)</sup>**

<sup>1)</sup> *Department of Food Engineering, Middle East Technical University, Turkey*

<sup>\*</sup> *Corresponding author - E-mail: mecit@metu.edu.tr*

The demand for highly nutritious and functional foods is increasing. Olive fruit is considered to have significant health benefits due to its high monounsaturated fatty acid and phenolic content, playing an essential role in the Mediterranean diet. In this regard, this study aims to produce a novel olive powder product.

Olive powder was produced using a high-pressure homogenizer (HPH) and freeze dryer. This method allowed the production of self-entrapped olive powder without the use of additives or the removal of the fruit's oil content. The pre-homogenized sample was processed through the HPH at two different pressure levels (600 and 1200 BAR) and with varying pass numbers (1 and 2 passes). Subsequently, the suspension was freeze-dried and ground. As control samples, olives were processed using the same procedure without HPH treatment, and directly lyophilized olives were also used.

The quality of powdered foods is crucial during storage. Therefore, bulk and tapped densities, Carr's Index, Hausner Ratio for flowability, and particle size analysis were conducted to assess the impact of applied pressure on the final product. Additionally, TD-NMR measurements, a non-destructive analytical technique, were performed to compare olive powders produced with HPH to the control groups.

Results indicated that the particle size of the final product influences its flow properties, and HPH can enhance the flowability of the powders. NMR measurements revealed two different relaxation times, which could be associated with different oil states. Moreover, changes in  $T_2$  relaxation times suggested that applying HPH could reduce the mobility of the oil within the powder.

In conclusion, a value-added olive powder can be produced using HPH followed by freeze-drying. The quality parameters of the final product were improved by applying pressure compared to the control samples.

**Keywords:** olive powder, high pressure homogenizer (HPH), TD-NMR, flowability

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**K38**

### **UNDERSTANDING TEMPERATURE-DEPENDENT CAKING MECHANISMS IN POWDERED DAIRY PRODUCTS USING TD-NMR RELAXOMETRY**

Ozan Tas<sup>(1)</sup>, Esmanur Ilhan<sup>(1)</sup>, Melis Cetin Karasu<sup>(1)</sup>, Selen Guner San<sup>(1)</sup>, Mecit Halil Oztop<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Engineering, Middle East Technical University, Turkey

\*Corresponding author - E-mail: mecit@metu.edu.tr

Caking is a common issue in many industries, especially during the production, storage, and transportation of powder products. Caked powders lead to higher costs, longer production times, and lower product quality. Temperature plays a vital role in caking, where lower temperatures increase the tendency, especially in fat-rich powders. Fat can melt as the temperature increases and form liquid bridges between particles, which solidify when the temperature drops, leading to caking. While many studies have explored caking using mathematical and experimental methods, these approaches often struggle to predict molecular changes and surface properties to understand caking. Time Domain Nuclear Magnetic Resonance (TD-NMR) relaxometry can be useful for studying the caking behavior at the molecular level, as it provides detailed insights into the internal structure and behavior of powders, offering a fast, non-destructive, and reliable approach. This study examined the effect of temperature on the caking of two demineralized whey powders (WPC) and three whole milk powders, known for their high caking tendency. The samples that had a moisture content of 3-4% were analyzed using TD-NMR at 4°C, 25°C, 35°C, and 60°C. T<sub>1</sub> and T<sub>2</sub> relaxation times were measured, and relative crystallinity and solid content values were calculated. The results showed that as temperature increased from 4°C to 60°C, solid content decreased, especially in whole milk powders, likely due to fat melting. T<sub>2</sub> times became longer, indicating that higher temperatures make fats more mobile and the matrix less rigid. T<sub>1</sub> relaxation times, characterized by short and long components, showed that with increasing temperature, the T<sub>1</sub> of the long component increased while the short component decreased, indicating stronger intramolecular interactions at lower temperatures. These findings suggest that powders are more prone to caking at lower temperatures due to the solidification of fats. This study demonstrates that TD-NMR relaxometry is an effective tool for investigating caking behavior, providing valuable insights into how temperature and fat content influence the caking process in powdered samples.

**Keywords:** caking, temperature influence, TD-NMR relaxometry, whey powders, whole milk powders

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### K39

#### CHARACTERIZATION OF ANTHOCYANIN GLYCOSIDES FROM BERRIES CULTIVATED IN KOREA BASED ON HIGH RESOLUTION MASS SPECTROMETRY (UPLC-DAD-QTOF/MS)

Heon-Woong Kim<sup>(1)</sup>, Ryeong Ha Kwon<sup>(1)</sup>, Hyemin Na<sup>(1)</sup>, Ju Hyung Kim<sup>(1)</sup>, So Ah Kim<sup>(1)</sup>, Eun-Suk Jung<sup>(1)</sup>, Hyo Jeong Hwang<sup>(1)</sup>, Chi-Do Wee<sup>(1)</sup>, Kwang-Sik Lee<sup>(1)</sup>, Young-Hee Park<sup>\*(1)</sup>

<sup>1)</sup> National Institute of Agricultural Sciences, South Korea

\*Corresponding author - E-mail: ypark@korea.kr

National Institute of Agricultural Sciences (NAS) have been developed comprehensively functional components (anthocyanin, flavonoid, phenolic acid, saponin, carotenoid, etc.) database from Korean agro-foods based on ultra-performance liquid chromatography-diode array detector with quadrupole time of flight/mass spectrometry (UPLC-DAD-QToF/MS). In the present study, anthocyanin glycosides from berries (blueberry, mulberry, and aronia) cultivated in Korea were characterized via mass fragmented interpretation with previously constructed LC-MS library. From 9 highbush blueberry cultivars, a total of 22 individual glycosides identified were composed of mono-glycosides moiety (galactose, glucose, arabinose) based on aglycones (cyanidin, delphinidin, malvidin, and peonidin). On the basis of these structure, 7 acetylated glycosides were additionally identified. The major anthocyanin of 15 mulberry cultivars was cyanidin 3-O-glucoside (71.7%), followed by cyanidin 3-O-rutinoside (26.6%), while pelargonidin 3-O-glucoside and pelargonidin 3-O-rutinoside were confirmed as their minor components (total of 1.7%). In addition, cyanidin 3-O-galactoside was found to be predominant glycoside in aronia fruits. In total anthocyanin content (mg/100 g, dry weight), blueberries were differently distributed by cultivars as the following order: New Hanover (1011.7), Patriot (910.4), Spartan (855.5), Draper (795.3), Suziblue (771.1), Legacy (725.4), Reka (635.9), Farthing (599.3), Hannah's choice (581.1). Mulberry cultivars were ranged from 471.5 (Su Hong) to 4700.2 (Gwa Sang2), and aronia fruits presented with contents from 2637.2 to 3781.3 by cultivated regions. Finally, our detailed anthocyanin profiles will support breeding of superior varieties as well as can be used as the fundamental data to contribute to related food industries and daily intake assessment.

**Keywords:** anthocyanin, blueberry, mulberry, aronia, UPLC-DAD-QToF/MS

**Acknowledgement:** This study was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2022-RD010364)" Rural Development Administration, Republic of Korea.

### K40

#### **MASS SPECTROMETRY IN THE FACTORY: AN ASSESSMENT OF RECENT DIELECTRIC BARRIER DISCHARGE IONISATION (DBDI) MASS SPECTROMETRY APPLICATIONS WITH IN-FACTORY ANALYSIS POTENTIAL**

Nurmeen Adil<sup>(1)</sup>, Natasha Logan<sup>(1)</sup>, Simon Haughey<sup>(1)</sup>, Chris Elliott<sup>(1)</sup>, Nicholas Birse<sup>\*(1)</sup>

<sup>1)</sup> *Queen's University Belfast, United Kingdom*

*\*Corresponding author - E-mail: n.birse@qub.ac.uk*

Mass spectrometry has developed in several food applications to be the 'Gold standard' method of confirming authenticity, detecting contaminants or otherwise assessing quality, with applications as diverse as mycotoxin quantification, allergen detection and lipid profiling all making use of some form of mass spectrometry for food safety, security and authenticity purposes.

These many varied applications typically are undertaken in a laboratory environment, rather than at-line, which causes delays in determining analysis outcomes, limits the potential throughput and creates additional sampling integrity issues, all of which could be significantly improved or overcome if mass spectrometry analysis could be undertaken in-factory (or at points close to primary production).

To assess whether a mass spectrometry platform could be deployed in a factory environment, an Agilent Ultivo compact triple-quadrupole instrument, fitted with a Plasmion SICRIT plasma based dielectric barrier discharge ionisation source was used to test a variety of different food products to determine whether such a system, potentially combined with chemometric modelling, could be installed at-line in a factory environment, and what considerations would be needed for such an installation.

**Keywords:** ambient mass spectrometry, dielectric barrier discharge, chemometrics

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### K41

#### THE EFFECT OF SELECTED ADDITIONS ON THE COMPOSITION OF FATTY ACIDS IN COTTAGE CHEESE MADE FROM GOAT'S MILK

**Beata Paszczyk**<sup>\*(1)</sup>, Joanna Klepacka<sup>(1)</sup>, Marta Czarnowska-Kujawska<sup>(1)</sup>

<sup>1)</sup> *Department of Commodity Science and Food Analysis, The Faculty of Food Science, University of Warmia and Mazury in Olsztyn, Poland, Poland*

*\*Corresponding author - E-mail: paszczyk@uwm.edu.pl*

Goat's milk has beneficial effects on health condition maintenance and physiological function of our organism. Goat's milk and products derived from it are becoming increasingly popular among consumers. In response to their needs, the range of products based on goat's milk is also expanding. The aim of the study was to determine the fatty acid composition of natural and enriched cottage cheeses from organic goat's milk. The experimental material consisted of: natural cottage cheeses and cottage cheeses with selected additives, such as: buckwheat husk, spirulina, chia seeds and linseed seeds. The fatty acid composition of the analysed products was determined using the gas chromatography method (GC-FID method).

The conducted study showed that the analyzed cottage cheeses were characterized by a varied content of fatty acids. The fatty acid composition of natural cottage cheeses from goat's milk was dominated by saturated fatty acids (SFA), which constituted 57.79% of the total fatty acid composition. The content of monounsaturated fatty acids (MUFA) in these cottage cheeses was 26.11%, short-chain fatty acids (SCFA) 15.17%, and polyunsaturated fatty acids (PUFA) 2.78% of the total fatty acid composition. The addition of buckwheat husk and spirulina did not cause bigger changes in the fatty acid composition of tested cheeses. Cottage cheeses with added chia seeds and linseed had a significantly higher ( $P < 0.05$ ) total content of PUFAs, 8.32% and 8.05% of the total fatty acid, respectively. Significantly higher ( $P < 0.05$ )  $n-3$  acids than the other analyzed cottage cheeses. The  $n-6/n-3$  ratio was significantly lower ( $P < 0.05$ ) in cottage cheeses with added chia seeds and linseed, which is important from the nutritional and health point of view. Studies have shown that goat cottage cheeses can be enriched with plant additives. These products may be a response to the consumer market's demand for innovative products containing natural ingredients with health-promoting properties.

**Keywords:** goat's milk, cottage, cheese, fatty acids, GC-FID

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### K42

## MONITORING ASPARAGINE CONTENT IN CEREALS: AN APPROACH TO REDUCE ACRYLAMIDE FORMATION IN BAKED GOODS

**Hana Pirova<sup>(1)</sup>, Lucie Stara<sup>(1)</sup>, Beverly Hradecka<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: hajslovj@vscht.cz*

Following the discovery of acrylamide in heat processed foods in 2002, acrylamide has become a food safety concern not only due to its probable carcinogenic properties (classified by the IARC as Group 2A), but also because of its genotoxic and neurotoxic effects [1,2]. Acrylamide formation occurs during the Maillard reaction at temperatures above 120 °C. Its main precursors are reducing sugars and free asparagine, which is the key precursor in cereals. Since asparagine is found in a very wide concentration range in grain, minimising its occurrence is therefore increasingly challenging [3]. To protect consumer health, possible strategies to reduce acrylamide formation continue to be explored.

As cereal products are one of the main sources of acrylamide in the diet of all consumer categories, it is necessary to identify factors that may contribute to high levels of asparagine in this type of products [4]. The nutritional value of bakery products is often enhanced by addition of bran that presents a good source of fiber but also minerals and vitamins (especially group B). Nevertheless, bran contains a significant amount of asparagine and may therefore increase acrylamide formation. The aim of this study was to perform baking experiments with varying percentage of bran (5, 10, 15 and 20%) added to the dough of crackers and cookies (containing only basic ingredients such as white wheat flour, oil, salt and sugar). The pastry was then baked for different times at 180°C. The products were subjected to acrylamide and free amino acid profile analysis employing validated analytical methods using ultra-high-performance liquid chromatography (U-HPLC) coupled to mass spectrometry (MS). The obtained data will contribute to the knowledge of the impact of bran on the chemical safety of these bakery products.

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**Keywords:** acrylamide, free asparagine, cereals, bakery products, bran

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### K43

## FROM BEANS TO BREWING: ENHANCING COFFEE QUALITY THROUGH DYNAMIC IMAGE ANALYSIS AND ZETA POTENTIAL MEASUREMENTS

**Henrique Brasil**<sup>(1)</sup>, Franz Miller Branco Ferraz<sup>(1)</sup>

<sup>1)</sup> Anton Paar GmbH, Austria

\*Corresponding author - E-mail: henrique.brasil@anton-paar.com

Dynamic Image Analysis (DIA) is a powerful technique with broad applications in food analysis, capable of precisely measuring particle size distribution in various food supplements such as coffee, milk, flour, sugar, and starch. In this study, we focus on the role of DIA in optimizing coffee production, a process that hinges on meticulous control of parameters from roasting to grinding and extraction. The particle size distribution, influenced by these processes, directly impacts coffee's extraction efficiency and final sensory profile.

Using Litesizer DIA, we analyzed particle size distributions in whole and ground coffee beans across different roasting profiles. The findings highlight how lighter roasts retain finer particles, while darker roasts reduce fines significantly, affecting flavor extraction during brewing. This ability to measure and control particle size is critical for coffee and other food supplements where consistency and quality are paramount.

Additionally, we employed zeta potential measurements using the SurPASS 3 instrument to assess the surface chemistry of coffee grounds. This analysis revealed the impact of roast levels on the hydrophobicity of coffee beans, which in turn affects the consistency and quality of the grind. Adjusting water content during grinding, based on zeta potential data, can optimize particle size distribution, reduce static-related issues, and enhance the overall coffee brewing experience.

A Design of Experiments (DoE) approach was also utilized to optimize brewing parameters such as water-to-coffee ratio and temperature. The results indicated that while temperature plays a role, the water-to-coffee ratio is a more critical factor in maximizing extraction efficiency and achieving the desired °Brix levels. These insights are valuable for fine-tuning brewing conditions for both light and dark roasts.

In conclusion, the application of DIA and zeta potential measurements offers a robust framework for improving the quality of not only coffee but a wide range of food supplements. By leveraging these advanced analytical techniques, manufacturers can ensure consistency, optimize processes, and deliver high-quality products that meet consumer expectations. This research demonstrates the versatility and effectiveness of DIA in food analysis, making it an indispensable tool in the food industry.

**Keywords:** dynamic image analysis, coffee, extraction, zeta potential, particle size

### K44

#### UNLOCKING THE POTENTIAL OF MOROCCAN VIRGIN OLIVE OILS FOR A POSSIBLE RECOGNITION BY THE EUROPEAN UNION GEOGRAPHICAL INDICATION THROUGH COMPREHENSIVE ANALYTICAL CHARACTERIZATION OF THEIR QUALITY AND COMPOSITION

Sofia Zantedeschi<sup>(1)</sup>, Matilde Tura<sup>(2)</sup>, Enrico Valli<sup>\*(2)</sup>, Sara Barbieri<sup>(1)</sup>, Alessandra Bendini<sup>(2)</sup>, Nouredine Mokhtari<sup>(3)</sup>, Fatima Zahrae El-Mossaïd<sup>(4)</sup>, Aadil Bajoub<sup>(4)</sup>, Abdelaziz Ait Elkassia<sup>(3)</sup>, Erika Ramanzini<sup>(5)</sup>, Ali Abidar<sup>(3)</sup>, Abdeslam Oughad<sup>(6)</sup>, Naima Basidi<sup>(5)</sup>, Marco Setti<sup>(1)</sup>, Tullia Gallina Toschi<sup>(2)</sup>

<sup>1)</sup> Department of Agricultural and Food Sciences, Alma Mater Studiorum - Università di Bologna, Italy

<sup>2)</sup> Department of Agricultural and Food Sciences and Interdepartmental Centre for Industrial Agrofood Research, Alma Mater Studiorum - Università di Bologna, Italy

<sup>3)</sup> Rural Economics Department, National School of Agriculture, Morocco

<sup>4)</sup> Laboratory of Food and Food By-Products Chemistry and Processing Technology, National School of Agriculture, Morocco

<sup>5)</sup> ONG CEFA - Comitato Europeo per la Formazione e l'Agricoltura - ETS and CEFA Maroc, Italy

<sup>6)</sup> Zouyout Dir Beni Mellal GIE, Morocco

\*Corresponding author - E-mail: enrico.valli4@unibo.it

Morocco is nowadays one of the world's leading producers of virgin olive oil, producing mainly monovarietal virgin olive oils from the "Picholine Marocaine" variety, which is predominant due to its high adaptation to the country's edaphoclimatic conditions. However, Moroccan extra virgin olive oils currently lack any Geographical Indications (GIs) recognized by the European Union. To fill this gap, the present study aims to assess the quality and composition of virgin olive oils produced in the Moroccan region of Beni Mellal, while investigating the potential for protection through a GI recognized by the European Union. For this purpose, 15 samples of virgin olive oil, produced from at least 80% of olives of the 'Picholine Marocaine' variety which accounts for over 90% of Moroccan plantations, were collected from 5 different cooperatives in the Beni Mellal region, with 3 samples taken from each cooperative. The analysis of the main quality criteria showed that the peroxide values were between  $11.7 \pm 0.2$  and  $16.8 \pm 0.1$  mEq O<sub>2</sub>/kg and the spectrophotometric coefficients in the ultraviolet range were within the standard limits set out in the EU Regulation 2022/2104. The total phenol content, determined by the Folin-Ciocalteu method, varied between  $236.2 \pm 14.6$  and  $303.1 \pm 13.0$  mg GA/kg. The fatty acid analysis showed a typical virgin olive oil profile with a percentage of oleic acid (C18:1 n-9) between 68.45% and 70.36%. The oxidative stability measured by the Rancimat method ranged from  $15.94 \pm 0.02$  to  $23.56 \pm 0.09$  hours. Free radicals evaluated by microESR using an innovative approach were found in moderate concentrations ranging from  $3.69 \pm 0.14$   $\mu$ M to  $10.57 \pm 0.57$   $\mu$ M after heating the oils at 80°C for 4 hours. Moreover, volatile profile analysis is ongoing to further contribute to a comprehensive characterization of these virgin olive oils, together with sensory analysis (Panel test), to highlight the presence of positive notes or defects; the aim is to valorize the peculiar aspects or improve the quality, and overall, to provide clear, targeted guidelines for producers in view of a possible GI. This final goal would offer recognition and protection while promoting regional identity and ensuring reproducible quality standards.

**Keywords:** virgin olive oil, picholine Marocaine, geographical indication, physico-chemical quality, sensory analysis

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## K45

### INFLUENCE OF TIME AND PRESSURE IN THE CHARACTERIZATION AND DEVELOPING OF PUFFED RICE

**Madalina-Oana Marincas**<sup>(1)</sup>, **Romina Marc**<sup>(1)</sup>, **Crina Muresan**<sup>(1)</sup>

<sup>1)</sup> *University of Agricultural Sciences and Veterinary Medicine, Romania*

*\*Corresponding author - E-mail: madalina-oana.marincas@student.usamvcluj.ro*

**Introduction:** White rice is the predominant type of rice cultivated on the world. These grains are commonly consumed for their gluten free properties, restore glycogen levels and is a low-residue food and easy to digest. Rice as expanded rice, has high carbohydrate content, making it a good energy source. In the expansion process, time and pressure play an important role in the results [1]. Some physiochemical properties that affect the quality of puffing are the amount of amylose, protein, moisture, and level of gelatinization [2].

**Aims:** We considering that expanding ratio is produced at higher pressure and duration of expansion. Among different strategies to increase production rate, we analyzed chemical and physical profile to compare the samples.

**Material and methods:** Expanded rice was made in puffing gun at Romanian company, Boromir. We analyzed chemical, physical profile and texture of the expanded product. The method used was to determinate dry substance, ash, crude fiber, expansion rate, expansion volume, total content of nitrogen, lipids and texture.

**Results:** Expanded rice samples have lower dry substance content compared to long grain rice and the control sample. This suggests that the puffing process reduces the density and solid content, making expanded rice lighter and possibly more moisture retentive. Expanded rice samples show a wide range in ash content. Expanded rice generally has low lipid content. Protein content is highly variable across the expanded rice samples. Expanded rice 2 has the highest protein content, whereas other expanded rice samples are very low in protein. The puffed rice samples differ in hardness and gumminess but are similar in terms of adhesiveness and chewiness. Rice generally has a good crude fiber content.

**Conclusion:** Expanded rice can be a good source of proteins, fiber and minerals, full filled with upper intake of carbohydrates which support energy. Economically and qualitatively speaking, time and pressure have a good influence on the final product. Furthermore, expanded rice must be studied to enhance the nutritional value by a heaty salty coated solution.

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[2] Kamaraddi V, Prakash J. Assessment of suitability of selected rice varieties for production of expanded rice. *Cogent Food & Agriculture*. 2015 Nov 18;1(1):1112675. <https://doi.org/10.1080/23311932.2015.1112675>.

**Keywords:** chemical composition, expanding ratio

### K46

## NATURAL PREFERMENTS: ENHANCING QUALITY AND SUSTAINABILITY IN BAKING

**Maria-Luissa Maino<sup>(1)</sup>, Romina Marc<sup>\*(1)</sup>, Crina Muresan<sup>(1)</sup>**

<sup>1)</sup> *Universitatea de Stiinte Agricole si Medicina Veterinara, Romania*

<sup>\*</sup>*Corresponding author - E-mail: romina.vlaic@usamvcluj.ro*

**Introduction:** This study explores the influence of sourdough preferments on colloidal processes and the quality of bakery products, focusing on both theoretical and experimental perspectives. The first section reviews the evolution of bread-making, from traditional methods to modern industrial practices, and their impact on bread quality. Special emphasis is placed on the colloidal interactions of flour components, gluten formation, and the transformation of dough into bread.

**Aims:** The aim of this research is to investigate the benefits of using natural preferments, particularly sourdough, in enhancing the quality and sustainability of bakery products. This includes evaluating their impact on nutritional value, flavor, texture, and overall consumer acceptability.

**Materials and Methods:** Various analytical methods, including high-performance liquid chromatography (HPLC), were employed to evaluate the organic acids, carbohydrates, and chemical composition of bread made with sourdough. Experimental designs included controlled fermentation trials and sensory analysis to assess the organoleptic properties of the final products.

**Results:** The study investigates the advantages of lactic acid fermentation over commercial yeast, showing that sourdough fermentation enhances both the nutritional and organoleptic properties of bread. The composition of the microbiota in sourdough and its role in improving bread's nutritional value are further analyzed. The findings demonstrate that sourdough preferments significantly improve the texture, flavor, and nutritional profile of bread compared to traditional yeast-based methods.

**Conclusion:** This research offers valuable insights for the baking industry, promoting healthier and higher-quality bread products through the use of preferments.

**Keywords:** preferments in baking, HPLC analysis, lactic acid fermentation, baking sustainability, sourdough

**Acknowledgement:** *We sincerely thank the Faculty of Food Science and Technology at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca for providing the essential resources and support needed to complete this research. Their commitment to fostering a rich academic environment has greatly contributed to our success. Our appreciation also extends to the laboratory staff for their invaluable assistance with the experimental procedures crucial to our findings. Their expertise was particularly beneficial in the analysis of sourdough fermentation through high-performance liquid chromatography (HPLC) and ash content determination. We are truly grateful for the encouragement and guidance received throughout this research journey, which has deepened our understanding of the role of natural preferments in enhancing the quality and sustainability of baking.*

## K47

### TRANS FATTY ACIDS CONTENT IN FOOD FOR INFANTS AND YOUNG CHILDREN

Hanna Mojska<sup>(1)</sup>, Bogumiła Krygier<sup>\*(1)</sup>, Edyta Jasinska-Melon<sup>(1)</sup>

<sup>1)</sup> National Institute of Public Health NIH - National Research Institute, Poland

\*Corresponding author - E-mail: bkrygier@pzh.gov.pl

Trans fatty acid (TFA) are defined as fatty acids with at least one non-conjugated (i.e. interrupted by at least one methylene group) carbon-carbon double bond in the trans configuration. TFA are classified as industrial (i-TFA) and natural (r-TFA). The main sources of r-TFA are milk and meat from ruminants, and i-TFA - partially hydrogenated vegetable oils and foods manufactured with use of this fats. TFA may have adverse effects on growth and development through interfering with long chain polyunsaturated fatty acids (LC-PUFA) metabolism. This may have a longer-term effect, for example increasing risk of developing allergy disease in infants and young children. For that reason, the dietary TFA intake should be as low as possible. Since 2021, in Poland, the Commission Regulation 2019/649 has been validated. According them i-TFA content in food are limited (max 2g/100g of fat).

The aim of this work was to determine trans fatty acids content in selected food for infants and young children.

Testing material included 92 samples of products from the category "Foods for infants and young children", including 31 processed cereal-based food with milk (milk porridges) and 61 baby food in jars. Fat content in the samples was determined according to PN ISO 1444:2000 *Meat and meat products-determination of free fat content by Soxhlet method* using the B-811 extraction apparatus with the B-411 pre-hydrolysis attachment by BÜCHI Labortechnik AG. Trans fatty acids were analysed in methyl ester form (FAME) by gas chromatography coupled with a mass detector, using the Hewlett-Packard 7890A gas chromatograph with the 5975C inert MS detector.

The median TFA content in milk porridges was 0.06% (range:< 0.02% to 1.36% wt/wt) and in baby food in jars 0.17% (range:< 0.00% to 3.77% w/w). These values calculated as grams, were as follows: 0.00-0.15g/100g, and 0.00-0.11g/100g. The highest median TFA content in the samples of foods for infants and young children were found in beef baby food in jars (Me = 0.02g/100g) and milk and rice porridges (Me = 0.01g/100g) respectively. In the rest samples, the TFA content did not exceed 0.06g/100g for baby food in jars and 0.03g/100g for porridges. Taking into account the labels of these products, it has been shown that the modified milk was source of TFA in the porridges, whereas in the baby food in jars it was ruminant meat and dairy products (including cream). No samples were found to be exceeded limit for TFA content.

It should be noted that there are a wide range of food products on the market. Also manufacturers are introducing new and/or reformulated products. It is important to regularly analysing TFA content in food, especially for infants and young children. Current data on TFA content in different food groups, including foods for infants and young children. Current data on TFA content in different food groups, including foods for infants and young children are available on e-Base TFA website (<https://izomery.pzh.gov.pl/>).

**Keywords:** trans fatty acids, food for children

**Acknowledgement:** This study has been carried out by the contract with the Ministry of Health (NIZP PZH-PIB/2021 /1094/1056PIB) under National Research Institute Task 5.8 Report of Poland preparation in terms of meeting the World Health Organization (WHO) requirements for obtaining the Certificate about elimination of i-TFA from foods.

## GENERAL FOOD ANALYSIS

**K48**

### **ASSESSING THE IMPLICATION OF SPAD AND NDVI VALUES IN NITROGEN APPLICATION AND GRAIN QUALITY OF SELECTED MAIZE HYBRIDS**

**Muhoja Sylvester Nyandi**\*<sup>(1)</sup>, **Peter Pepo**<sup>(1)</sup>

<sup>1)</sup> *University of Debrecen, Hungary*

\**Corresponding author - E-mail: smuhoja2012@gmail.com*

Crop production faces increased stresses due to climate change and land degradation. With the increasing population, food security has become a global issue. Being a versatile crop used as food, feed, and raw materials for industries, Maize (*Zea mays L.*) contributes significantly to global food systems. However, abiotic stresses such as drought and soil fertility constrain its production. Among the amelioration techniques include fertilization, which maintains optimum nutrition, leverages nutrient deficiency conditions, optimizes maize growth and consequent yield. Modern precision agricultural tools such as chlorophyll meters are vital for rapid and non-destructive chlorophyll assessment and nitrogen status. An experiment was conducted in 2022 and 2023 spring at the Látókép research site of the University of Debrecen to evaluate the effect of nitrogen fertilization and maize genotypes on the agro-physiological parameters and grain quality using three nitrogen regimes (0, 90, and 150 Kg/ha) and three maize hybrids (P9610-FAO 340, DKC4590-FAO360 and GKT376-FAO360). Results showed that SPAD and NDVI significantly ( $p < 0.05$ ) positively correlated with grain quality and yield. Likely, the nitrogen applied significantly ( $p < 0.05$ ) influenced SPAD with its application. However, there were insignificant ( $p < 0.05$ ) between N90 and N150 for SPAD and protein content. Maize hybrids and levels with better chlorophyll content indicated maximum yield at N90 by 9%, equivalent to 1.1 tons, compared to control for N level and 11.6% for the hybrid P9610, equivalent to 1.9 tons to GKT376. Likewise, protein content was highest at higher SPAD and NDVI values. Therefore, SPAD values could be a potential means to analyze the N nutrient requirements of maize under field conditions to estimate the grain yield.

**Keywords:** SPAD, NDVI, chlorophyll content, grain yield, grain quality

**K49**

### **CHARACTERIZATION OF SECONDARY METABOLITES OF DYER'S ALKANET (ALKANNA TINCTORIA) POSSESSING ANTIMICROBIAL ACTIVITY**

**Tereza Jaegerova**<sup>\*(1)</sup>, Jana Hajslova<sup>(1)</sup>, Hana Sykorova<sup>(2)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

<sup>2)</sup> *University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: jeagerot@vscht.cz*

The roots of *Alkanna tinctoria* were used mainly as a source of natural red pigments, however, several research papers pointed towards various bioactive properties, such as antimicrobial, anti-inflammatory, and antioxidant. Nonetheless, the chemical profile of the roots has not been studied in detail yet. Our study aimed to investigate the antimicrobial activity of different root extracts against 7 pathogens and identify the bioactive compounds. According to the results, the aqueous root extract did not show antimicrobial activity at all, while the ethanolic and isopropanol-hexane extracts showed comparably strong inhibition of the growth of Gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Cutibacterium acnes*. Using these extracts, a fractionation method on preparative HPLC was developed and the obtained fractions were further tested on the inhibitory activity. Bioactive fractions were then analyzed by targeted screening using UHPLC-ESI-QTOF-MS/MS. While no phenolic compounds were identified in the fractions, the analysis revealed the presence of several naphthoquinones, of which alkannin and  $\beta,\beta$ -dimethylacrylalkannin were confirmed by a certified reference standard. The inhibitory activity of *Alkanna tinctoria* roots against *Cutibacterium acnes* is reported for the first time.

**Keywords:** LC-MS

# GENERAL FOOD ANALYSIS

## K50

### ASSESSMENT OF THE PERFORMANCE OF PARTICIPANTS IN CAFFEINE ANALYSIS PROFICIENCY TESTS IN SAUDI FOOD LABORATORIES

Turki Abuhamrh\*<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: tkhamrh@sfd.gov.sa

Background: The Laboratory Proficiency Department within the Laboratory Development Center (LDC) and Reference Laboratories of the Saudi Food and Drug Authority (SFDA) organizes proficiency testing programs. Accredited by the Saudi Accreditation Center (SAAC) to ISO/IEC 17043:2023 standards, these programs are indispensable quality assurance tools for laboratories, mandated by ISO 17025. To enhance the quality of testing outcomes in Saudi Arabia's food and drug laboratories, the proficiency testing program encompasses food chemistry, microbiology, pharmaceuticals, cosmetics, and herbal preparations. This research focuses on food chemistry, particularly caffeine analysis in soft drinks, examining participant numbers and performance since 2020.

Objectives: This article aims to provide an overview of the proficiency testing program conducted to screen laboratories testing caffeine in soft drinks and to examine data regarding laboratories' participation and performance in the proficiency testing program.

Methodology: Registered laboratories received 100 ml soft drink samples following rigorous preparation, homogeneity, and stability assessments. The statistical approach for data analysis was determined in accordance with ISO 13527 guidelines for food chemistry reference laboratories. Participating laboratories analyzed the samples and submitted their results. This proficiency test employed a Consensus value approach to calculate the Assigned value, standard deviation, and uncertainty.

Results: A total of 42 laboratories participated in the proficiency testing program over a four-year period from 2020 to 2024. The average success rate among participants was 88%. Notably, the number of participating laboratories grew by 61% over the same period. Performance analysis revealed that 88% of laboratories consistently achieved excellent results, while no laboratories attained very unsatisfactory or unacceptable levels. The average standard deviation across the four years was calculated as 10.60 mg/kg.

Conclusion: Since 2020, four rounds of proficiency testing have been conducted to evaluate participant competency in caffeine analysis within soft drink samples. The program successfully demonstrated the analytical capabilities of participating laboratories, with all participants achieving satisfactory Z-scores. The PT program underscores the efficacy of quality control measures implemented within the laboratory.

**Keywords:** proficiency testing, quality assurance, caffeine, analysis, ISO/IEC 17043:2023, Saudi Food and Drug Authority (SFDA)

## K51

### DYNAMIC CHARACTERISATION OF PERMEATION PROCESSES IN POLYMER PACKAGING FILMS

**Andreas Stenzel**<sup>(1)</sup>, **Jonathan Beauchamp**<sup>(2)</sup>, **Y Lan Pham**\*<sup>(2)</sup>

<sup>1)</sup> *Department of Sensory Analytics and Technologies, Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany; Chair of Aroma and Smell Research, Department of Chemistry and Phar, Germany*

<sup>2)</sup> *Department of Sensory Analytics and Technologies, Fraunhofer Institute for Process Engineering and Packaging IVV, Freising, Germany, Germany*

\*Corresponding author - E-mail: [y.lan.pham@ivv.fraunhofer.de](mailto:y.lan.pham@ivv.fraunhofer.de)

Packaging represents the largest area of application for plastics in Europe (EU27+3). In 2022, around 21 Mt of plastic material were converted [1]. With its properties as a functional barrier, the purpose of packaging materials is to protect the product inside from the loss of valuable ingredients and to inhibit the migration of external contaminants [2], minimising spoilage processes and maintaining the product quality [3,4]. A key parameter affecting the barrier property is the diffusion constant of the permeant within the packaging material, which can be determined by performing lag-time experiments [2]. An established method based on gas chromatography-flame ionisation detection (GC-FID) developed by Ewender and Welle [5] allows the lag-times to be predicted. Initially applied for permeation tests of n-alkanes through polyethylene terephthalate (PET) films, this method can also be used for permeation studies of other volatile organic compounds (VOCs). However, with a data acquisition rate of 1.5 h<sup>-1</sup> it can be challenging to investigate fast permeation processes.

The aim of the present work is to develop a method for real-time monitoring of permeation by means of proton transfer reaction-mass spectrometry (PTR-MS) to dynamically evaluate the barrier function of packaging materials to individual VOCs. This novel approach has the potential to broaden the range of analysed compounds and to serve as a basis for establishing screening procedures.

A micro-chamber/thermal extractor ( $\mu$ CTE) with inserts for permeation analysis (MARKES International Ltd., Bridgend/UK) was coupled to a PTR-MS instrument (IONICON Analytik GmbH, Innsbruck/AT) via a customised adapter [6]. The VOCs of interest were injected into the lower chamber of the permeation insert that was separated from an upper chamber by a high-density polyethylene (HDPE) film. After migrating through the polymer film, the VOCs were constantly purged from the upper chamber into the PTR-MS, allowing for quantitative analysis in real-time.

In initial experiments performed on HDPE films of 300 and 500  $\mu$ m film thickness, the gas phase concentration of 2,3-butanedione, as a selected target compound, increased with a lag-time of 40 and 100 min after injection, respectively. Reaching a maximum permeation rate at detectable gas phase concentrations within 300 and 500 min after injection, respectively, this novel approach was capable of dynamically monitoring the permeation processes that take place in the range of a few hours. Besides, this method could be advantageous to evaluate the barrier properties of other materials, or against odours, e.g., to assess odour-inhibiting additives.

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[2] Gruber & Beauchamp, in: Food Contact Materials Analysis 2019, 105-138.

[3] Poudel *et al.*, Adv. Hort. Sci. 2022, 36(2).

[4] White & Lockyer, Nutr. Bull. 2020, 45(1).

[5] Ewender & Welle, Packag. Technol. Sci. 2014, 27(12).

[6] Pham *et al.*, Anal. Chem. 2022, 94(50).

**Keywords:** novel approach for permeation studies, dynamic characterisation of barrier properties, real-time monitoring of fast permeation processes by proton transfer reaction-mass spectrometry, permeation of VOCs through polymer packaging films, quantitative VOC analysis in real-time

**Acknowledgement:** This work was funded by the Fraunhofer Cluster of Excellence Circular Plastics Economy CCPE.

### K52

#### COMPARATIVE PHARMACOKINETICS OF NANOFORMULATED CBD AND CBDA IN APOE KO MICE: A THREE-MONTH STUDY

Zuzana Binova<sup>\*(1)</sup>, Frantisek Benes<sup>(1)</sup>, Matej Maly<sup>(1)</sup>, Petr Kastanek<sup>(2)</sup>, Monika Cahova<sup>(3)</sup>, Milena Stranska<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> University of Chemistry and Technology, Prague, Czech Republic

<sup>3)</sup> Institute for Clinical and Experimental Medicine, Czech Republic

\*Corresponding author - E-mail: bobkovaz@vscht.cz

Phytocannabinoids, particularly cannabidiol (CBD) and cannabidiolic acid (CBDA), are gaining attention for their therapeutic potential. Number of CBD preparations are available at different markets. Understanding their pharmacokinetics is crucial for optimizing their use in medical treatments. This study compared the pharmacokinetics of nanoformulated CBD and CBDA in ApoE KO mice, a model organism prone to atherosclerosis, making it suitable for studying human cardiovascular diseases.

A total of 32 ApoE KO mice (16 males and 16 females) were divided into groups that received either nano-CBD, nano-CBDA delivered through drinking water (1% solution with Kolliphor® EL emulsifier) or water without nanoformulation and active compounds as a control group for three months. The animals had *ad libitum* access to the water.

Pharmacokinetic profiles were assessed based on the analysis of CBD, CBDA and their metabolites in plasma sampled at four-week intervals, for three months. Ultra-high performance liquid chromatography coupled to tandem mass spectrometry (U-HPLC-HRMS/MS) was developed and validated for this purpose. The study revealed significant differences in the bioavailability of CBD and CBDA, with CBDA showing a notably higher plasma concentration across all time points. Specifically, CBDA reached plasma levels tenfold higher than CBD in both male and female mice.

The obtained results suggest that nanoformulated CBDA may offer more effective systemic delivery compared to CBD, potentially enhancing its therapeutic efficacy. This study underscores the importance of further exploring CBDA's pharmacokinetics in therapeutic applications, particularly in the context of cardiovascular diseases where enhanced bioavailability could play a critical role in treatment outcomes.

**Acknowledgement:** This work was supported by the Czech Science Foundation (GAČR) - 22-20860S.



### K53

#### OPTIMIZING PROFICIENCY TESTING SAMPLES IN FOOD MICROBIOLOGY

Loreto Gallego<sup>(1)</sup>, Vicente Catalan<sup>(1)</sup>, Elena Penaranda<sup>(1)</sup>, Ana De Pablo<sup>(1)</sup>, Luis Maria Gallego<sup>\*(2)</sup>

<sup>1)</sup> Labnova Distribuciones Agroalimentarias S.L, Spain

<sup>2)</sup> Analiza Control de Calidad S.L, Spain

\*Corresponding author - E-mail: luisg@analizacalidad.com

Proficiency testing (PT) is widely recognized as an essential tool for demonstrating the competence of conformity assessment bodies and generally involves the use of interlaboratory comparisons for the evaluation of laboratory performance. A PT scheme offers participating laboratories a valuable way to obtain an external and independent assessment of their performance in conducting specific tests or measurements.

PT has increased in food microbiology laboratories in response to ISO 17025 accreditation requirements, regulatory focus, customer needs, internal quality control processes and method validation activities.

Although all the general requirements for the competence of a PT providers are well defined in ISO 17043 standard and there are many accredited PT providers, one of the main technical challenges remains in some cases the preparation of items that match in terms of matrix and concentrations, as closely as practicable, to the type of items or materials found in routine tests. It is very common to find artificial spiked samples, reference materials or irradiated foods. This raises the question of whether these samples are suitable because the equivalence or commutability between results obtained on artificial and authentic food samples is not always clear.

This presentation will provide a comprehensive perspective on the efforts and strategies employed by PT providers to prepare more suitable samples, including examples of innovative approaches and technologies implemented, such as the preparation of customized matrices that replicate the physical and chemical properties of actual food products or use freeze-drying and vacuum sealing and modified atmosphere packaging (MAP) methods applied to naturally contaminated matrices.

All of these advancements will ensure that laboratories receive high-quality, reliable samples that mimic real-world conditions, ensuring that they provide meaningful and reliable results for laboratory assessment, ultimately leading to more accurate food safety testing.

**Keywords:** proficiency testing, food microbiology, sample optimization

### K54

#### FROM SPELT SEED TO FUNCTIONAL FOOD: IMPROVING BIOACCESSIBILITY OF PHENOLICS THROUGH BIOPROCESSING TECHNIQUES

**Marjeta Mencin**\*<sup>(1)</sup>, **Maja Mikulic Petkovsek**<sup>(2)</sup>, **Petra Terpinc**<sup>(2)</sup>

<sup>1)</sup> *Jožef Stefan Institute, Slovenia*

<sup>2)</sup> *Biotechnical faculty, University of Ljubljana, Slovenia*

\*Corresponding author - E-mail: marjeta.mencin@ijs.si

Phenolics are the major antioxidants in cereal seeds, occurring in extractable (free, soluble conjugated) and bound forms. Spelt (*Triticum spelta* L.) is an important source of dietary fiber and phenolics bound to it, with strong antioxidant activity. Due to the poor bioaccessibility of bound phenolics, which limits their potential health benefits, our study investigated methods to release phenolics from insoluble bound forms using bioprocessing techniques (germination, fermentation, and enzymatic treatment), both individually and in combination. We evaluated their impact on bioaccessibility of total and individual phenolics from spelt seeds after *in vitro* gastrointestinal (GI) digestion using LC-MS/MS. Our results showed that combining bioprocessing techniques significantly increased the bioaccessibility of phenolics compared to single technique. In particular, the "germination + fermentation" combination was the most effective method, increasing the content of total bioaccessible phenolics by more than 2-fold compared to untreated spelt seeds. However, the highest bioaccessibility of *p*-coumaric and *trans*-ferulic acids was observed in seeds treated with "enzymatic treatment + fermentation". These results underline the potential of combined bioprocessing techniques to improve the nutritional value of *Triticum* seeds and to develop functional food.

Therefore, we used bioprocessed spelt flour in bread production to evaluate its nutritional value, as well as its technological and sensory properties, in comparison to control wheat bread. In this study, the bioaccessibility of total and individual phenolics from wheat bread enriched with bioprocessed spelt flour was determined for the first time. After digestion, bioprocessed breads, especially those enriched with 5% "germinated + fermented" spelt flour, contained a significantly higher content of bioaccessible *trans*-ferulic acid, up to 283% compared to control bread. Breads containing up to 5% "germinated + fermented" spelt flour showed a higher bioaccessibility of *p*-coumaric, *trans*-ferulic, and *cis*-ferulic acids as well as more acceptable technological and sensory properties than other digested bioprocessed breads. However, addition of more than 5% bioprocessed flour in bread recipe resulted in unacceptable technological and sensory properties. Overall, "germinated + fermented" spelt flour is a promising ingredient for the formulation of bakery products, avoiding enzymatic improvers and promoting consumer acceptance and »clean label« adoption. Nevertheless, many of the bread phenolics remain indigestible after GI digestion, reaching the colon where they can be metabolized. In the future, the bioaccessibility of phenolics after colon fermentation needs to be investigated to provide more complete understanding of their bioaccessibility in human body.

**Keywords:** bioprocessing techniques, functional food, spelt seeds, phenolic bioaccessibility, *in vitro* digestion

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# GENERAL FOOD ANALYSIS

## K55

### EFFECT OF BUCKWHEAT HUSK ADDITION ON SENSORY PROPERTIES OF BREAD

Wajeeha Mumtaz<sup>(1)</sup>, Marta Czarnowska-Kujawska<sup>\*(1)</sup>, Joanna Klepacka<sup>(1)</sup>

<sup>1)</sup> *Department of Commodity Science and Food Analysis, The Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland*

*\*Corresponding author - E-mail: marta.czarnowska@uwm.edu.pl*

Buckwheat husk is a by-product of the production of buckwheat groats. This underrated product is most often used as a filling for mattresses or pillows. Meanwhile, buckwheat husk used in ground form can be applied as a food additive, due to its high content of various health promoting components. These include fiber, minerals and B vitamins. The product also contains different polyphenols, such as flavonoids, especially rutin. In addition, buckwheat husk does not contain gluten proteins, making it an ideal product for people with gluten intolerance. However, products enriched with husk, in order to gain consumer recognition, must, in addition to improved nutritional value, also present an attractive and acceptable sensory properties.

The purpose of the study was to evaluate sensory properties of breads enriched with buckwheat husk. The research material consisted of toasted and wholemeal breads with the addition of ground buckwheat husk at the level of 0% (control sample), 1.5%, 3.0% and 4.5%. Sensory evaluation was carried out by differential profiling method using a bipolar linear scale (from -3 the least intensity, to +3 the highest intensity) by 15 panellists. Enriched breads properties were compared to control samples, which sensory quality corresponded to a 0 score on the scale.

The addition of buckwheat husk changed the analyzed sensory characteristics of both wholemeal and toasted bread. All analyzed breads with the addition of husk differed in color from the control breads. The darkest (2.9 and 2.8 for toasted and wholemeal breads, respectively) were breads with the highest, 4.5%, husk addition. In all breads, the increasing addition of the husk masked the taste and aroma typical for toasted and wholemeal breads, while the taste and aroma of the husk became more noticeable. The husk was the most perceptible during chewing in breads with its highest addition (2.1 and 2.3 for toasted and wholemeal breads, respectively). In the case of wholemeal bread, husk addition clearly affected its elasticity, reaching the highest value of 1.9 for bread with 4.5% husk. This bread was the most flexible, soft and had the least compact texture. All husk-enriched breads achieved a higher overall acceptability than the control samples. This encourages further research into the enrichment of staple foods, such as bread, with buckwheat husk, in order to offer to the consumer a product that is not only rich in ingredients of a functional nature that are beneficial from a nutritional point of view, but, above all, sensorially attractive.

**Keywords:** breads, buckwheat, sensory analysis

**K56**

### **CHANGES IN THE SENSORY PROPERTIES, PHYSICO-CHEMICAL CHARACTERISTICS AND AROMAS OF SMOKED CHEESE IN DIFFERENT RIPENING STAGES**

**Anamaria Bolbos<sup>(1)</sup>, Romina Marc<sup>(2)</sup>, Crina Muresan<sup>\*(2)</sup>**

<sup>1)</sup> *University of Agricultural Sciences and Veterinary Medicine, Romania*

<sup>2)</sup> *University of Agricultural Sciences and Veterinary Medicine Cluj Napoca, Romania*

*\*Corresponding author - E-mail: crina.muresan@usamvcluj.ro*

**Introduction:** The study underscores the complexity of cheese aroma formation and perception, emphasizing that cheese composition (specifically fat, salt, and whey lactose levels) and microbial activity are crucial factors (Mureşan C., 2021).

**Aims:** The descriptive sensory assessment provided a detailed and objective evaluation of the cheese samples, allowing the researchers to characterize how different composition parameters affect aroma compound production, release, and perception during ripening.

**Materials and Methods:** Descriptive Sensory Analysis

**Results:** The study demonstrated that the flavor and texture of smoked cheese improved significantly with storage time due to the metabolic processes occurring during ripening. The use of QDA allowed for a detailed and objective assessment of these changes, highlighting the dynamic nature of flavor and texture development in cheese. The data gathered from sensory evaluations and chemical analyses provide valuable insights for optimizing cheese production and storage practices to enhance sensory attributes and overall product quality.

**Conclusion:** In the present research work, we demonstrated that the intricate relationship between cheese composition and aroma profile, highlighting the significant roles of fat content, protein, salt, and smoke. Each of these factors contributes uniquely to the microstructure, flavor composition, and release dynamics of aroma compounds during ripening.

**Keywords:** flavour, sensory, profiles, smoked cheese

**Acknowledgement:** *Lacto Sinelli SRL*

## K57

### PROFILING OF MILK TRIGLYCERIDS BY UHPLC-MS

**Jean-Michel Romnee**<sup>(1)</sup>, **Mohamed El Morabit**<sup>(2)</sup>, **Virginie Decruyenaere**<sup>(3)</sup>, **François Stevens**<sup>(2)</sup>

<sup>1)</sup> CRAW - Valorisation of Agricultural Products Department, Belgium

<sup>2)</sup> CRAW - Animal Production Department, Belgium

\*Corresponding author - E-mail: j.romnee@cra.wallonie.be

**Introduction:** The defining characteristic of fat is its fatty acid profile. The triglyceride profile is employed with less frequency, despite its potential to furnish valuable insight into technological processes.

The methodology presented in this study is founded upon UHPLC-MS.

**Materials and Methods:** The milk samples (n = 39) were collected at the experimental dairy farm of the Walloon agricultural research center. A representative sample was obtained from each cow during the milking process and stored in melting ice until analysis. Following homogenisation at 40°C, the butterfat was extracted using a butanol/methanol/chloroform mixture (3:5:4). The extract was separated on an Acquity CSH C18 column (55°C) in gradient mode. Mass spectra were acquired on a Quatro Premier XE (ESI+ and ESI-, 90 to 1500 amu). The data is analysed in terms of and spectral volume.

**Principal component analysis (PCA)** was applied on the total ion current (TIC) chromatograms. As a novel approach, PCA was also applied on the spectral volumes. In this case, each combination of mass and RT was considered as a variable. Only those variables exhibiting the highest variability were retained, in order to keep tractable computation times and highlight the most relevant information. To link the triglyceride profile to the cows, several parameters were projected onto the PCA results, including the day in milk, the date of birth of the cow, the volume of milk produced, the genealogy of the cow (father and maternal grandfather).

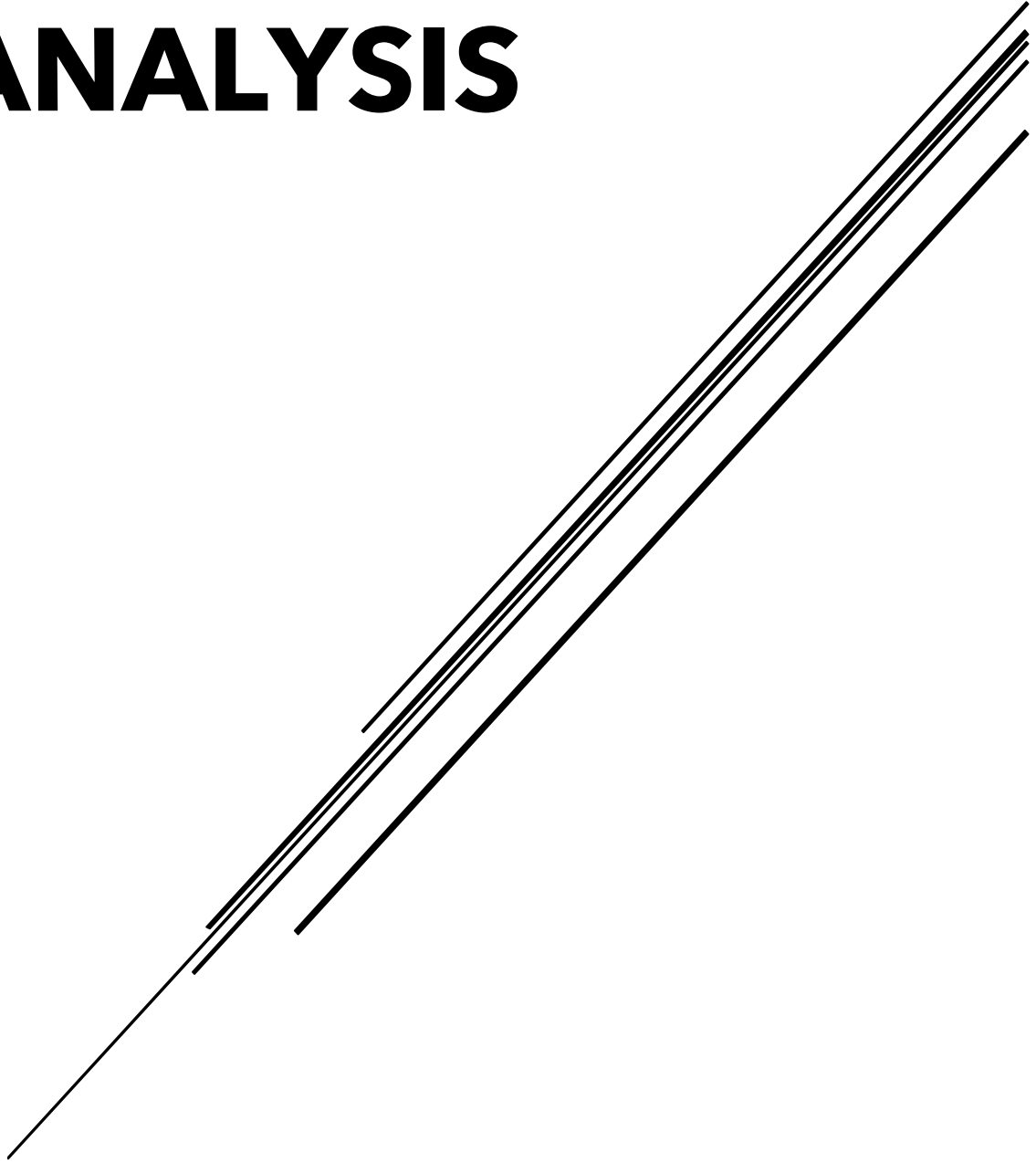
**Results:** The chromatographic method enabled the isolation of over 150 triglycerides, based on their retention time, mass (500 to over 1000 amu), number of carbons, and unsaturations. However, the use of a simple quadrupole does not provide any information regarding the structure of the detected triglyceride (nature and position of the fatty acids and of the unsaturations).

On the TIC chromatograms, the PCA scores yielded the identification of distinct groups of cows. Similar results were obtained for the PCA on the spectral volumes. The date of birth and the genetic appear to be two significant parameters. In contrast to the TIC, the analysis of the loading for the spectral volumes allows identifying a group of triglycerides that could be held responsible for the observed trends and clusters.

**Conclusions:** The method, which was developed and applied to milk samples obtained from the CRAW dairy farm, was employed to obtain a triglyceride profile based on the number of carbons and unsaturations. PCA of TIC chromatograms or spectral volumes demonstrated that the age of the cow and its genetics exert a significant influence on the observed discrimination. A reduction in the data set to those exhibiting the greatest variability revealed the triglycerides potentially responsible for discrimination.

**Keywords:** triglyceride, milk, UHPLCMS

# **GREEN FOOD ANALYSIS**



## M1

### DES-TINATION: A SUSTAINABLE JOURNEY TO ACCURATE AFLATOXIN ANALYSIS IN PISTACHIOS

**Andrea Schincaglia<sup>(1)</sup>, Alberto Cavazzini<sup>(1)</sup>, Luisa Pasti<sup>(1)</sup>, Giorgia Purcaro<sup>(2)</sup>, Marco Beccaria<sup>\*<sup>(1)</sup></sup>**

<sup>1)</sup> *University of Ferrara, Italy*

<sup>2)</sup> *Gembloux Agro-Bio Tech, University of Liège, Belgium*

*\*Corresponding author - E-mail: marco.beccaria@unife.it*

Aflatoxins, recognized as carcinogenic mycotoxins by the International Agency for Research on Cancer, significantly threaten global food safety. Commonly found in various foods including wheat, rice, corn, milk, spices, and nuts, these toxins require strict regulatory controls and thorough pre-trade analyses. Traditional aflatoxin analysis methods generate substantial waste due to the use of organic solvents. This study proposes a novel, sustainable method employing Deep Eutectic Solvents (DES) for aflatoxin extraction and quantification in pistachios, a valuable food consumed as it is or used as an ingredient in pastry. DES are a class of alternative solvents, consisting of a hydrogen bond donor and acceptor in specific ratios. These solvents are often non-toxic, biodegradable, and effective for extracting various target compounds. The extraction process was optimized using a Design of Experiment, and the complex extracts were cleaned and concentrated by dilution with water and passage through a C-18 solid phase extraction cartridge. The trapped aflatoxins were subsequently eluted with a minimal quantity of methanol and analyzed using UHPLC-FLD equipped with a partially porous C18 column. This method demonstrated robustness and reliability, achieving inter-day repeatability below 6.4% CV% for each analyte, with limits of quantification under 0.71 ng/g. Recoveries were 97.5% for AFB<sub>1</sub>, 98.5% for AFB<sub>2</sub>, 105.2% for AFG<sub>1</sub>, and 92.9% for AFG<sub>2</sub>. Environmental sustainability was evaluated using two distinct metrics, namely AGREEPrep and White Analytical Chemistry, confirming the method's eco-friendly nature and analytical performance. This innovative approach supports the shift towards sustainable analytical practices in food safety.

**Keywords:** deep eutectic solvents, pistachios, aflatoxins, sample preparation, validation

### M2

## USE OF UNIQUE GC-TOFMS TECHNOLOGY FOR ENHANCED & INCREASED THROUGHPUT SCREENING OF GINGER OILS - METHOD TRANSFER FROM HELIUM TO HYDROGEN CARRIER GASES

Nick Jones<sup>(1)</sup>, Sebastiano Panto<sup>(1)</sup>, Pavel Jiros<sup>(1)</sup>, Lena Dubois<sup>\*(1)</sup>

<sup>1)</sup> LECO EATC, Germany

\*Corresponding author - E-mail: lena\_dubois@leco.com

The origin of and quality of natural flavour ingredients, as well as their aroma/flavour properties, often varies tremendously. Therefore, the ability to confidently understand and control these variables with efficient but in-depth analysis is vital, in order to both guarantee the quality of numerous consumer products, and to perform research and development of new products and processes. Laboratories are analysing large numbers of raw materials, process development and finished goods daily. Consequently, robust, fast and cost-effective approaches, which deliver high quality data outputs are needed.

In this study, we analysed several ginger oils - a widely used and potent ingredient in many food and beverage products using both helium and hydrogen carrier gases. The transferral of methods to hydrogen carrier gas is attractive, as it is green and so much cheaper than helium to produce and can allow fast analysis to be performed. However, it has previously been problematic due to limitations and challenges associated with some types of mass spectrometry technology used. In particular, the mass spectral quality & fragmentation, in comparison with that obtained using helium, as well system sensitivity and also, system robustness and acquisition rate are important factors which can differ and negatively affect results. In this study we have investigated the use of a particular type of GC-TOF-MS technology in regard to overcoming these issues and to elevate the analysis and comparison of the different ginger oil grades. The method transfer approach, enriched data quality and processed statistical output are demonstrated.

**Keywords:** ginger oils, green analysis, consumer product quality, high through put, enhanced data

**Acknowledgement:** LECO Europe Application Development.



### M3

#### **RECOVERY OF PROTEINS AND BIOACTIVE COMPOUNDS FROM A TURMERIC CURCUMA LONGA WASTE USING SUBCRITICAL WATER EXTRACTION AND HIGH INTENSITY FOCUSED ULTRASOUNDS. IDENTIFICATION OF COMPOUNDS BY RP-UPLC-ESI-QTOF**

**Carlos Rubio Marin<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>, Maria Concepcion Garcia<sup>\* (1)</sup>**

<sup>1)</sup> *Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain*

*\*Corresponding author - E-mail: concepcion.garcia@uah.es*

Turmeric (*Curcuma longa*) is a spice increasingly consumed due to its outstanding composition and its association with healthy properties. It is employed in the production of many foods including juices resulting in big amounts of waste. This waste constitutes a cheap and sustainable source of compounds whose recovery has never been proposed despite its potential. However, the exploitation of this waste requires the development of efficient and environmentally friendly methods that are not available so far. This work proposes the development of methods using subcritical water extraction (SWE) and high intensity focused ultrasounds (HIFU), as sustainable extraction techniques, and water, as green solvent, for the recovery of proteins and compounds with antioxidant and antibacterial properties (against *Escherichia coli* and *Staphylococcus aureus*) from turmeric. The Box Behnken experimental design was employed to predict the most favourable extraction conditions in every case while minimizing the formation of potentially harmful Maillard products during extraction. Extraction of antioxidant compounds was promoted when using water under subcritical conditions in comparison with the use of water under normal conditions. Extracts highlighted by their capacity to scavenge free radicals and to avoid the formation of hydroxyl radicals, among the different mechanisms against oxidation. On the other hand, the capability of ultrasounds with water was higher than SWE for the extraction of proteins. Moreover, this yield significantly increased when combining UAE with enzyme assisted extraction using the polysaccharidase enzyme Celluclast. Extracts presented antimicrobial capacity, especially against *S. aureus* Gram-positive bacteria. The extracts showing the highest antioxidant and antimicrobial activity were analyzed by ultrahigh performance liquid chromatography coupled to high resolution tandem mass spectrometry for the detection of main compounds responsible for the observed activities.

**Keywords:** turmeric, antioxidant, antimicrobial, waste, phenolic compounds

**Acknowledgement:** *This work was supported by the Spanish Ministry of Science and Innovation (ref. PID2020-114891RB-I00), the Comunidad de Madrid (Spain) and European funding from FSE and FEDER programs (S2018/BAA-4393, AVANSECAL-II-CM).*

### M4

#### COMPARISON OF GREEN STRATEGIES FOR MAXIMIZING THE EXTRACTION OF PROTEINS AND COMPOUNDS WITH ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY FROM A GINGER WASTE

Carlos Rubio Marin<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>, Maria Concepcion Garcia<sup>\* (1)</sup>

<sup>1)</sup> Universidad de Alcalá, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid Barcelona Km. 33.600, 28871 Alcalá de Henares (Madrid), Spain, Spain

<sup>\*</sup>Corresponding author - E-mail: concepcion.garcia@uah.es

The residue from ginger juice extraction contains valuable substances, such as phenolic compounds and proteins. This residue is underutilized and the development of suitable and sustainable extraction techniques could enable its valorization. In this work, two low environmental impact methods have been developed for the extraction of these substances: one using high-intensity focused ultrasounds (HIFU) and another one using subcritical water (SWE). In both cases, water has been used as extracting solvent and a Box-Behnken experimental design was employed to optimize the recovery of proteins and antioxidant and antimicrobial compounds. The antioxidant activity of extracts was evaluated based on their ability to reduce oxidizing compounds, to scavenge free radicals, and to inhibit the formation of hydroxyl radicals. The extract obtained using SWE showed high antioxidant activity, with a slight improvement observed when using 2 cycles instead of one, or assisting the extraction with either a polysaccharide or a proteolytic enzyme. The highest protein content was observed in the HIFU extract. This recovery was improved when assisting the extraction with an enzyme, regardless a polysaccharidase or protease was employed. The HIFU extract was exhibited a higher antimicrobial activity, particularly against *Staphylococcus aureus*. Optimal extracts were analysed by RP-UPLC-ESI-QTOF being possible the identification of numerous phenolic compounds, peptides, and other compounds that could be contributing to the observed activities.

**Keywords:** ginger, antioxidant, antimicrobial, waste, sustainable extraction

**Acknowledgement:** This work was supported by the Spanish Ministry of Science and Innovation (ref. PID2020-114891RB-I00), the Comunidad de Madrid (Spain) and European funding from FSE and FEDER programs (S2018/BAA-4393, AVANSECAL-II-CM).

### M5

#### MICROWAVE-ASSISTED EXTRACTION AND SAPONIFICATION TO INCREASE THE THROUGHPUT IN FOOD QUALITY ANALYSIS

Donatella Ferrara<sup>(1)</sup>, Nicola Ruin<sup>(2)</sup>, Marco Beccaria<sup>(2)</sup>, Chiara Emilia Irma Cordero<sup>(3)</sup>, Giorgia Purcaro<sup>\*(4)</sup>

<sup>1)</sup> University of Liege, Gembloux Agro-Bio Tech, Belgium, Belgium

<sup>2)</sup> University of Ferrara, Department of Chemical, pharmaceutical and agricultural sciences, Italy

<sup>3)</sup> Department of Drug Science and Technology, University of Turin, Italy

<sup>4)</sup> Gembloux Agro-Bio Tech, University of Liège, Italy

\*Corresponding author - E-mail: gpurcaro@uliege.be

The sample preparation step often represents the most time-consuming step in the whole analytical workflow, although it remains the most important to prevent contamination, improve accuracy, and minimize the risk of results distortion. Despite that, most of the methods, especially when dealing with highly fatty foods are still long, solvent and time-consuming. This is particularly true when saponification is involved to favorize the enrichment of minor compounds. To simplify and speed up this step, microwave-assisted solvent extraction (MASE) and microwave-assisted saponification (MAS) offer a reliable and efficient alternative. Several processes can take advantage of microwave heating, reducing time and solvent, enabling the lab to have a greener and more cost-effective approach. This work presents the optimization of a rapid MAS method for the robust analysis of sterols in lipids. Moreover, the tedious TLC purification to isolate sterols is replaced by a more practical and faster SPE purification, followed by derivatization prior to the final GC(×GC)-FID analysis.

**Keywords:** microwave-assisted extraction, saponification, green analysis, food quality

## M6

### PLANT-BASED BEVERAGES IN NUTRITION

**Bogumiła Krygier<sup>(1)</sup>, Edyta Jasinska-Melon<sup>(1)</sup>, Hanna Mojska<sup>(1)</sup>**

<sup>1)</sup> National Institute of Public Health NIH - National Research Institute, Poland

\*Corresponding author - E-mail: bkrygier@pzh.gov.pl

Plant-based beverages are becoming increasingly popular around the world as an alternative to milk. Plant-based products, including alternatives to milk and dairy, are also growing in popularity in Poland. In the last 12 months, products from this category were present in 4.7 million Polish households, representing 34.6% of all households in our country. Reasons for the increase in consumption of plant-based beverages include: an increase in the number of cases (diagnosed or suspected) of lactose intolerance (leading to gastrointestinal symptoms), new dietary trends (reduced consumption of meat products) and allergy to ruminant milk protein.

The aim of this study was to compare the nutritional value of different plant-based beverages available on the Polish market with cow's milk.

The plant-based beverages were purchased from various conventional and online shops. The inclusion criteria were nutrition declaration according to *Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers* (energy value, fat, saturated fatty acids, carbohydrates, sugars, protein and salt expressed per 100 g or 100 ml) and the absence of flavourings. The nutritional values of tested plant-based beverages were compared to the composition of cow's milk using data from the Table of Composition and Nutritional Value of Foods.

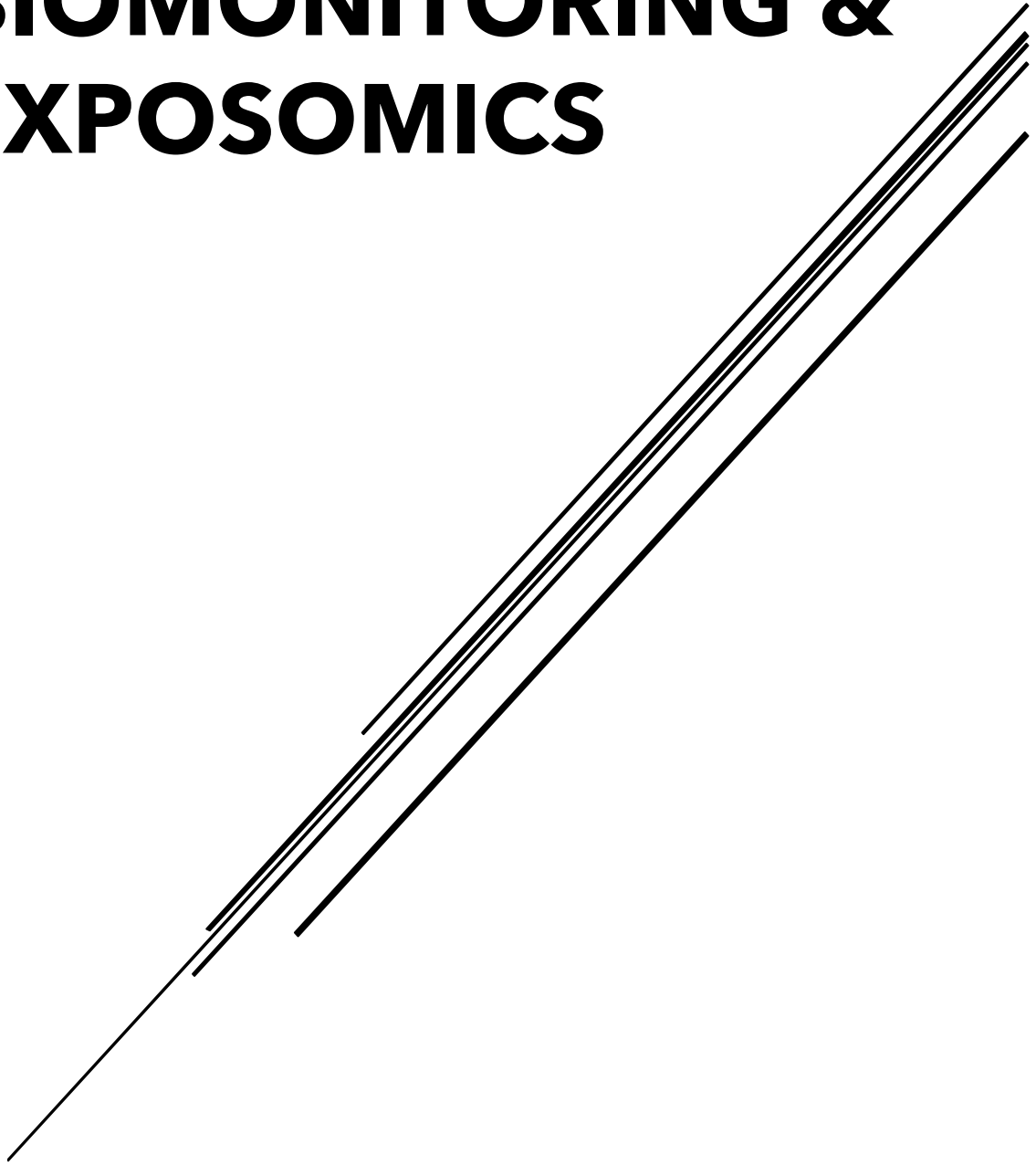
According to the used criteria, a total of 34 beverage samples were purchased in six categories: soy (6), oat (6), rice (5), almond (6), coconut (5) and other nuts (6). All of the analysed beverages were lower in energy, fat, saturated fat and protein than cow's milk. The exception was soya beverages with a similar protein content. Carbohydrate content varied and depended on the main ingredient and added sugars. All of the beverages contained a small amount of dietary fibre.

Plant-based beverages should not be considered as an alternative to milk, but as a different product with its own nutritional and functional values. Their inclusion in a varied, balanced diet can provide useful nutrients, such as easily digestible fibre or unsaturated fatty acids, which can contribute to the health of the population.

**Keywords:** plant-based beverages, nutritional value

**Acknowledgement:** *The project was financed from MEiN subsidies granted for the realisation of the statute scientific research FZ-3/2024 entitled: 'Research on the temporal trends of changes in the content of selected process pollutants in food, diet and body fluids in relation to the risk of development of nutrition related diseases,*

# **HUMAN BIOMONITORING & EXPOSOMICS**



## N1

### MYCOTOXINS IN HUMAN URINE OF ADULTS FOLLOWING HEALTHY AND SUSTAINABLE DIETS

**Octavian Augustin Mihalache**<sup>(1)</sup>, **Raquel Torrijos**<sup>(1)</sup>, **Chiara Dall'Asta**<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

\*Corresponding author - E-mail: [augustinoctavian.mihalache@unipr.it](mailto:augustinoctavian.mihalache@unipr.it)

The aim of this study was to develop and validate an ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method for the determination of 24 mycotoxins and metabolites in human urine samples. The targeted mycotoxins were aflatoxins (AFB1, AFB2, AFG1, AFG2), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), fumonisins (FB1 and FB2), HT-2/T-2, ochratoxin A (OTA), ochratoxin alpha (OT $\alpha$ ), zearalenone (ZEN), alpha-zearalenol ( $\alpha$ -ZEL), beta-zearalenol ( $\beta$ -ZEL), zearalanone (ZAN), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM), enniatins (ENN, ENNB, ENNB1, ENNA, ENNA1), and beauvericin (BEA). We obtained satisfactory method characteristics with recoveries >70% and precision with relative standard deviations (RSDs) <20%. The developed method was applied to 300 human urine samples from Italian adults. The participants followed several healthy and sustainable diets such as the Mediterranean diet and vegetarian diet. The results of this study will provide new insights regarding mycotoxin exposure and dietary patterns. Since the ongoing shift towards alternative diets can lead to an increased exposure to contaminants it is crucial to keep evaluating the food safety implications of such transitions.

**Keywords:** human biomonitoring, mycotoxin, biomarker, risk, healthy diet

**Acknowledgement:** This research was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No. 101110615 (PRISMA project).

## N2

### MINERAL CONTENT IN SICILIAN WOMEN'S BLOOD BY ICP-MS

**Irene Maria Spanò<sup>(1)</sup>, Caterina Faggio<sup>(2)</sup>, Ambrogina Albergamo<sup>(1)</sup>, Miriam Porretti<sup>(2)</sup>, Giuseppa Di Bella<sup>(1)</sup>**

<sup>1)</sup> Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy., Italy

<sup>2)</sup> Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM), University of Messina, Messina, Italy., Italy

\*Corresponding author - E-mail: irene.spano@studenti.unime.it

Humans can be exposed to various contaminants not only through ingestion of contaminated food and water, but also through inhalation (of indoor and outdoor air) and direct skin contact [1]. Exposure can lead to their accumulation in tissues and organs and ultimately to various adverse health effects [2]. Among the various pollutants, exposure to mineral elements, especially cadmium, lead, and mercury, is widespread and associated with various diseases. The human physiological state and many diseases (osteoporosis, cardiovascular diseases, breast cancer) also influences the mineral content [3]. Based on our previous study evaluating the possible presence of plasticisers and bisphenols in 75 blood samples taken from healthy women of different ages (20-60 years) living in Sicily (Italy) [4], the present study focused on a screening the mineral content of these biological matrices, to determine the variation in mineral levels in relation to the different physiological states (reproductive, pre-menopausal, menopausal) of the women studied. Mineral elements were determined using ICP-MS and all samples were analysed in triplicate. The content of macro-elements (Na, Ca, Mg) was higher in fertile women and decreased with age. In general, the order of abundance was Na > Ca > Mg. Among the essential trace elements, iron and zinc had the highest levels, followed by copper and selenium. Among the toxic and potentially toxic elements, aluminium was the highest. In particular, the trend was the opposite to that previously observed: the concentrations of Al and of Pb, Cd, Hg, As and Ni increased with age.

As this study shows, aluminium is the element most commonly found in women's blood and this can be a problem. Aluminium is an extremely effective antiperspirant; it is usually present in deodorants during heavy sweating and this effectiveness probably results in an immediate and high reserve of aluminium on the surface of the skin [5]. As reported in several studies in the literature, it is believed that antiperspirant, if used abundantly and continuously, can permeate the skin and enter the bloodstream, as high aluminium contents have been found in the axillary region and near the breast region. As the breast region is very delicate and at risk for women to develop breast cancer, it is necessary to keep an eye on the aluminium content [6]. It is hoped that this study will provide insights related to the mineral element content of women's blood in consideration of age and fertility.

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**Keywords:** mineral content, human monitoring, women's blood

### N3

#### DETERMINATION OF 30 PFAS IN BLOOD SERUM IN A CROSS-SECTIONAL STUDY IN SWITZERLAND AND LINKING THE RESULTS TO EXPOSURE SOURCES

**Alexandra Jaus**<sup>(1)</sup>, **Celine Fragniere Rime**<sup>(2)</sup>, **Julien Riou**<sup>(3)</sup>, **Murielle Bochud**<sup>(3)</sup>, **Beat Bruschweiler**<sup>(4)</sup>, **Natalie von Gotz**<sup>(5)</sup>

<sup>1)</sup> Division of Chemistry and Biology, Federal Institute of Metrology, Bern, Switzerland, Switzerland

<sup>2)</sup> Health Protection Directorate, Federal Office of Public Health FOPH, Switzerland

<sup>3)</sup> Center for Primary Care and Public Health Unisanté, Department of Epidemiology and Health Systems, University of Lausanne, Switzerland

<sup>4)</sup> Knowledge Foundations Division, Federal Food Safety and Veterinary Office FSVO, Bern, Switzerland, Switzerland

<sup>5)</sup> Health Protection Directorate, Federal Office of Public Health FOPH, Bern, Switzerland, Switzerland

\*Corresponding author - E-mail: alexandra.jaus@metas.ch

Per- and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals mainly used for their water and grease repellent properties. They have been widely employed in industrial and consumer products like firefighting foams, ski waxes, non-stick cookware, waterproof clothing, food packaging and cosmetics. Due to their chemical and thermal stability, PFASs accumulate in the environment and the human body. Current research has established a link between PFAS exposure and adverse health effects, including metabolic alterations, reproductive issues, immunotoxicity, cardiovascular diseases and cancer. It is, therefore, urgent to gain insight into PFAS levels of the general population and the multifaceted sources contributing to PFAS exposure.

The Swiss Health Study aims to provide an overview of the health status, chemical exposure and nutritional status of the general population in Switzerland. As part of the pilot phase, 30 PFASs including legacy PFASs and polyfluorinated replacement substances were determined in blood serum samples of 630 participants. A robust and efficient sample workup was developed and PFAS levels were determined by HPLC-MS/MS with a limit of quantification of 0.1 ng/mL serum for all analytes.

PFOS, PFOA and PFHxS were detected in all samples and contributed almost 90% to the median total PFAS body burden determined. Other PFASs, namely PFNA, PFDA, PFUnDA and PFHpS were also present in quantifiable amounts in more than 50% of the samples.

Participants were stratified by age and sex, and the most likely sources of exposure were investigated by statistical methods.

**Keywords:** PFAS, LC-MS/MS, human biomonitoring, Swiss health study, PFOS, PFOA

**Acknowledgement:** We thank all the participants of the Swiss Health Study pilot phase for their valuable contributions and FSVO, FOPH and Unisanté for funding.



## N4

### PFAS IN HUMANS: INSIGHTS FROM HUMAN BIOMONITORING IN THE CZECH REPUBLIC

Darina Dvorakova<sup>(1)</sup>, Tomas Gramblicka<sup>(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Veronika Svobodova<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: darina.dvorakova@vscht.cz

Per- and polyfluoroalkyl substances (PFAS), often classified as "forever chemicals," are a group of persistent, environmental, and bioaccumulative contaminants that have raised significant public health concerns due to their adverse effects on reproduction and development. As these chemicals accumulate in the environment and in the human body, understanding their occurrence in the population is crucial for assessing potential health risks and for policy decisions. Human biomonitoring, the measurement of various environmental chemicals or their metabolites in appropriate human biological samples, allows direct assessment of human exposure to these substances.

This poster provides a comprehensive overview of the results of biomonitoring of PFAS, mostly represented by C4-C14 perfluorocarboxylic acids (PFCAs), C4-C10 perfluorosulfonic acids (PFSA) and four ether-PFAS used as substitutes, in the Czech Republic over the last 20 years. For this purpose, data from the National Institute of Health in Prague [1], which is responsible for the organisation of biomonitoring at national level, as well as results from the Healthy Ageing in the Industrial Environment (HAIE) project [2] will be comprehensively discussed. The Department of Food Analysis and Nutrition at the University of Chemistry and Technology, Prague, has contributed its analytical expertise to many of these activities. The employed analytical methodologies demonstrate compliance with sensitivity requirements for ultratrace analysis, achieving quantification limits ranging from single-digit to tens of pg/mL, while maintaining relatively low sample consumption (0.5 mL of serum and 5 mL of breast milk). In total, results from 949 serum and 1,032 breast milk samples will be included, and data analysis will also focus on temporal trends to assess changes in PFAS concentrations over time. For example, a decreasing trend was observed for PFOA and PFOS in breast milk, i.e. median PFOS/PFOA concentrations in pg/mL continue to decrease: 45/75 pg/mL in 2006 and 9/12 pg/mL in 2019-2021. In addition, a risk assessment using the current tolerable weekly intake level established by the European Food Safety Authority (EFSA) for four PFAS (represented by PFOA, PFNA, PFHxS, and PFOS) [3] and a calculation of PFAS intake in breastfed infants will be performed. The data will also be compared with the results of PFAS monitoring as part of the Human Biomonitoring for Europe Initiative (HBM4EU) [4].

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**Keywords:** PFAS, breast milk, serum, Czech Republic, human biomonitoring

**Acknowledgement:** This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities.

## N5

### SILICONE WRISTBANDS AS A TOOL FOR ASSESSING HUMAN EXPOSURE TO VARIOUS ENVIRONMENTAL POLLUTANTS

**Tomas Gramblicka**<sup>(1)</sup>, **Ondrej Parizek**<sup>(1)</sup>, **Jana Pulkrabova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: gramblit@vscht.cz*

People are constantly exposed to various toxic substances from the environment throughout their lives. Many methods are currently used to assess external or internal exposure, focussing on the use of non-invasive matrices and approaches. Silicone wristbands are a suitable tool for detecting long-term or short-term (depending on the duration of wear) personal inhalation or dermal exposure to contaminants and understanding their effects on human health [1,2].

This study focuses on assessing human exposure to certain polycyclic aromatic hydrocarbons (PAHs; n=19) and organophosphorus flame retardants (OPFRs; n=17). The cohort analysed consisted of 57 individuals. The silicone wristbands were decontaminated, i.e. rinsed with various solvents [3], and then worn. Seven people wore two wristbands, one of which they wore continuously for seven days and the second of which they removed before personal hygiene. The analytical procedure for OPFRs consisted of ultrasound-assisted triplicate extraction in methanol followed by instrumental analysis by ultra-high performance liquid chromatography coupled with tandem mass spectrometry. PAHs were extracted by ultrasound-assisted triple extraction into a mixture of *n*-hexane:dichloromethane (1:1; v/v) and purified to 1 g silica. The PAHs were analysed by gas chromatography in combination with tandem mass spectrometry. From the group of OPFRs, tributyl phosphates, tris(2-butoxyethyl) phosphate and tris(1,3-dichloro-2-propyl) phosphate were found in 100% of the samples at concentrations of 0.503-94.3, 0.239-22.9, 1.82-254, and 0.995-121 ng/g, respectively. In the case of halogenated OPFRs, higher levels were found in wristbands worn at all times than in wristbands taken off (influence of personal hygiene). Three- and four-ring PAHs, in particular fluorene, phenanthrene, pyrene, and fluoranthene, were found in more than 73% of the samples in concentrations <0.025-104, <0.025-810, <0.025-264 and <0.025-100 ng/g, respectively. Carcinogenic benzo[a]pyrene was found in 42% of the samples with concentrations <0.025-15.3 ng/g. Higher amounts of PAHs were found in the wristbands of smokers and in workers at vehicle emission control stations. Our data are comparable with similarly oriented foreign studies.

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[3] O'Connell, S. G., Kincl, L. D. & Anderson, K. A. Silicone Wristbands as Personal Passive Samplers. *Environ. Sci. Technol.* 48, 3327-3335 (2014).

**Keywords:** silicone wristbands, human biomonitoring, polycyclic aromatic hydrocarbons, organophosphorus flame retardants, exposome

**Acknowledgement:** *This work was supported from the grant of Specific university research - grant No A1\_FPBT\_2024\_006.*

## N6

### USE OF SILICONE WRISTBANDS TO STUDY DERMAL AND INHALATION EXPOSURE TO CHLORINATED PARAFFINS

Denisa Parizkova<sup>(1)</sup>, Ondrej Parizek<sup>(1)</sup>, Jakub Tomasko<sup>(1)</sup>, Jana Pulkrabova<sup>\*(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: pulkrabj@vscht.cz*

Chlorinated paraffins (CPs) are among the chemicals categorised as persistent organic pollutants. CPs are commonly used as flame retardants and plasticisers. Human exposure to chlorinated paraffins occurs mainly through food. Skin contact and inhalation of contaminated dust can be another significant source of exposure. It is very important to carry out an assessment of personal exposure to CPs as they are potentially harmful to health. Silicone wristbands (SWs) have proven to be an effective tool to determine personal inhalation or dermal exposure to airborne contaminants, whether long-term or short-term, depending on the duration of wear, and to better understand their impact on human health.

Passive sampling with silicone wristbands provides a time-adjusted average exposure to absorbed substances. When SWs are worn on the wrist in contact with the skin, organic substances from the environment are adsorbed to the SWs together with those on the human skin.

This study estimates human exposure to short-chain (SCCPs, C10-C13) and medium-chain (MCCPs, C14-C17) chlorinated paraffins. The cohort comprises 57 participants, mainly students and academic staff from the University of Chemistry and Technology in Prague. The SWs were worn for seven days, with seven individuals wearing two SWs and removing one for personal hygiene. CPs were isolated from the SWs by ultrasound-assisted triple extraction in a mixture of n-hexane:dichloromethane (1:1; v/v). Subsequently, the extracts were purified with 1 g silica and a mixture of n-hexane:dichloromethane (3:1; v/v). Instrumental determination was performed using chromatography coupled with high-resolution mass spectrometry in the negative chemical ionization. The concentration range for SCCPs ranged from LOQ to 139 218 ng/g SW weight, and for MCCPs from LOQ to 67 634 ng/g SW weight. The results were compared with detailed questionnaires of the study participants. The questionnaire included information on personal hygiene, household cleaning, employment and most frequent place of residence.

**Keywords:** chlorinated paraffins, silicone wristbands, human exposure

**Acknowledgement:** *This work was financially supported from a specific university research A2\_FPBT\_2024\_064.*

## N7

### STUDY OF THE EFFECT OF ORGANIC DIET THROUGH HUMAN BIOMONITORING OF MYCOTOXINS AND PESTICIDES

Jose A. Gallardo-Ramos<sup>(1)</sup>, Jesus Marín-Sáez<sup>(2)</sup>, Vicente Sanchis<sup>(1)</sup>, Laura Gamiz-Gracia<sup>(3)</sup>, Maykel Hernandez-Mesa<sup>(3)</sup>, German Cano-Sancho<sup>(4)</sup>, Ana M. Garcia-Campana<sup>\*(3)</sup>

<sup>1)</sup> Department of Food Technology, Engineering and Science. Applied Mycology Group, AGROTECNIO-CERCA Center, University of Lleida, Spain

<sup>2)</sup> Department of Chemistry and Physics, Research Centre for Mediterranean Intensive Agrosystems and Agri-Food Biotechnology (CIAIMBITAL), University of Almeria, Spain

<sup>3)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain

<sup>4)</sup> ONIRIS, INRAE, LABERCA, France

\*Corresponding author - E-mail: amgarcia@ugr.es

Pesticides and mycotoxins are commonly found in plant-based foods, contributing to their presence in the human body as evidenced by biomonitoring programs. However, there is limited knowledge about their co-occurrence patterns. While intervention studies have demonstrated that organic diets can significantly reduce pesticide levels, their impact on mycotoxin exposure has been overlooked. This study pursued two objectives: (i) to characterize the simultaneous presence of mycotoxins and pesticides in human urine samples using biomarkers of exposure; and (ii) to investigate the influence of organic foods on these co-exposure patterns. This pilot study involved 20 healthy volunteers consuming either exclusively organic or conventional foods during a 24-h diet intervention. Their first-morning urine samples were collected, minimally treated, and analyzed using LC-Q-ToF-MS by means of a multitargeted method to detect the presence of selected pesticides and mycotoxins.

Among the 52 screened compounds, four mycotoxins and seven pesticides were detected in over 25% of the samples. Deoxynivalenol (DON) and the non-specific pesticide metabolite diethylphosphate (DEP) exhibited the highest frequency rates (100%) and the highest median concentration levels among detected pesticide biomarkers (18.7 µg/g creatinine creatinine-adjusted or 23 µg/mL non-adjusted creatinine). Correlations were observed between urine levels of mycotoxins (DON, ochratoxin alpha [OT $\alpha$ ], and enniatin B [ENNB]) and organophosphate pesticide metabolites DEP and 2-diethylamino-6-methyl-4-pyrimidinol (DEAMPY). The study suggested a reduction in ENNB and OT $\alpha$  levels and an increase in  $\beta$ -zearalenol levels in urine after a short-term replacement with organic food. However, due to the reduced sample size and short duration of the study, more research is needed to fully understand the human exposome and to refine chemical risk assessment.

**Keywords:** mycotoxins, pesticides, foods, diet, human biomonitoring

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## N8

### TRANS FATTY ACIDS IN BREAST MILK AS AN INDICATOR OF DIETARY EXPOSURE TO THESE COMPOUNDS

**Edyta Jasinska-Melon**<sup>\*(1)</sup>, **Hanna Mojska**<sup>(1)</sup>

<sup>1)</sup> National Institute of Public Health NIH - National Research Institute, Poland

\*Corresponding author - E-mail: [ejasinska@pzh.gov.pl](mailto:ejasinska@pzh.gov.pl)

**Introduction.** Trans fatty acids (TFA) can be formed through industrial processes (i-TFA) or naturally (r-TFA). TFA are associated with a number of diseases, such as atherosclerosis, cardiovascular and neurodegenerative disease, or cancer. Excessive dietary intake of trans fatty acid contributes to impaired synthesis of long-chain polyunsaturated fatty acids (LC-PUFA). The disturbed metabolism of LCPUFA by TFA in early life may have a longer-term effect. This can lead to impaired development of the nervous system and an increased risk of developing asthma or atopic dermatitis in infants and young children. As TFA are not synthesised *de novo* in the human body, their content in breast milk may be a good marker (indicator) to assess the dietary intake of these compounds by breastfeeding mothers and, indirectly, the TFA exposure of breastfed infants.

**Objective.** The aim of this study was to evaluate the relationship between the TFA content in breast milk and the intake of these acids from the diet of breastfeeding mothers.

**Materials and methods.** The material for the study consisted of 10 samples of breast milk and 10 samples of daily whole food rations reconstituted on the basis of a 10x of the 24-hour dietary recall. Fat content was determined according to PN ISO 1444:2000 *Meat and meat products-determination of free fat content* by Soxhlet method using the B-811 extraction apparatus with the B-411 pre-hydrolysis attachment by BÜCHI Labortechnik AG. Trans fatty acids were analyzed in methyl ester form (FAME) by gas chromatography coupled with a mass detector, using the Hewlett-Packard 7890A gas chromatograph with the 5975C inert MS detector.

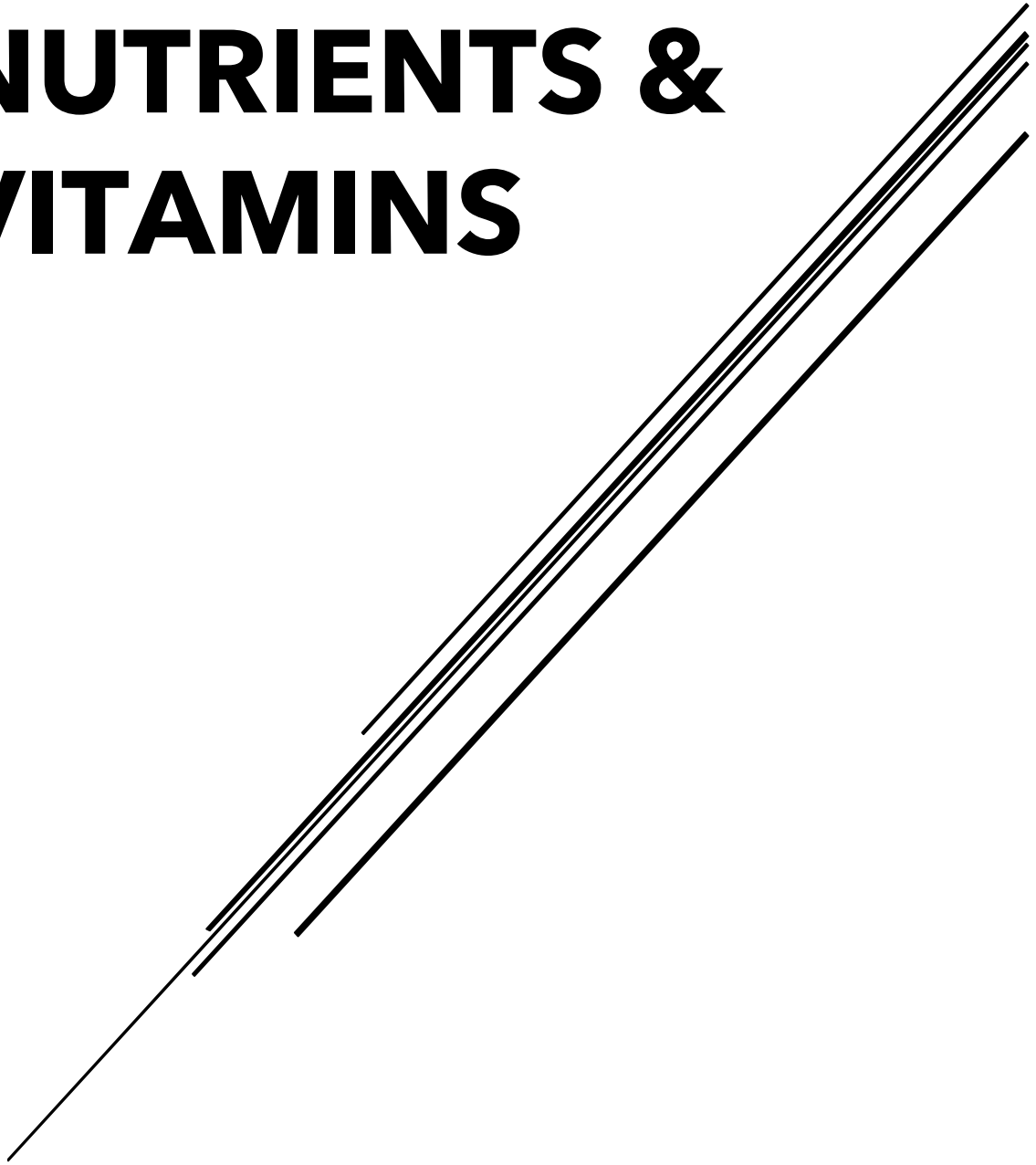
**Results.** The median (Me) TFA content of the breast milk samples was 0.96% wt/wt (0.04 g/100 ml), ranging from 0.58 to 1.13% wt/wt (0.02 - 0.07 g/100 ml). The median TFA content of daily whole food rations was 0.97% wt/wt (range: 0.50 - 2.11% wt/wt). Expressed as g/100 g or g/day diet, the values were 0.02 g (range: 0.01 - 0.03 g) and 0.32 g (range: 0.11 - 0.52 g). A strong significant ( $p < 0.05$ ) positive correlation was found between the TFA content in breast milk, both in % wt/wt and in g/100 ml, and the TFA content in the daily whole food rations ( $r = 0.83$  and  $r = 0.92$ ).

**Conclusions:** Results of the correlation between milk and diet confirmed that the TFA content in breast milk can be used as an indicator of maternal dietary sources containing trans fatty acids.

**Keywords:** TFA, human milk, diet, exposure

**Acknowledgement:** The study was carried out in the framework of Task 1/FZMŁ/2023 entitled "Study of the possibilities of using different dietary analysis techniques to estimate dietary intake of trans fatty acids"

# **MAJOR NUTRIENTS & VITAMINS**



## MAJOR NUTRIENTS & VITAMINS

### O1

#### APPLICATION OF FT-NIR SPECTROSCOPY COUPLED WITH CHEMOMETRICS FOR RAPID QUANTIFICATION OF MACRONUTRIENTS IN CEREAL-BASED BABY FOOD

**Alexandra Nanou**<sup>\*(1)</sup>, **Sofia Koutroumpi**<sup>(1)</sup>, **Evangelia Krystalli**<sup>(1)</sup>

<sup>1)</sup> *Yiotis S.A., Greece*

*\*Corresponding author - E-mail: a.nanou@hriclabs.gr*

A rapid, non-destructive, and cost-effective method was developed for the simultaneous quantification of macro components - total sugars (including fructose, glucose, sucrose, maltose, and lactose), protein and fat - in cereal-based baby food using Fourier transform near-infrared (FT-NIR) spectroscopy coupled with chemometric techniques. NIR spectra were acquired across the range of 4000 to 12500  $\text{cm}^{-1}$  from 158 samples containing varying concentrations of the seven target components. Spectral pre-processing procedures included first derivative transformation, vector normalization, and smoothing. An optimized spectral region for the quantification of each component was selected. Partial least squares regression (PLSR) was employed to construct the calibration models. Reference values for each component were obtained through independent, validated chemical analyses. Model performance was assessed based on the determination coefficient ( $R^2$ ) and root mean square error of calibration (RMSEC). The calibration ranges for each component were: Fructose, glucose, maltose: 0-11% (g/100g), Sucrose: 0-18% (g/100g), Lactose: 10-21% (g/100g), Fat: 0-11% (g/100g), Protein: 14-19% (g/100g). RMSEC values for protein, fat, fructose, glucose, sucrose, maltose, and lactose were spanning from 0,075 to 0,285. The corresponding  $R^2$  values ranged from 91,32 to 99,52%. Moreover, the model robustness was confirmed through both cross-validation and external validation procedures. For the external verification and evaluation of the models, 10 unknown baby food samples, that were randomly selected and excluded from the initial development of the new calibration curves, were analyzed. Quantification of these samples was conducted using the newly established curves and the obtained results were then compared to the reference values. The findings confirmed that FT-NIR spectroscopy is an accurate, efficient and rapid method for the quantitative analysis of protein, fat, and sugars, demonstrating its applicability for the accurate determination of these components in cereal-based baby foods.

**Keywords:** FT-NIR, chemometrics, macronutrients, cereal-based, baby food

### O2

## DEVELOPMENT OF A NEW ECO-FRIENDLY ULTRASOUND-ASSISTED EXTRACTION METHOD TO QUANTIFY TRYPTOPHAN IN WILD MUSHROOMS AND DETERMINATION OF ITS BENEFICIAL PROPERTIES

**Alejandro R. Lopez**<sup>\*(1)</sup>, Elena Ortega-Caneda<sup>(1)</sup>, Estrella Espada-Bellido<sup>(1)</sup>, Nuria Chinchilla<sup>(1)</sup>, Miguel Palma<sup>(1)</sup>, Maria Jose Aliano-Gonzalez<sup>(1)</sup>, Gerardo F. Barbero<sup>(1)</sup>, Ceferino Carrera<sup>(1)</sup>

<sup>1)</sup> University of Cadiz, Spain

\*Corresponding author - E-mail: alejandro.ruizlopez@alum.uca.es

Several studies have shown that mushrooms, in addition to their peculiar culinary qualities, have numerous beneficial properties for the proper functioning of the body. These benefits are largely due to the bioaccumulation of essential metals carried out by mushrooms, such as K, Na, Ca, Mg, P or Fe. However, it has been demonstrated that mushrooms, through their secondary metabolism, are able to synthesize different bioactive compounds involved in numerous anti-inflammatories, antibacterial or anticarcinogenic processes. For this reason, in recent years, the consumption of mushrooms by the population has increased, specifically wild mushrooms, due to their high demand as they are seasonal foods. Thus, this work has determined the concentration of a main bioactive compound (tryptophan) present in a total of twenty-six samples of wild mushrooms of high gastronomic value, belonging to the genus *Lactarius* and *Boletus*, collected in different locations in southern Andalusia and northern Morocco. For this purpose, firstly, an identification of the different bioactive compounds present in the mushroom samples was carried out, using ultra high-performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer (UHPLC-QToF-MS). Subsequently, the optimization of an ultrasound-assisted extraction (UAE) method was carried out by applying a Box-Behnken (BBD) response surface design of experiments with five independent variables (%EtOH, temperature, amplitude, cycle and ratio) and one response variable (concentration of tryptophan). Repeatability and intermediate precision studies were also carried out, obtaining coefficients of variation lower than 5%, thus indicating the precision of the method. Next, the concentration of bioactive compounds in the samples was quantified using ultra high-performance liquid chromatography coupled to a diode-array detector and a fluorescence detector (UHPLC-DAD-FLR). The results indicate that the analysed mushroom samples show good levels of tryptophan. On the other hand, the different health benefits of the extracts obtained were evaluated by determining the antioxidant capacity using the DPPH and ABTS spectrophotometric methods, as well as a bioassay to determine the acetylcholinesterase (AChE) inhibitory activity. Both studies of mushroom extracts showed good results of antioxidant capacity, as well as inhibitory activity of the enzyme acetylcholinesterase. Finally, a hierarchical cluster analysis was performed showing that tryptophan plays a key role in the properties of the extracts obtained.

**Keywords:** bioactive compounds, tryptophan, mushrooms, antioxidant capacity, acetylcholinesterase inhibitory activity

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### O3

#### EVALUATION OF THE MINERAL COMPOSITION OF YERBA MATE (*ILEX PARAGUARIENSIS*)

Justyna Osko<sup>\*<sup>(1)</sup></sup>, Aleksandra Bojarowska<sup>(1)</sup>, Kornelia Kadac-Czapska<sup>(1)</sup>, Małgorzata Grembecka<sup>(1)</sup>

<sup>1)</sup> Medical University of Gdańsk, Poland

<sup>\*</sup>Corresponding author - E-mail: justyna.osko@gumed.edu.pl

For years, consumers mainly consumed locally available products. Today, goods that were available only in certain parts of the world are being exported to previously inaccessible foreign markets. An example of such a commodity is yerba mate. It is an infusion obtained from the Paraguayan holly tree (*Ilex paraguariensis* A.St.-Hil.). Yerba mate was only available in South American countries such as Brazil, Argentina, Paraguay, and Uruguay. Currently, it is increasing in popularity, mainly among Europeans and North Americans. An increase in yerba mate imports has also been observed in Poland. Therefore, it is necessary to evaluate the quality of this product in view of its compounds and minerals.

The purpose of this study was to determine the content of 14 macro- and microelements in 30 yerba mate products from Argentina, Brazil, Uruguay, and Paraguay. The safety of the product was also assessed in terms of toxic metal content, and the data obtained were chemometrically processed. The elemental composition was determined using atomic absorption spectrometry (FAAS), which was previously validated, with satisfactory accuracy and precision (89-112% and 0.02-10.2%, respectively).

It was found that yerba mate from Uruguay had the highest content of the analyzed macroelements, i.e., Na (3.01 mg/100 g), Ca (814 mg/100 g), Mg (603 mg/100 g), and P (182 mg/100 g) compared to products from Paraguay, Brazil, and Argentina. Among the microelements analyzed, the highest concentration was found for Mn (100-153 mg/100 g). Samples from Paraguay were characterized by the highest content of Cr (0.07 mg/100 g), Fe (32.2 mg/100 g), and Zn (9.27 mg/100 g). In all samples analyzed, the Pb content was below the limit of detection (LOD=0.02 mg/100 g), so the permissible Pb concentration (0.06 mg/100 g) established for mate by the Brazilian Health Regulatory Agency (ANVISA) was not exceeded. In contrast, the majority of yerba mate products from Brazil (75%) and Paraguay (60%) had Cd concentrations higher than the level permitted by ANVISA (0.04 mg/100 g). The use of multivariate chemometric techniques such as factor analysis and cluster analysis made it possible to prove that the concentration of individual elements in the raw material depends on the country of origin, soil type, cultivation method, and harvesting period. The applied chemometric methods revealed similarities in the elemental composition of the raw material originating from yerba mate plants in Argentina, Paraguay, and Brazil.

Yerba mate can be successfully used as a food additive, cosmetic ingredient, and raw material in the pharmaceutical industry. However, its composition should always be studied before usage due to the danger of exceeding toxic metals in the dried product.

**Keywords:** yerba mate, macroelements, microelements, F-AAS

### O4

#### FATTY ACID PROFILE OF CLAMS FROM SICILIAN TRANSITIONAL WATER ZONES

Salvatore Giacobbe<sup>(1)</sup>, Benedetta Sgro\*<sup>(2)</sup>, Federica Litrenta<sup>(3)</sup>, Giuseppa Di Bella<sup>(3)</sup>, Vincenzo Lo Turco<sup>(3)</sup>

<sup>1)</sup> Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM), University of Messina., Italy

<sup>2)</sup> Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM), University of Messina. Department BIOMORF, University of Messina., Italy

<sup>3)</sup> Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy

\*Corresponding author - E-mail: benedetta.sgro@studenti.unime.it

Clams are species of great interest because they have been collected for food since ever. They are rich in high-quality nutrients and bioactive compounds that significantly impact human health. In particular, clams provide polyunsaturated fatty acids (PUFAs) especially of n-3 series such as EPA (eicosapentaenoic acid, C20:5 n-3) and DHA (docosahexaenoic acid, C22:6 n-3) [1]. In this work, the fatty acid profile was evaluated by gas chromatography coupled to flame ionization detector (GC-FID) in three different clam species of commercial interest, *R. decussatus*, *C. glaucum* and *P. aureus*, collected during the 2023 and 2024 winter seasons in the urbanized lagoon of Capo Peloro and the pristine one of Oliveri-Tindari, transitional water zones where land, fresh and salt waters mix, forming a natural transition between terrestrial, freshwater and marine ecosystems [2]. The aims were to highlight the differences between clams collected in two different years and zones and to evaluate the lipid nutritional quality. The results show that saturated fatty acids (SFAs) predominate in both years. C16:0 (33.07-21.75%) and DHA (9.14-17.72%) are the main fatty acids. The 2024 clams have a higher content of SFAs (41.50-51.70%) and PUFAs (32.34-44.04 %), than the 2023 clams, which have a higher content of monounsaturated fatty acids (MUFAs, 18.59-21.63%). Moreover, the clams from Capo Peloro have a higher content of SFAs (42.38-51.70%) than in clams from Oliveri-Tindari (38.65-48.79%), excepted for *P. aureus* collected in February 2024 (41.50%). MUFAs in clams from Capo Peloro are lower in samples collected in 2024 (13.39-16.00%) and higher in samples collected in 2023 (18.93-21.49%) than clams from Oliveri-Tindari (17.44-22.24% and 18.59-21.63%, respectively). PUFAs in clams from Oliveri-Tindari (32.45-41.72%) are higher than in clams from Capo Peloro (31.47-44.04%). PUFAs n-3 are highest in 2024 in Oliveri-Tindari (24.85-28.83%) excepted for *P. aureus* from Capo Peloro (29.56-31.06%). In terms of lipid nutritional quality, all samples have very low n6:n3 fatty acid ratio (<0.50), atherogenic (<1.10) and thrombogenic (<0.70) indexes as a result of the high content of PUFAs, so their consumption is associated with human health benefits. However, h:H ratio is low because of the high C16:0 content. Although the lipid profile depends on many factors, including species, reproductive cycle, diet and climatic and environmental conditions, the results show a product with a good lipid profile in both years and transitional water zones.

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**Keywords:** clams, lipid profile, fatty acids, polyunsaturated fatty acids

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## O5

### SYNBIOTIC PROPERTIES OF BREAST MILK

**Katarzyna Łubiech**\*<sup>(1)</sup>, **Magdalena Twaruzek**<sup>(1)</sup>, **Elena Sinkiewicz-Darol**<sup>(2)</sup>, **Dorota Martysiak-Zurowska**<sup>(3)</sup>, **Barbara Kusznierevicz**<sup>(3)</sup>

<sup>1)</sup> Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30, 85-064 Bydgoszcz, Poland, Poland

<sup>2)</sup> Kazimierz Wielki University, Faculty of Biological Sciences, Department of Physiology and Toxicology, Chodkiewicza 30, 85-064 Bydgoszcz, Poland; Human Milk Bank, Ludwik Rydygier' Provincial Polyclinical Hospital in Torun, Torun, 53-59 St. Jozef St., 87-100 Torun, Poland, Poland

<sup>3)</sup> Department of Chemistry, Technology and Biotechnology of Food, Chemical Faculty, Gdansk University of Technology, G. Narutowicza 11/12 Str., 80-233 Gdansk, Poland

\*Corresponding author - E-mail: [katarzyna.lubiech@ukw.edu.pl](mailto:katarzyna.lubiech@ukw.edu.pl)

Breast milk is the optimal food for children in the early stages of life which is characterized by nutritional properties adapted to the needs of the developing organism. It provides basic nutrients and factors with biological activity that support the child's health in many ways. These factors include the breast milk microbiota, prebiotic factors, which include breast milk oligosaccharides and many other components. Among the bacteria inhabiting the environment of breast milk, strains with potential probiotic effects can be identified. These include, among others: bacteria from the *Lactobacillaceae* family, the presence of which in mother's milk depends on many variable factors. These include maternal, perinatal and environmental factors. The microbiological composition also changes qualitatively and quantitatively.

The aim of the study was to determine the frequency of occurrence of microorganisms belonging to the *Lactobacillaceae* family and to examine the content of oligosaccharides in the breast milk of Polish breastfeeding women. The study included 63 samples of breast milk from breastfeeding women at various stages of lactation who expressed their willingness to participate in the study, confirmed by written consent. All participants of the study were informed about the scope and course of the scientific research, which was approved by the Bioethics Committee.

The methodology of the conducted study included microbiological analyses, performed based on culture methods with subsequent identification of microorganisms using MALDI-TOF/MS mass spectrometry. The studies focused on the content of oligosaccharides in breast milk, were conducted using ultra-high-performance liquid chromatography (UHPLC) combined with high-resolution mass spectrometry (HRMS).

The obtained results confirmed the presence of bacteria belonging to the *Lactobacillaceae* family in 22.2% of the analyzed samples of breast milk. A statistically insignificant relationship was observed between the occurrence of *Lactobacillaceae* bacteria and the content of HMOs in human milk. Human milk is a rich source of oligosaccharides with prebiotic activity and can also be a source of microorganisms with a potential probiotic character.

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### O6

#### CHANGES IN THE FOLATE CONTENT OF KOMBUCHAS DERIVED FROM MILK AND PLANT DRINKS

**Marta Czarnowska-Kujawska<sup>\*1)</sup>, Joanna Klepacka<sup>(1)</sup>, Beata Paszczyk<sup>(1)</sup>**

<sup>1)</sup> *Department of Commodity Science and Food Analysis, The Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland*

*\*Corresponding author - E-mail: marta.czarnowska@uwm.edu.pl*

Traditional kombucha is a sweetened infusion of black or green tea fermented with the use of SCOBY (Symbiotic Cultures of Bacteria and Yeasts) which is composed of various acetic acid-producing bacteria, lactic acid-producing bacteria and yeasts. This functional beverage gains growing popularity due to its antioxidant, antimicrobial and anticancer potential as well as the ability to reduce inflammation and cholesterol levels. These health promoting properties result from the presence of various bioactives such as phenolic compounds, vitamins (B, C) or organic acids, which contents can be modified during SCOBY fermentation. Folates, B vitamins, are naturally occurring food derivatives of folic acid, which under favorable conditions can be synthesized during fermentation by yeast and lactic acid bacteria (LAB).

The aim of the study was to evaluate the effect of fermentation with SCOBY on the content of folates in kombuchas prepared on the basis of cow's milk and plant drinks. Test material consisted of cow's milk (3.2% fat), lactose-free cow's milk (3.2% fat), almond drink, coconut drink and SCOBY fermented kombuchas prepared from them. Folate forms were analyzed with the use of high performance liquid chromatography (HPLC) method with the fluorescence and the photodiode array detectors.

Apart from kombucha fermented coconut drink, where twofold decrease in total folate content was observed, all beverages after fermentation with SCOBY showed significantly ( $P < 0.05$ ) higher folate levels. The spectacular increase in the total folate content from 2.2 up to 9.0  $\mu\text{g}/100\text{ g}$  was observed in kombucha derived from almond drink. The total folate content of the analyzed both cow's milk after fermentation increased twofold, from 5.4 to 12.6  $\mu\text{g}/100\text{ g}$  in kombucha prepared from regular cow's milk, and from 6.5 to 11.6  $\mu\text{g}/100\text{ g}$  in kombucha made from lactose free cow's milk. The results obtained in the current study proved the potential of SCOBY to synthesize folate. Such an effect is the result of the interaction of bacteria and yeast during fermentation with SCOBY, and not, as in the case of traditional yogurt starters, bacteria alone. On the other hand, observed decrease in total folates in kombucha based on coconut drink, indicates the need for selection of the favorable food product composition for efficient folate production in SCOBY fermented kombucha type beverages.

**Keywords:** SCOBY, kombucha, folates, drinks, HPLC

**Acknowledgement:** *Project funded under the designated subsidy of the Minister of Science Republic of Poland, task entitled: The Research Network of Life Sciences Universities for the Development of the Polish Dairy Industry - Research Project.*

### O7

## THE INFLUENCE OF BUCKWHEAT HUSK ADDITION ON THE ANTIOXIDANT PROPERTIES OF BREAD

**Joanna Klepacka<sup>(1)</sup>, Wajeeha Mumtaz<sup>(1)</sup>, Marta Czarnowska-Kujawska<sup>(1)</sup>**

<sup>1)</sup> *Department of Commodity Science and Food Analysis, The Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland*

*\*Corresponding author - E-mail: klepak@uwm.edu.pl*

Buckwheat husk is a product obtained in large quantities during the processing of buckwheat seeds into groats, which are popular in many countries. Despite its high nutritional value resulting primarily from the high content of fiber (approx. 80%), phenolic compounds (5-7%), proteins (4-6%) and minerals (approx. 3%), it is mainly used for non-food purposes. Currently, buckwheat hull is available on the market in micronized form, which makes it easier to dose to various food groups, compared to the less fragmented form that was previously available. Due to the high number of diseases caused by free radicals and the search for naturally occurring antioxidants, the aim of this study was to determine how the addition of buckwheat husk affects the antioxidant properties of bread.

The research material were wholemeal and toasted breads baked in laboratory conditions, to which buckwheat husk was added in the amount of 1.5%, 3% and 4.5% (control samples without husk were also prepared). The photochemiluminescence method was used to determine the ability of bread extracts to scavenge the superoxide anion radical ( $O_2^{\bullet-}$ ). Samples were diluted either with methanol in the case of antioxidants soluble in lipids or buffer in the case of antioxidants soluble in water, and based on the obtained results, the total antioxidant activity of the tested extracts was calculated.

It was shown that both types of breads contained almost twice as many lipid-soluble antioxidant components as water-soluble ones. With the increasing addition of buckwheat husk, the total antioxidant activity of both types of breads systematically increased from 3.84  $\mu\text{mol/g}$  determined in the control sample of wholemeal bread to 5.62  $\mu\text{mol/g}$  in the sample with the highest (4.5%) husk addition. In the case of toasted bread, this level changed from 1.46 to 4.01  $\mu\text{mol/g}$ , respectively. Expressing the obtained results in percentages in relation to the control samples, a significantly higher rate of change was observed in toasted bread, in which 1.5% addition of buckwheat husk caused an increase of antioxidant activity by over 50%, while 3% and 4.5% addition of buckwheat husk increased the antioxidant potential by 123% and 174%. These changes in the wholemeal bread were almost 3 times lower and the rate of increase of this parameter was 13%, 28% and 46%, respectively.

Based on the obtained results, it should be stated that the addition of buckwheat husk significantly increases the antioxidant activity of bread and the rate of changes depends on the amount of the addition and the type of bread. It is worth considering commercial enrichment of bread with buckwheat husk, which will give it the status of functional food sought by many consumers.

**Keywords:** bread, by-products, buckwheat husk, antioxidants, functional food

O8

### THE IMPACT OF INDUSTRIAL ROASTING OF COFFEE BEANS ON THE LEVEL OF PHENOLIC COMPOUNDS

Joanna Klepacka<sup>\*(1)</sup>, Marta Czarnowska-Kujawska<sup>(1)</sup>, Beata Paszczyk<sup>(1)</sup>

<sup>1)</sup> *Department of Commodity Science and Food Analysis, The Faculty of Food Sciences, University of Warmia and Mazury in Olsztyn, Poland, Poland*

*\*Corresponding author - E-mail: klepak@uwm.edu.pl*

Coffee is a beverage consumed willingly on a large scale. The method of roasting is of crucial importance in the processing of coffee beans, which is carried out in various hydrothermal conditions. These processes affect not only the sensory characteristics but also the chemical composition of coffee infusions, especially the level of saccharides, caffeine and antioxidant phenolic compounds. The aim of this study was to determine how industrial coffee beans roasting affects the content of phenolic compounds occurring in their infusions.

The research material consisted of Arabica coffee beans from Guatemala (Central America). They were roasted by one of the Polish coffee roasteries using different parameters, most frequently used by this roastery (190°C/12 min 15 sec - lightly roasted beans; 194°C/12 min 40 sec - medium roasted beans; 205°C/13 min 10 sec - hard roasted beans). The process was carried out in a gas-fired drum oven. Coffee infusions were prepared using both deionized and tap water. The total content of phenolic compounds was determined by the colorimetric method with Folin's reagent and sodium carbonate.

The obtained results indicated that the roasting process affected the content of phenolic compounds, and the rate and direction of changes depended not only on the roasting parameters, but also on the type of water used to prepare the infusions. When they were made using deionized water, the content of polyphenols increased from 18.86 mg/100 ml (unroasted beans treated as a control sample) to 25.16 mg/100 ml (lightly roasted beans), and then decreased to 23.22 mg/100 ml (medium roasted beans) and slightly increased to 24.14 mg/100 ml in hard roasted beans. Also in the case of infusions prepared with tap water, an initial increase in the level of polyphenols was observed from 19.72 mg/100 ml (unroasted beans) to 26.50 mg/100 ml (lightly roasted beans), and then their content decreased to 23.22 mg/100 ml and 21.30 mg/100 ml determined in medium- and hard-roasted beans.

The rate and scope of changes observed upon coffee beans roasting are primarily due to the chemical structure of phenolic compounds and their susceptibility to thermal changes. Increased temperature may, on the one hand, cause the degradation of their free forms and, on the other hand, their additional release from glycosidic and ester connections they form with other coffee components. The type of water used to prepare infusions had the greatest impact on the extraction of polyphenols from the most intensively roasted beans, for which it was much more advantageous to use deionized water without minerals.

**Keywords:** coffee, infusions, roasting, phenolics, antioxidants

O9

### OPTIMISATION OF SAMPLE PREPARATION PROCEDURE FOR THE DETERMINATION OF FOLATE IN VEGETABLES

Lenka Libenska\*<sup>(1)</sup>, Jana Pulkrabova<sup>(1)</sup>, Lucie Drabova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\**Corresponding author - E-mail: Lenka.Libenska@vscht.cz*

Vitamin B9, also known as folate or folic acid, is a group of hydrophilic essential substances known for their importance for the correct development of the foetus, especially in the early stages of pregnancy. However, this vitamin is essential for all people in the population.

The first aim of this work was to optimise a method for the determination of folates using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). Since folate occur in nature as polyglutamate forms, the hydrolysis had to be optimised. The parameters of the extraction buffer and the enzymatic hydrolysis were decisive for the result. The combination of acetate buffer and trienzymatic hydrolysis was selected as optimal.

The second aim of this work was to determine the folate content in fresh and processed spinach, beetroot, asparagus and green peas. In this part, the effect of boiling, baking, heat-drying and freeze-drying on the content of the most abundant forms of folate (5-methyltetrahydrofolate (5-MTHF), 5-formyltetrahydrofolate (5-formyl-THF) and 10-formylfolic acid (10-formyl-FA)) was investigated. The most abundant form of folate in the samples was 5-MTHF and the total folate content changed significantly after most culinary treatments.

**Keywords:** folate, vegetables, extraction, LC-MS/MS, culinary treatment

**Acknowledgement:** *This work was supported by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2023064) including access to its facilities and by grants from specific university research: No: A1\_FPBT\_2023\_002, A1\_FPBT\_2024\_006 and A2\_FPBT\_2023\_075.*

### O10

#### EVALUATION OF THE VITAMIN (A, D AND E) STATUS OF CZECH MOTHERS AND THEIR NEWBORNS: DIFFERENCES BETWEEN PRE-TERM AND FULL-TERM LABORS (PILOT STUDY)

**Veronika Gomersall<sup>(1)</sup>, Lenka Libenska<sup>(1)</sup>, Radim J. Sram<sup>(2)</sup>, Jiri Dusek<sup>(3)</sup>, Jana Pulkrabova<sup>(1)</sup>, Lucie Drabova<sup>\*(1)</sup>**

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> Institute of Experimental Medicine CAS, Videnska 1083, 142 20 Prague 4, Czech Republic, University of South Bohemia, Faculty of Health and Social Sciences, J. Boreckého 27, 370 05 České Budějovice, Czech Republic, Czech Republic

<sup>3)</sup> Gynaecology and obstetrics department and Department of Neonatology of hospital Ceske Budejovice a.s., B. Němcové 585/54, 370 01 České Budějovice, Czech Republic, Czech Republic

\*Corresponding author - E-mail: lucie.drabova@vscht.cz

The fat-soluble vitamins (FSV) A, E and D are essential micronutrients that are metabolised into their biologically active forms in the human body for various physiological processes. They play a particularly important role in the growth and development of the foetus.

This study aimed to investigate the relationship between pre-term birth and the concentrations of vitamins A, E and D in maternal blood and cord blood, as well as the relationship between the concentrations of these vitamins in maternal blood plasma, breast milk and cord blood plasma.

The study included two groups of mothers and their newborns: 17 full-term newborns and 22 pre-term newborns. Isolation of FSV from blood plasma samples involved precipitation of the proteins followed by 3× repeated liquid-liquid extraction (LLE) with *n*-hexane. Breast milk samples were prepared using a procedure involving precipitation of proteins followed by alkaline hydrolysis and 3× repeated LLE (*n*-hexane). Identification and quantification of target analytes was performed by ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The performance characteristics of the validated methods obtained by analysing (i) SRM 1950 (Metabolites in Frozen Human Plasma) and (ii) SRM 1849a (infant/adult milk-based formula), both in 6 replicates, were as follows: recovery (i) 78-113% and (ii) 109-125% with repeatability expressed as relative standard deviation (RSD) (i) 6-16% and (ii) 7-11% and limits of quantification 4.45-29.4 ng/mL of plasma and 0.05-10.9 ng/mL of milk.

In more than half of the mothers, plasma vitamin A and vitamin D levels were below the threshold indicating deficiency (vitamin A <0.7 µmol/L, vitamin D <50 nmol/L). The group of full-term infants had significantly higher vitamin A levels in cord blood plasma compared to the group of pre-term infants (median: 608 vs. 242 µmol/L). Significantly higher levels of 25-hydroxycholecalciferol were found in the cord blood plasma of full-term infants compared to pre-term infants (median: 34 vs. 20 nmol/L).

We have obtained unique data on vitamin A, E and D status from Czech mothers who were delivering at full-term and pre-term. The results of this study should later be incorporated into the data used to optimise nutritional supplementation, especially of vitamins A and D, for Czech mothers in the prenatal period and their newborns.

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## MAJOR NUTRIENTS & VITAMINS

### O11

#### UHPLC-Q-ORBITRAP-MS-BASED LIPIDOMIC STUDY OF SWINE SAUSAGE PRODUCED BY ADDING NATURAL INGREDIENTS TO REPLACE NITRITE AND NITRATE ADDITION

Annalisa Mentana<sup>(1)</sup>, Andrea Chiappinelli<sup>(1)</sup>, Maria Campaniello<sup>(1)</sup>, Rosalia Zianni<sup>(1)</sup>, Giovanna Berardi<sup>(1)</sup>, Marco Langianese<sup>(1)</sup>, Valeria Nardelli<sup>(1)</sup>, Marco Iammarino<sup>\*(1)</sup>

<sup>1)</sup> Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy, Italy

\*Corresponding author - E-mail: marco.iammarino@tin.it

Nitrites and nitrates are preservatives used in meat products stabilization especially for their specific action against dangerous bacteria such as *Clostridium botulinum*. On the other side, it is well-known that high intake of these additives is linked to methemoglobinemia and gastric cancer.

The main objective of the M.A.R.I. project in Italy is the evaluation of the possible synergistic effect of food irradiation, a proven treatment to stabilize foods, coupled to natural ingredients addition to replace nitrite and nitrate against *C. botulinum* growth.

The first step of this project was the optimization of a meat sausage formulation obtained by adding natural ingredients such as extra virgin olive oil, rosemary essential oil and lemon albedo, since their effect in food stabilization has already been proven [1]. According to a Design of Experiments (DoE), 12 different formulations were tested preparing swine meat sausage samples added with extra virgin olive oil (from 0% to 1.5%), rosemary essential oil (from 0% to 0.15%) and dried lemon albedo (from 0% to 7%), together with two samples with (NN) and without (no-NN) nitrite and nitrate. All prepared products were then seasoned following a traditional procedure, lasting 24 days at controlled temperature and humidity, and then evaluated by means of lipidomic approach. The lipid extraction was obtained by using Folch's extraction procedure, and the extracts were analyzed by an Ultimate 3000 UHPLC system combined to heated electro-spray ionization (HESI) Q-Exactive Focus Orbitrap Mass Spectrometer. Lipid identification from raw data was performed by Thermo Fisher LipidSearch™ 5.0.63.8 software [2].

For understanding the effects of the stabilization procedure of meat products based on natural ingredients addition, in this work we focused on diacylglycerols (DG) and oxidized triacylglycerols (OX\_TG) contents. Comparing samples prepared at central conditions of DoE to NN and no-NN samples, respect to all other samples, DG showed a decreasing in no-NN ones together with increasing of the most of OX\_TG. These two phenomena were probably due to both lipid degradation and oxidation, naturally occurring phenomena in non-treated cured meat.

Resuming, the results highlighted the stabilizing effect of natural antioxidants in seasoned sausages on lipid oxidation and degradation, somehow comparable to that obtained by adding nitrite and nitrate. Applied to this study, lipidomic approach proves to be a powerful tool useful for evaluating new food preservation techniques. The optimized formulation identified through this study will be used in the following tests of the M.A.R.I. project, coupled to food irradiation, in order to evaluate the possible inhibition of *C. botulinum* growth in absence of nitrite and nitrate addition [1].

[1] Chiappinelli et al (2024) Ital J Food Safety 13(3), 51-52.

[2] Tomaiuolo et al (2023) J Food Sci Technol 57(2), 484-494

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### O12

#### ENHANCED VITAMIN ANALYSIS: THE POWER OF IMMUNOAFFINITY CLEAN-UP AND AUTOMATION

Lochlann Walford<sup>(1)</sup>, Naomi McLachlan<sup>(1)</sup>, Carol Donnelly<sup>(1)</sup>, Claire Milligan<sup>(1)</sup>, Elizabeth Mannaing<sup>\*(1)</sup>

<sup>1)</sup> R-Biopharm Rhone Ltd, United Kingdom

\*Corresponding author - E-mail: Elizabeth@r-biopharmrhone.com

In today's fast-paced laboratory environments, maximising efficiency is of utmost importance. With advancements in automation technology, labs can now significantly increase throughput, streamline workflows, and improve overall productivity.

To meet the demands of the industry, Gilson and R-Biopharm Rhône are collaborating to combine excellence in clean-up with the added benefits of automation. R-Biopharm Rhône are a trusted manufacturer and supplier of clean-up solutions in the food and feed industry for over 35 years. Gilson, globally recognised for reliability and performance, have been manufacturing purification and liquid handling instruments for decades, and offers more than 65 years of expertise. This collaboration means we are now able to offer customers options with regards to complete end-to-end solutions for accurate and reliable clean-up.

Vitamins are analysed to ensure product quality, safety, and compliance with nutritional standards in food, pharmaceuticals, and supplements. Accurate vitamin analysis is essential for verifying label claims, assessing nutritional content, preventing deficiencies or overdoses, and supporting health research. It also helps monitor degradation during storage or processing.

Combining immunoaffinity clean-up with automation in vitamin analysis offers several advantages and together these techniques enhance the speed, accuracy and reliability of vitamin analysis.

In this study, EASI-EXTRACT® VITAMIN B12 immunoaffinity columns were validated on a Gilson ASPEC® 271 automation system for the analysis of infant food, vitamin supplements and pet foods. Recoveries were in the range of 85 - 110 % and %RSD <5 %.

In conclusion, automation significantly optimises technicians' time in the lab by handling routine analysis, allowing them to focus on more complex or value-added activities. Automated systems such as the Gilson ASPEC® 271 operate continuously, even after work hours, ensuring that equipment and analysis processes remain productive overnight. This round-the-clock operation increases lab throughput without requiring additional manpower, ultimately saving time and reducing labour costs. By maximising equipment utilisation and minimising downtime, automation not only boosts efficiency but also leads to significant cost savings, making it a highly valuable investment for any laboratory.

**Keywords:** automation, streamline workflow, fast, reduce labour

### O13

#### HEPARIN AFFINITY COLUMN: ANALYSIS OF LACTOFERRIN IN INFANT FORMULA

Siobhan Kelly<sup>(1)</sup>, Helen Cameron<sup>(1)</sup>, Carol Donnelly<sup>\*(1)</sup>, Claire Milligan<sup>(1)</sup>

<sup>1)</sup> R-Biopharm Rhone Ltd, United Kingdom

<sup>\*</sup>Corresponding author - E-mail: Carol@r-biopharmrhone.com

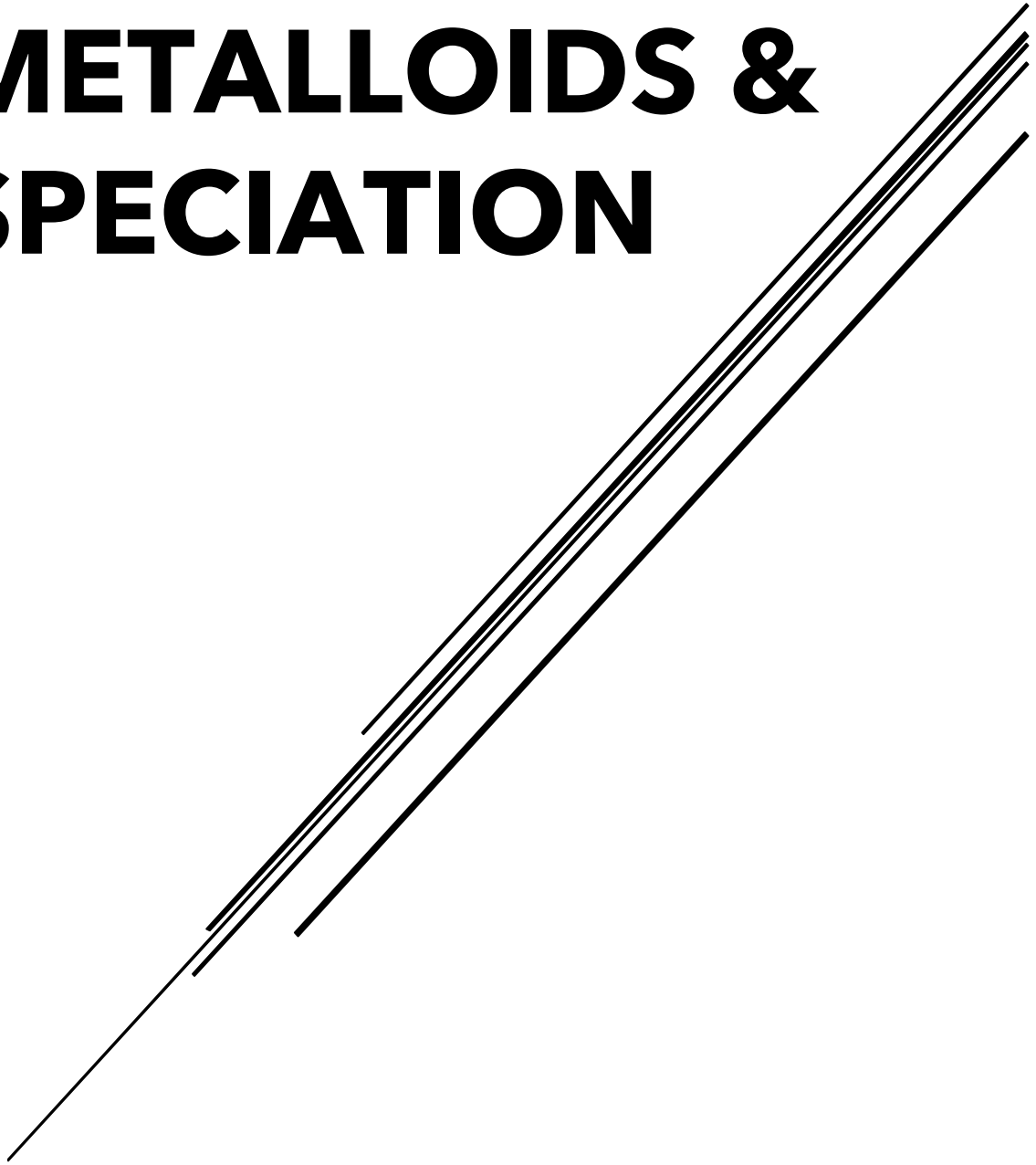
With the newly published GB China Standard and the AOAC Official Method in draft for quantification of lactoferrin in infant formula, R-Biopharm has developed a heparin affinity column that can be used for both methods. After sample clean-up using the new EASI-BIND® LACTOFERRIN heparin column the eluent is analysed using reverse phase UV-HPLC at 280 nm wavelength.

The validation with the GB China Standard method has been completed and shown that the EASI-BIND® LACTOFERRIN column can be used up to 10 times. To date, a variety of infant formulas have been tested including both solid and liquid formats. The composition of the infant formulas varies which can lead to varied performance in heparin columns due to blocking and back pressure. The EASI-BIND® LACTOFERRIN column shows good performance even with the more difficult infant formulas. The validation of the column using the draft AOAC official method is now underway.

The validation work was carried out with the vacuum tank which increased the speed of the process by allowing 12 columns to be run simultaneously and repeatedly. With the increasing need for automated high throughput analysis testing has also been carried out with the EASI-BIND® LACTOFERRIN column on the Gilson ASPEC GX-271 system.

**Keywords:** infant formula, Gilson APECT, bovine lactoferrin

# **METALS AND METALLOIDS & SPECIATION**



## P1

### FRONTAL CHROMATOGRAPHY-ICP-MS FOR ANTIMONY SPECIATION IN PET ADDITIVES

**Alejandro R. Lopez**<sup>(1)</sup>, **Davide Spanu**<sup>(2)</sup>, **Damiano Monticelli**<sup>(2)</sup>, **Sandro Recchia**<sup>(2)</sup>, **Gilberto Binda**<sup>(2)</sup>, **Gianluca Roncoroni**<sup>(2)</sup>

<sup>1)</sup> *Scuola Universitaria Superiore Pavia, Italy*

<sup>2)</sup> *Università degli Studi dell'Insubria, Italy*

\*Corresponding author - E-mail: [alejandro.ruiz@iusspavia.it](mailto:alejandro.ruiz@iusspavia.it)

Antimony (Sb) contamination from anthropogenic activities presents serious environmental and health challenges, particularly through its leaching from widespread polyethylene terephthalate (PET) materials into water sources. As a matter of fact, PET contains hundreds of mg kg<sup>-1</sup> of Sb because it is used as a catalyst during the polymerization process. This study aims to address the critical need for precise speciation analysis of Sb. Notably, distinguishing between Sb(III) and Sb(V) is crucial given their differing carcinogenicities, with Sb(III) generally considered more toxic than Sb(V). However, existing methodologies primarily focus on total Sb content only. We have recently introduced a novel approach utilizing frontal chromatography coupled with inductively coupled plasma mass spectrometry (FC-ICP-MS) for rapid and accurate speciation analysis of Sb(III) and Sb(V) in mineral water [1]. In this project we optimized this strategy with specific application to Sb migration from PET additives.

We developed and optimized the FC-ICP-MS method through a systematic multivariate data analysis to achieve rapid and sensitive Sb speciation. The method employed a short low-pressure column with a strong cation-exchange resin as the stationary phase and HNO<sub>3</sub> as the eluent. Key parameters, including column dimensions, HNO<sub>3</sub> concentration, and sample flow rate, were optimized to minimize analysis time and maximize resolution. The optimized method was then applied to investigate Sb leaching from various PET samples, including colored, virgin, recycled, and environmental plastics.

The optimized FC-ICP-MS method achieved a remarkably short analysis time of 150 seconds with a detection limit below 1 ng Kg<sup>-1</sup> for both Sb species. The method effectively separated Sb(III) and Sb(V), demonstrating high sensitivity and a wide linear range from 0.01 to 5 µg kg<sup>-1</sup>. Application to PET leaching studies revealed significant differences in Sb species migration depending on plastic aging and manufacturing processes. Environmental samples exhibited higher leaching rates compared to virgin and recycled PET, with Sb(V) consistently dominating the leachates across all samples.

The FC-ICP-MS approach proved to be a highly effective, rapid, and cost-efficient method for Sb speciation in water, particularly concerning PET leaching. This method offers substantial advantages over existing techniques, including shorter analysis times, lower detection limits, and simpler instrumentation. The findings underscore the impact of PET aging and manufacturing on Sb species migration, providing valuable insights for environmental monitoring and risk assessment. The integration of green analytical chemistry principles further enhances the method's suitability for widespread environmental and health applications.

[1] Spanu et al., (2023). *Spectrochimica Acta Part B: Atomic Spectroscopy*, 207, 106762.

**Keywords:** antimony, chromatography, speciation, PET

## P2

### RAPID AND SENSITIVE DETERMINATION OF METHYLMERCURY IN FISH AND HUMAN HAIR FOR COMPREHENSIVE BIOMONITORING

**Davide Spanu**<sup>(1)</sup>, **Sandro Recchia**<sup>(1)</sup>, **Damiano Monticelli**<sup>(1)</sup>

<sup>1)</sup> *University of Insubria, Department of Science and High Technology, Italy*

*\*Corresponding author - E-mail: davide.spanu@uninsubria.it*

Methylmercury (MeHg) is a highly toxic neurotoxin that bioaccumulates in organisms, primarily through fish consumption, posing significant health risks to humans. Effective biomonitoring should require selective analysis of MeHg rather than total mercury (Hg) alone, as traditional methods often prioritize. However, such approaches can limit our understanding of MeHg's fate and accurate exposure assessment.

To address this issue, we developed a novel, simplified method using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for selective and sensitive determination of MeHg in fish tissues and human hair [1,2]. Human hair is preferred over blood as a biomarker due to its higher MeHg accumulation (up to 250 times), ease of handling and less invasive sampling.

The developed ICP-MS strategy avoids the need for a High-Performance Liquid Chromatography (HPLC) system to separate Hg species by simply positioning a custom low-pressure column packed with anion exchange resin between the peristaltic pump and the nebulizer of the ICP-MS setup. Based on a frontal chromatography configuration, this approach continuously feeds the sample through the column allowing the complete blocking of inorganic Hg (iHg) species and the selective elution of MeHg. The method features a straightforward, 15-minute ultrasound-assisted extraction using an optimized HCl-HBr-thiourea mixture, achieving a very low detection limit of 2.8  $\mu\text{g kg}^{-1}$  (based on a 20 mg solid sample mass) with minimal interference from inorganic Hg. Validation studies using certified reference materials, including human hair (NIMD-01), phytoplankton (BCR 414), fish muscles (BCR-463 and ERM-CE464), and liver (DOLT-5), confirmed the method's accuracy across diverse biological matrices.

Application to the marine trophic chain in Djibouti (from zooplankton to scalloped hammerhead and milk shark) revealed a high trophic magnification factor (TMF = 13.5) for MeHg, highlighting the significant risk associated with MeHg biomagnification. In human hair samples, the developed method demonstrated a strong correlation between MeHg levels and individual fish and seafood intake, underscoring the link between dietary habits and MeHg exposure.

This streamlined method enables high-throughput monitoring of MeHg levels in various biological matrices, supporting comprehensive risk assessment and mitigation efforts.

[1] Spanu et al., (2022). *Anal. Chim. Acta*, 1206, 339553.

[2] Spanu et al., (2024). *Talanta*, 270, 125612.

**Keywords:** methylmercury, biomonitoring, ICP-MS, speciation, trace elements

### P3

#### ELEMENTAL PROFILING IN BRAZILIAN ARTISAN CHOCOLATES CONTAINING VARYING LEVELS OF COCOA

Giovanna Santana<sup>(1)</sup>, Romulo Silveira Rodrigues<sup>(2)</sup>, Geisamanda Pedrini Brandão<sup>(1)</sup>, Vinicius Câmara Costa<sup>(2)</sup>, Maria Tereza Weitzel Dias Carneiro\*<sup>(2)</sup>

<sup>1)</sup> Universidade Federal Do Espírito Santo, Brazil

<sup>2)</sup> UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO, Brazil

\*Corresponding author - E-mail: mariacarneiro@hotmail.com

Brazil is one of the major cocoa producers of the world being Bahia, Pará, and Espírito Santo the higher producers. The great quality of the cocoa bean increases the production market of artisanal chocolate. Artisan chocolate production is distinguished by adopting a characteristic and manual process beyond the higher cocoa contents compared to the large-scale production. This work aimed to evaluate the influence of cocoa content and the chocolate production region from Brazil on the elemental profiling. Ca, Cd, Fe, K, Mg, Mn, Na, P, Pb, and Zn were determined in artisan chocolate samples with different contents of cocoa from two different suppliers (A and B). Samples from brand A are artisanal chocolate produced in Linhares, Espírito Santo state, located in the Southeast region of Brazil. On the other hand, samples from brand B are artisanal chocolate produced in the Gramado, Rio Grande do Sul state, located in the South region of Brazil. Both brand samples were divided into six classes according to the amount of cocoa: class 1, 35-40% cocoa (1a, 1b, 1c, and 1d); class 2, 45-65% cocoa (2a, 2b, and 2c); class 3, 70-80% cocoa (3a, 3b, 3c, and 3d); class 4, 80-90% cocoa (4a and 4b); class 5, 100% cocoa; and class 6, cocoa content not informed by the supplier (6a, 6b, and 6c). All samples were left in the freezer for 24 h before pre-processing, passed through a plastic grater and were stored in plastic bags in a refrigerator before analytes extraction. The analytes were extracted using diluted nitric acid and sonication for 10 min at 50 °C. The quantification of them was done by ICP-OES and ICP-MS and the results were assessed by principal component analysis. Samples of brand A were influenced mainly by the presence of Mg, Cd, K, P, Fe, Zn, Mn, and Fe while samples of brand B were influenced by Ca, Na, and Pb. Chocolates from brand A, manufactured in Linhares/ES, sited in the Atlantic Forest region and with cocoa cultivated in alluvial soil may explain the influence of K, P, and Mg elements on samples A. It was also noted that despite the existence of two groups, samples of different brands with the same range of cocoa content tend to be closer, demonstrating the possible influence of the cocoa content on the element concentration. The influence of milk in formulations is noted by the higher Ca and Na concentrations. Although, samples of class 6 do not have information about cocoa content, they are craft milk chocolate samples with significant influence of Ca and Na. The results show the influence of the production place of the craft chocolates in the composition of micronutrients present in it, besides correlating the amount of cocoa with the concentration of the analyte. This demonstrates influence of cocoa planting location and cocoa content in the elemental profiling.

**Keywords:** artisan chocolate, elemental profiling, principal components analysis

**Acknowledgement:** FAPES, CNPq, PPGQ-UFES, LabPetro

## P4

### EXTRACTION OF IMPURITIES FROM AMORPHOUS SILICON DIOXIDE BY DILUTED HYDROCHLORIC ACID

**Daniela Montalvo**<sup>(1)</sup>, **Loic Bandinelli**<sup>(1)</sup>, **Noa Olluyn**<sup>(1)</sup>, **Jan Mast**<sup>(1)</sup>, **Karlien Cheyns**<sup>(1)</sup>

<sup>1)</sup> *Sciensano, Belgium*

*\*Corresponding author - E-mail: daniela.montalvogrijalva@sciensano.be*

Synthetic amorphous silica (SAS) is a permitted food additive authorized as E551 in the European Union under Regulation (EC) No 1333/2008. The forms of SAS used as food additive include fumed, hydrous, precipitated and gel silica. Depending on the manufacturing process, toxic elements may be present in the final product as impurities, coming from contaminants in the raw materials used for its production. In the context of assessing the risks for human health to toxic elements in food additives, total element concentrations are determined. Analysis of the total content of toxic elements in SAS requires a complete digestion of the material. Harsh digestion methods that include the use of hydrofluoric acid (HF) are usually needed to decompose silicious matrices. For SAS, a partial extraction of toxic elements with diluted hydrochloric acid (HCl) can be an approach conservative enough to assess exposure to those impurities; with the advantage of waiving the use of toxic HF. A general protocol for determining impurities soluble in HCl is described in the FAO-JECFA Monograph 20, however no standard method is available which can be used by testing laboratories. The objective of this work was to assess the influence of liquid/solid (L/S) ratio and extraction temperature on the extraction efficiency of six toxic elements (Al, As, Cd, Cr, Ni, Pb) present in SAS as impurities. The representative test materials, JRC NM-200 and NM-203 were extracted with 0.5 M HCl solution at a L/S ratio 40, 100 and 200 (volume/weight) for 30 min at 100°C. The extracts were let to cool down and the supernatants filtered through 0.2 µm membrane filters and analyzed by ICP-MS. The influence of temperature was investigated in additional samples where the extraction of impurities was conducted at room temperature (22°C). Results showed that increasing the L/S from 40 to 100 significantly ( $P < 0.05$ ) increased Cr, Ni, Pb and Al extracted concentrations. The higher L/S ratio (200) resulted in a statistically significant increase in the extracted concentration for Cr only. For As, no statistically significant ( $P < 0.05$ ) differences were observed in the extracted concentrations with L/S ratios of 40, 100 or 200. As to the effect of temperature, extractions conducted at 100°C yielded higher concentrations compared to extractions conducted at room temperature (~22°C). Overall, comparing the acid extractable fraction to total element concentration (determined after HF digestion), > 50% of Pb, Ni, and As leached with 0.5 M HCl solution at 100°C. For Al and Cr the extractable fraction represented 25% and 30%, respectively of the total concentration present in SAS. These results highlight the importance of a standardized extraction method when determining the concentrations of toxic elements in SAS.

**Keywords:** food additive, E551, metals, contaminants, impurities, ICP-MS

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P5

### DETERMINATION OF ESSENTIAL METALS (Fe, Mg, K, Ca, and Na) IN CULTIVATED AND WILD EDIBLE MUSHROOMS FROM THE SOUTH OF SPAIN AND NORTH OF MOROCCO

Oscar R. Taracena-Zepeda<sup>(1)</sup>, Alejandro Ruiz-Lopez<sup>(1)</sup>, Elena Ortega-Caneda<sup>(1)</sup>, Estrella Espada-Bellido\*<sup>(1)</sup>, Miguel Palma<sup>(1)</sup>, Gerardo Fernandez-Barbero<sup>(1)</sup>

<sup>1)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Cadiz, Agrifood Campus of International Excellence (ceiA3), IVAGRO, Spain

\*Corresponding author - E-mail: [estrella.espada@uca.es](mailto:estrella.espada@uca.es)

Among all the edible mushrooms available to the consumer, cultivated and wild-growing mushrooms are characterized by accumulating a high level of essential metals which are found in the fruiting soil. Some of these essential metals, like Fe, Mg, K, Ca or Na, are crucial for the proper functioning of the human body, due to their participation in numerous functions and biological changes in the organism. Due to the lack of information related to the content of essential metals provided by edible mushrooms, it is important to determine the total content of these essential metals in mushroom samples.

Different genus and species of cultivated and wild edible mushrooms were analyzed, comprising the genus: *Agaricus*, *Pleurotus* and *Macrolepiota*, from the south of Spain and north of Morocco. To determine the total metallic content, acid digestion must be carried out, being microwave assisted digestion the most used technique. Therefore, this study aims to first optimize and validate a microwave assisted digestion to determine the total content of five essential metals, Fe, Mg, Na, K, and Ca, applying milder conditions. The optimization employed a Box-Behnken design with a response surface methodology and statistics show that nitric acid volume and digestion time were minimized, with the optimal values being 5 mL and 10 min, respectively, and 198 °C for the temperature. Once applied the digestion method, the samples were analyzed by FAAS and ICP-OES with results showing that K was the major metal found in mushroom samples. Additionally, due to the importance of bring to light information about the nutritional profile of mushrooms and its beneficial properties to the organism, the percentage of the recommended daily intake (RDI) of each metal covered by the intake of 300 g of fresh mushroom was calculated. It can be observed that RDI levels are just exceeded for Fe. Then, despite these beneficial properties, mushroom consumption should be moderate due to the presence of high levels of Fe, which may pose health risks.

**Keywords:** mushrooms, essential metals, microwave assisted digestion, recommended daily intake

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### P6

## SPECIATION OF INORGANIC ARSENIC AND METHYL MERCURY IN FOODSTUFFS USING LC-ICP-MS

**Ben Watkin**\*<sup>(1)</sup>

<sup>1)</sup> Fera Science Ltd., United Kingdom

\*Corresponding author - E-mail: [ben.watkin@fera.co.uk](mailto:ben.watkin@fera.co.uk)

Arsenic and mercury are naturally occurring substances, which are present in the Earth's crust. They can be released into the environment by natural activity, such as volcanic eruptions, and certain anthropogenic processes, such as mining, metal smelting, and the use of fossil fuels, herbicides and pesticides. Arsenic is absorbed by all plants, but is more concentrated in leafy vegetables, apple and grape juice, and seafood. Being grown in water-logged paddy fields, rice can absorb significant amounts of arsenic from contaminated water. Methylmercury is formed from inorganic mercury by microbial action in aquatic. Most fish readily absorb the fat-soluble methylmercury but excrete it very slowly, leading to its bioaccumulation and biomagnification along the food chain.

Due to the varying toxicities of the different species, it's important to be able to identify and quantify the most toxic forms. Many of the organic forms of arsenic are considered non-toxic, whereas inorganic arsenic is classified as toxic, dangerous for the environment, and several inorganic arsenic compounds are recognized as carcinogens. Organic mercury is fat-soluble and can cross the blood-brain barrier. Exposure to high levels of mercury can cause harm to the brain, lungs, kidneys, and immune system.

The previous method for determining these compounds, involved solubilisation for 3-4 hours in strong hydrohalic acid, followed by extraction with organic solvents. The samples required mixing and centrifugation before the organic layer is removed, to be back-extracted into ICP-compatible acid solutions. The solutions required further mixing and centrifugation before removing the acid layer for analysis. This method is not entirely selective; in particular, monomethyl arsonic acid is extracted, when present.

Therefore, a more selective method was developed, involving a single, shorter extraction phase, with the separation of compounds being carried out using LC with a strong anion exchange (SAX) column, coupled to the ICP-MS. The new method removed the use of chlorinated organic solvents, along with the laborious separation of organic and inorganic layers during extraction. The LC separation also allowed for the identification and quantification of other species present.

Primary development was carried out on shellfish and seafood samples, but the method is also suitable for rice-based food, seaweed, infant formula, and various other foods, such as meats, eggs, milk, and fruit juices. Development is continually being done to improve peak separation and detection limits, and to ensure maximum compatibility with a variety of food and feed matrices.

**Keywords:** inorganic arsenic, methylmercury, speciation, LC-ICP-MS, heavy metals

### P7

#### ELEMENTAL COMPOSITION OF CZECH AND SLOVAK HONEY SAMPLES

**Anna Simankova<sup>(1)</sup>, Zuzana Prochazkova<sup>(1)</sup>, Lucie Drabova<sup>(1)</sup>, Richard Koplík<sup>(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: simankoa@vscht.cz*

Honey is one of the delicacies and food ingredients that are sometimes subject to adulteration. In addition to established criteria of honey quality such as 5-hydroxymethylfurfural content, specific conductivity, the profile of sugars, acids, phenolic compounds and fragrant compounds, the elemental composition of honey can be a useful marker of the type of honey and its geographical or botanical origin. This contribution summarizes the results of an initial study focused on honey samples from the Czech Republic and Slovakia.

We determined 24 chemical elements in 31 samples of blossom honeys, mostly mixed, originating from Slovakia (15 samples mainly from Bratislava) and various places in the Czech Republic (16 samples). The honey samples were prepared for elemental analysis by microwave pressure digestion with nitric acid. Sodium, potassium, magnesium and calcium were determined by flame atomic absorption spectrometry. The elements lithium, boron, aluminium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, arsenic, rubidium, strontium, molybdenum, cadmium, caesium, barium, thallium and lead were determined by inductively coupled plasma mass spectrometry.

The median values of the content of each element decrease in order: K (713 mg/kg), Ca (60.1 mg/kg), Mg (18.3 mg/kg), Na (6.71 mg/kg), B (6.09 mg/kg), Fe (0.532 mg/kg), Rb (0.522 mg/kg), Zn (0.484 mg/kg), Mn (0.372 mg/kg), Al (0.345 mg/kg), Cu (0.143 mg/kg), Sr (0.114 mg/kg), Ba (0.056 mg/kg) and Ni (0.023 mg/kg). The contents of Li, Pb, Mo, Cr, Co, Cs, Cd and Tl ranged from tenths to units of µg/kg. In the case of vanadium, most of the results were below the limit of detection or between the limit of detection and the limit of quantification (only five samples were properly quantified). Only three samples were exactly quantified for arsenic, the others were below the limit of quantification or even below the limit of detection.

Significant differences in the elemental content between Czech and Slovak honey samples were especially evident in the boron content, which was noticeably higher in the Czech samples. Higher concentrations of cobalt, nickel and thallium were also found in the Czech samples. On the other hand, the Slovak samples had a higher content of molybdenum, but this difference was not statistically significant. The differences in elements between the Czech and Slovak samples were further analysed using multivariate statistical analysis, namely PCA and PLS-DA methods.

**Keywords:** honey, elemental analysis, ICP-MS, FAAS

### P8

#### **SURVEY OF LEAD AND OTHER TRACE ELEMENTS IN DRIED SPICES SOURCED FROM THE DANISH MARKET**

Lou Estines<sup>(1)</sup>, Dooa Diaaeldin Ahmed M Abouelenein<sup>(2)</sup>, **Heidi Amlund**\*<sup>(2)</sup>

<sup>1)</sup> ENSCR, Ecole Nationale Supérieure de Chimie de Rennes, France

<sup>2)</sup> National Food Institute, Technical University of Denmark, Denmark

\*Corresponding author - E-mail: haml@food.dtu.dk

Dried spices may contain elevated levels of lead (Pb). Since 2022, levels of Pb exceeding the maximum limits (MLs) in dried spices have been reported eight times through the European Union (EU) Rapid Alert System for Food and Feed (RASFF). Different MLs are set for Pb in dried spices, depending on the type of spice [1]. The current MLs for Pb in dried spices vary from 0,60 mg/kg in fruit spices to 2,0 in bark spices [1].

We conducted a survey of Pb in dried spices sourced from the Danish market. A total of 37 samples were collected, covering all categories of dried spices included in the EU food legislation. These categories include seed spices, fruit spices, bark spices, root and rhizome spices, bud spices, and flower pistil spices. The survey also included other elements of interest: aluminium (Al), arsenic (As), cadmium (Cd), mercury (Hg) and nickel (Ni). Samples were homogenised if needed and analysed as is. The element levels were determined by inductively coupled plasma-mass spectrometry (ICP-MS) after microwave-assisted acid digestion. The certified reference material DORM-5 was included in the analysis to ensure the accuracy and reliability of the measurements.

Selected results show that Pb, Al, Cd and Ni were found in all samples, while As was present in most samples (4 samples < LOQ) and Hg was present in just half the samples (17 samples < LOQ). The level of Pb ranged from 0,023 to 3,9 mg/kg, and the MLs were exceeded in two of the samples. Both exceedances were for samples of cinnamon (bark spices). The samples contained 2,4 and 3,9 mg/kg, respectively, exceeding the ML of 2,0 mg/kg for bark spices.

High levels of Pb in dried spices, particularly in turmeric (also known as curcuma), have been linked to food adulteration [2], where lead chromate (PbCrO<sub>4</sub>) was suspected to have been added as a colourant to the spices to increase the intensity of their yellow colour. A molar ratio of Pb:Cr of close to one, suggestive of PbCrO<sub>4</sub>, was determined in two of the cinnamon samples. The Pb:Cr molar ratio was lower for all other samples and within a range of 0.01 to 0.52.

A detailed overview of the survey will be presented and discussed.

[1] EU Commission regulation 2023/915, and amendments

[2] A. Maquet, A. Lievens, V. Paracchini, G. Kaklamanos, B. de la Calle, L. Garland, S. Papoci, D. Pietretti, T. Zdiniakova, A. Breidbach, J. Omar Onaindia, A. Boix Sanfeliu, T. Dimitrova, F. Ulberth, Results of an EU wide Coordinated Control Plan to establish the prevalence of fraudulent practices in the marketing of herbs and spices, EUR30877EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-79-42979-1, doi:10.2760/309557, JRC126785.

**Keywords:** arsenic, cadmium, lead, mercury, ICP-MS

P9

### TRACE ELEMENT ANALYSIS OF GOURMET AND REFINED TABLE SALTS BY ICP-OES AND ICP-MS

Paulin Mouly<sup>(1)</sup>, Daniel Clayton-Cuch<sup>(2)</sup>, Heidi Amlund\*<sup>(2)</sup>

<sup>1)</sup> ENSCR, Ecole Nationale Supérieure de Chimie de Rennes, France

<sup>2)</sup> National Food Institute, Technical University of Denmark, Denmark

\*Corresponding author - E-mail: haml@food.dtu.dk

The World Health Organization (WHO) recommends limiting daily salt intake to 5 grams of NaCl to mitigate associated health risks. Gourmet and refined table salts contain trace elements that can have both beneficial and harmful effects. The European Commission Regulation 2023/915 and amendments sets maximum limits for certain elements in salts, including arsenic (0,5 mg/kg), mercury (0,1 mg/kg), cadmium (0,5 mg/kg), and lead (1 mg/kg for fleur de sel or 2 mg/kg for grey salts). Therefore, it is critical to develop precise analytical methods to determine the elemental compositions of various salt samples, which is challenging due to the high concentrations of sodium. This study employs inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) to analyze the content of 11 elements (As, Hg, Cd, Pb, K, Ca, Cu, Mg, P, S, Se) in both gourmet and refined table salts, including the four elements regulated by the European Commission. Significant variations in elemental composition were observed, depending on the type and origin of the salts. Preliminary data suggests that the regulated elements (Pb, Hg, As, Cd) can be detected in varying concentrations within the samples chosen for this study.

Due to the high concentrations of sodium, an easily ionizable element, the analysis of trace elements in gourmet and/or table salts can be difficult. Generally, the high concentration of sodium results in greater intensity of neutral lines and reduced intensity of ionic lines. Method optimization is therefore critical to obtain precise analytical data, shifting parameters such as viewing modes, RF power and minimizing physical interferences (changes in viscosity etc. of the solution) are some examples that can be optimized for.

In addition, the analysis of elements such as Hg by ICP-OES have been implicated with poor detection limits, high carryover effects and sample instability. In this study, we present a method of sample preparation for ICP-OES Hg analysis in refined and gourmet salt samples, which minimizes these effects. This research lays a foundation for future studies and underscores the importance of regulating and analyzing the trace element content of gourmet salts to protect public health.

**Keywords:** arsenic, cadmium, lead, mercury

**P10**

### **PROFILING THE ELEMENTAL COMPOSITION OF WHISKY USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)**

**Matthew Cassap<sup>(1)</sup>, Bhagyesh Surekar<sup>(1)</sup>, Daniel Kutscher<sup>(1)</sup>**

<sup>1)</sup> Thermo Fisher Scientific, United Kingdom

\*Corresponding author - E-mail: matthew.cassap@thermofisher.com

The main whisky producing countries are Scotland, Ireland, and the US, have controls over geographic origin to maintain product integrity. Due to the large profit range especially for aged and rare whisky from specific manufacturers or regions, adulteration or incorrect labelling causes issues. There are a series of marker compounds that can be analysed using gas chromatography-mass spectrometry to identify whisky adulteration, but the elemental composition can also provide relevant insights. There are certain elements that are known to vary in soils and may be used as indicators for geographical origin or to characterize relevant and unique steps in manufacturing. However, due to the distillation process, they may be depleted significantly in the final spirit compared to the original grain. This means highly sensitive instrumentation is needed to detect potentially small differences in concentrations.

The direct analysis of whisky by ICP-MS is challenging and often not possible, this is due to the alcohol content of around 40%. Reducing the alcohol content by dilution makes the analysis possible without major changes to the instrumentation, specifically the sample introduction system. However, even if successful sample introduction spectral and physical interferences still need to be considered. First, the remaining alcohol content changes the viscosity of the sample solutions and will therefore affect flow rates and nebulization efficiency in the sample introduction system. This can be addressed by preparation of matrix-matched calibration standards. Another reason to use matrix-matched calibration standards is the fact that analytes like arsenic and selenium are prone to signal enhancement in the presence of carbon and may therefore lead to false positive results if this is not compensated for. Last but not least, carbon can contribute to the formation of polyatomic interferences, such as  $^{40}\text{Ar}^{12}\text{C}^+$  on  $^{52}\text{Cr}$ .

This poster will explore how these analytical challenges can be address with the latest ICP-MS technology, to produce a robust method that can aid in elemental profiling of whisky samples to help to distinguish differences according to their geological origin as well as in the processing methods.

**Keywords:** ICP-MS, geographical origin, whisky, metals, elemental profiling

### P11

#### TRACE METALS ANALYSIS IN BABY FOOD USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS)

**Matthew Cassap**<sup>(1)</sup>, Laurent Naels<sup>(1)</sup>

<sup>1)</sup> Thermo Fisher Scientific, United Kingdom

\*Corresponding author - E-mail: matthew.cassap@thermofisher.com

Infants and young children up to the age of three are particularly vulnerable to various illnesses and the risk of potential lifelong neurological damage due to exposure to toxic heavy metals. To reduce risk of infant exposure to heavy metals, numerous countries have implemented regulations and guidelines to ensure the safety and quality of baby food products. These regulations are typically grounded in scientific research and risk assessments carried out by health and food safety authorities. Toxic heavy metals, including lead, arsenic, cadmium, and mercury, are among the various contaminants subject to screening.

Since January 2022, baby food manufacturers are required to comply with maximum levels for toxic elements by testing their final products. This approach is necessary because testing only the ingredients tends to significantly underestimate the toxic heavy metal levels in the final product. Furthermore, manufacturers must publish the results of their product testing online twice annually. Similarly, while the FDA is developing a "Closer to Zero" plan, the European Union has already established regulations and maximum limits for various contaminants, including toxic elements, in baby food.

ICP-MS (inductively coupled plasma mass spectrometry) is the widely used analytical technique for measuring trace elements in various samples, including baby food. This poster will assess the latest ICP-MS technology and its suitability for the testing of baby for heavy metals.

**Keywords:** ICP-MS, baby food, heavy metals, elemental analysis

# **MIGRANTS FROM FOOD CONTACT MATERIALS**





## Q1

### INVISIBLE THREAT – MICROPLASTICS RELEASE FROM FEEDING BOTTLES

**Kornelia Kadac-Czapska<sup>\*(1)</sup>, Beata Bochentyn<sup>(2)</sup>, Karolina Jucewicz<sup>(1)</sup>, Sebastian Mahlik<sup>(3)</sup>, Małgorzata Grembecka<sup>(1)</sup>**

<sup>1)</sup> Medical University of Gdańsk, Poland

<sup>2)</sup> Gdańsk University of Technology, Poland

<sup>3)</sup> University of Gdańsk, Poland

\*Corresponding author - E-mail: kornelia.kadac@gumed.edu.pl

Plastics are materials that are used in daily life and in many different economic sectors. As products made of them might come into contact with food, they shouldn't leak any contaminants. Regretfully, microplastic particles (MPs) are among the undesired foreign bodies detected in food, with sizes varying from 0.1 to 5000 µm. Microplastics may come out of packaging or dishes when they are washed, heated, or come into contact with liquids at different temperatures. Plastic baby feeding bottles that are frequently used are an example of such a product.

The analysis aimed to investigate the release of MPs from bottles used for child feeding. Eighteen types of bottles available on the Polish market were examined. The tested products were made of polypropylene (PP) and polyamide (PA). The premise that plastic samples or products made of it interact with a food-like simulant at a time and temperature that match actual use conditions served as the foundation for the analyses. In the tests, ultrapure redistilled water, whose MPs content had been confirmed, was used as the model fluid. The effect of various temperatures (70 – 100 °C) and time (5 – 15 min) on the release of MPs from baby feeding bottles was examined. The applied protocol was based on the filling method and a vacuum filtration system. The recovery rate for PP was 96.0%, with a relative standard deviation (RSD) of 1.8%. Similarly, for PA, the recovery rate was 96.0%, and the RSD was 2.0%. The size, shape (optical microscopy), and chemical composition (µ-Raman spectroscopy) of the isolated plastic particles were evaluated. Furthermore, Scanning Electron Microscopy (SEM) surface shape analyses were carried out on the bottles in order to evaluate potential damage.

All of the tested baby feeding bottles released MPs. In PP bottles, the average number of particles isolated was  $2.0 \pm 1.3$ , whereas in PA bottles, it was  $1.4 \pm 0.8$ . In most of the cases, the isolated MPs were shaped like fragments or films, varying in width from 8 to 189 µm and length from 17 to 3178 µm.

This analysis made it possible to partially characterize the gastrointestinal route of infant exposure to MPs, including the sources of this contamination. The data unequivocally demonstrates that children may be exposed to MPs through feeding bottles.

**Keywords:** microplastics, polymer, plastic contamination, feeding bottle, infant

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# MIGRANTS FROM FOOD CONTACT MATERIALS

## Q2

### MIGRATION OF PLASTICISERS FROM LID GASKETS INTO FATTY FOODS: A CONTINUING CONTAMINATION ISSUE

**Christoph Czerwenka**<sup>\*(1)</sup>, Daniela Reil<sup>(1)</sup>, Bettina Schmolka<sup>(1)</sup>

<sup>1)</sup> Group for Contaminant and Special Analysis, Austrian Agency for Health and Food Safety, Austria

\*Corresponding author - E-mail: christoph.czerwenka@ages.at

The migration of plasticisers from food contact materials into food products resulting in an undesired and sometimes toxicologically problematic contamination has been a known issue for a number of years. The use of a certain group of plasticisers, the phthalates, has been restricted and partially banned in the European Union. As a consequence, other types of plasticisers are being used nowadays, several of which have been assigned an individual or group migration limit in EU Regulation 10/2011.

In the course of a recurring control scheme fatty foods packaged in glass jars from the Austrian market were analysed for a broad range of approximately 50 plasticisers by using a GC-MS/MS multimethod with a simple extraction step for (ter)phthalates, adipates, sebacates and citrates and as well as a transesterification GC-MS/MS method for epoxidised soybean oil. In a second step it was assessed whether the lid gaskets were the source for the found plasticisers by analysing extracts of the gaskets. The investigated products encompassed oil-pickled vegetables, pestos and oily pastes and were from local producers, EU manufacturers as well as from third countries.

The obtained data on plasticiser contamination will be presented in detail. They clearly show that plasticisers are still a problematic food contamination issue sometimes reaching the 100 mg/kg range, despite long-established regulations and technological alternatives. The data further highlight the usefulness of a broad-scope multimethod and provide information on lid gaskets as source for plasticiser contamination. More than every fifth sample (22%) exceeded EU migration limits and/or contained plasticisers not allowed in this type of food contact material. Although less than half of the investigated food samples were from non-EU countries, they accounted for more than 75% of non-compliant products. This indicates that future controls should place a special focus on foods imported into the EU from third countries.

### Q3

#### ALTERNATIVE PLASTIC ADDITIVES IN A SPANISH GROCERY BASKET: ARE UNPACKED FOODSTUFFS LESS HARMFUL?

Julio Fernandez-Arribas<sup>\*(1)</sup>, Teresa Moreno<sup>(1)</sup>, Ethel Eljarrat<sup>(1)</sup>

<sup>1)</sup> IDAEA-CSIC, Spain

\*Corresponding author - E-mail: jfaqam@cid.csic.es

The use of plastic materials in food sales is a significant concern due to the migration of chemical additives from these materials into the food through direct contact. Traditionally, additives such as phthalates (PAEs) and organophosphate esters (OPEs) have been used as plasticizers and flame retardants, respectively. These have been substituted with ostensibly safer alternatives like acetyl tributyl citrate (ATBC) and di-2-ethylhexyl adipate (DEHA). Nevertheless, the long-term toxicity attributed by a continuous exposure through diet remains under scrutiny.

In Spain, fresh food items make up to 37.5% of the food consumed at home. This category mainly includes fruits and vegetables, followed by meat, fish, cereals, and dairy products, among others. These items are purchased both in large retail stores and traditional markets, where unpackaged food is sold in bulk. The purpose of this study is to assess the occurrence of both alternative and conventional plasticizers across different groups of foodstuffs that comprise a typical Spanish grocery selection. A total of 109 samples were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS), detecting 20 out of 35 targeted plasticizers. ATBC was detected in 41% of the samples, mostly in baby foods and cereals, legumes, and sweets, whereas DEHA was determined in 31% of foodstuffs, highlighting its presence in meat, dairy products and eggs, and fish.

Purchased samples were sold in different packaging categories, such as can, glass, plastic, and cardboard or paper materials. In addition, unpacked bulk food items were distributed in either plastic or paper wraps. Significant differences ( $p \leq 0.001$ ) were observed among these packaging types concerning the presence of ATBC and DEHA. ATBC was predominantly found in glass containers, likely due to the plastic coating on metal caps, particularly in baby foods. In contrast, DEHA appeared to be associated with wraps used for fresh foods. This raises questions regarding whether the consumption of fresh foods from market stalls leads to lower exposure to plastic-associated additives than plastic-sealed products.

The findings of this study have led to an assessment of estimated daily intake (EDI) among adults aged over 18 years, infants aged 6-12 months, and toddlers aged 1-3 years, aiming to identify vulnerable groups within the population. Infants represent the most exposed demographic to alternative plasticizers (1670 ng/kg body weight (bw)/day) and PAEs (843 ng/kg bw/day), approaching to a risk threshold where daily intake of these substances poses potential health risks. These results might contribute to more strictly legislation about the use of plastic additives in food contact materials.

**Keywords:** ATBC, DEHA, food packaging, dietary intake, infant population

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## Q4

### ANALYSIS OF POLYETHYLENE TEREPHTHALATE OLIGOMERS IN PLANT-BASED MEAT ALTERNATIVES

**Elnora Abdullajeva<sup>(1)</sup>, Bina Bhattarai<sup>(1)</sup>, Elena Hakme<sup>(1)</sup>, Lene Duedahl-Olesen<sup>\*(1)</sup>**

<sup>1)</sup> Technical University of Denmark, Denmark

\*Corresponding author - E-mail: lduo@dtu.dk

Novel plant-based meat alternatives are advertised as a reduced carbon footprint alternative to conventional meat. To ensure proper transport and storage conditions, these products are packaged in low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polyethylene terephthalate (PET) plastic materials. Due to nuances of synthesis processes, PET oligomer chains often are not fully incorporated into the packaging material and migrate from the food packaging, despite being relatively inert. Migrating PET oligomers vary in monomer units (chemical identity and ratios between single units), chain lengths (from dimers to more high molecular weight complex oligomers) and chemical structures (linear or cyclic). Toxicological data on these substances is largely missing, still, they were identified as belonging to Cramer Class III, meaning high potential toxicity [3]. The EU regulation 1935/2004 requires that materials that come into contact with food must be stable enough to prevent any harmful substances from being transferred to food. Concerns about PET migration intensified when recycled packaging was studied using food simulants [1]. At this time, there are only a handful of studies that address presence of PET oligomers in food matrices [2,4].

The aim of the study is to assess the presence of first series PET oligomers in commercially available plant-based meat alternatives. For the analysis of the first series cyclic dimer to heptamer, as well as linear trimer, sample preparation method presented by Tsochatzis et al. (2021) [4] was used as starting point. In brief, the method is based on extraction with a mixture of organic solvents and subsequent sorbent (activated alumina and PSA) clean-up. Instrumental analysis was carried out on LC-ESI(+)-qTOF using data dependent acquisition mode (DDA). To assess recovery values, several types of plant-based meat alternative samples were spiked at three concentration levels (10 ng/mL, 100 ng/mL and 500 ng/mL). The results showed high matrix effects that negatively affected the recovery, namely for oligomers in higher molecular weight ranges. Therefore, a freezing-out step was added to mitigate matrix effects, resulting in the successful validation of the method. Developed method will allow quantitative analysis of food contact material components in plant-based meat alternatives available on the market. This research will ensure a safe transition towards plant-based diets and packaging solutions, enhancing consumer protection.

[1] Bhattarai, B., Kusano, Y., Cederberg, T. L., Jensen, L. K., Granby, K., & Pedersen, G. A. (2024). Chemical characterization of virgin and recycled polyethylene terephthalate films used for food contact applications. *European Food Research and Technology*, 250(2), 533-545. <https://doi.org/10.1007/s00217-023-04400-z>.

[2] Diamantidou, D., Tsochatzis, E., Kalogiannis, S., Alberto Lopes, J., Theodoridis, G., & Gika, H. (2023). Analysis of Migrant Cyclic PET Oligomers in Olive Oil and Food Simulants Using UHPLC-qTOF-MS. *Foods*, 12(14), 2739. <https://doi.org/10.3390/foods12142739>.

[3] Schreier, V. N., Çörek, E., Appenzeller-Herzog, C., Brüscheweiler, B. J., Geueke, B., Wilks, M. F., Schilter, B., Muncke, J., Simat, T. J., Smieško, M., Roth, N., & Odermatt, A. (2023). Evaluating the food safety and risk assessment evidence-base of polyethylene terephthalate oligomers: A systematic evidence map. *Environment International*, 176, 107978. <https://doi.org/10.1016/j.envint.2023.107978>.

[4] Tsochatzis, E. D., Alberto Lopes, J., Gika, H., Kastrup Dalsgaard, T., & Theodoridis, G. (2021). Development and validation of an UHPLC-qTOF-MS method for the quantification of cyclic polyesters oligomers in pasta by applying a modified QuEChERS clean-up. *Food Chemistry*, 347, 129040. <https://doi.org/10.1016/j.foodchem.2021.129040>.

**Keywords:** polyethylene terephthalate oligomers, plant-based meat alternatives, LC-qTOF-MS, method validation

## Q5

### SUSPECT SCREENING ANALYSIS OF CONTAMINANTS IN FOOD CONTACT MATERIALS USING SUPRAMOLECULAR SOLVENTS

Laura Garcia-Cansino<sup>(1)</sup>, Noelia Caballero-Casero<sup>\*(2)</sup>, Maria Angeles Garcia<sup>(1)</sup>, Maria Luisa Marina<sup>(1)</sup>, Soledad Rubio<sup>(2)</sup>

<sup>1)</sup> University of Alcalá, Spain

<sup>2)</sup> University of Córdoba, Spain

\*Corresponding author - E-mail: a42caasn@uco.es

The European Food Safety Authority (EFSA) has approved the use of various organic compounds in the production of food contact materials (FCMs), conferring properties such as UV filters, opacity, and light stabilizers. Beyond these intentionally added substances (IAS), FCMs may also contain non-intentionally added substances (NIAS), arising from degradation reactions or impurities. This study aims to identify both IAS and NIAS in FCMs to better understand the potential chemical risks associated with food packaging.

To achieve this, various supramolecular solvents (SUPRASs) synthesized from different amphiphilic molecules and coacervation conditions were tested for their efficacy in extracting contaminants from FCMs. The optimal SUPRAS synthesis condition was a ternary mixture of 10% 1-decanol, 20% ethanol, and 70% water. The extraction procedure and acquisition method, as well as the subsequent data-mining process, were optimised using signals from isotopically labelled compounds selected as representative of the main IAS and NIAS. In addition, analytical parameters of the method (e.g. Extraction efficiency, matrix effect, etc.) were evaluated for the isotopically labelled compounds. Detected features were filtered against an in-house suspect list and further annotated and tentative identified against public mass spectra databases such as MassBank, based on several criteria including mass accuracy (mass difference below 5 ppm), isotopic patterns, and MS/MS spectra.

The study analysed 16 types of food containers, categorised by material type and whether they were made from recycled materials. 26 compounds were tentatively identified with confidence levels ranging from 2 to 4, with flame retardants being the most frequently detected chemical class. The findings underscore the necessity of thorough screening for both IAS and NIAS in FCMs to elucidate potential chemical exposures of the population from food packaging, thereby contributing to enhanced food safety protocols.

**Keywords:** supramolecular solvents, food contact materials, non-target analysis, emerging contaminants, suspect screening

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### Q6

#### **BISPHENOLS IN FOOD AND FOOD CONTACT MATERIALS: TWO-YEAR-OBSERVATION AFTER THE RISK ASSESSMENT REVISION BY EFSA**

**Thomas Behnke**<sup>(1)</sup>, **Jörg Konetzki**<sup>(1)</sup>

<sup>1)</sup> *Mérieux NutriSciences, Germany*

*\*Corresponding author - E-mail: Thomas.Behnke@mxns.com*

After the first publication announcing a revision of the risk assessment for bisphenol A (BPA) by the European Food Safety Authority in December 2021 and even more after the final statement to decrease the TDI by a factor of 20.000 in April 2023 the need for analyses of bisphenols in food as well as in various kinds of food contact materials (FCM) with a much lower limit of quantification (LOQ) than required before increased. The use of BPA in FCM and during the production of food is expected to be banned from the end of 2024.

Bisphenols are used, among other purposes, in the production of food contact materials made of polycarbonate plastics, epoxy resin coatings of cans, inks or adhesives and they can migrate from the food packaging into the product. The increasing amount of recyclates, e.g. polycarbonate or thermal paper, used in packaging material is expected to further increase the risk of these contamination. Bisphenols can also contaminate food directly during the production process if it comes into contact with the ingredients or the final product.

In order to enable an appropriate risk assessment Mérieux NutriSciences validated a method for the quantification of BPA, BPF and BPS in various food matrices and FCM with LOQs in the ppt range, which allows us to identify contamination which could not be detected before. Several different sample preparation approaches have been developed, compared and validated to take account of the great variety of matrices. This includes both extraction and, in case of food contact materials, also migration procedures. Due to the great variety of applications we also implemented a large portfolio of methods and simulants. A big challenge during the implementation of these methods was always the ubiquitous occurrence of bisphenols. Sample preparation procedures had to be kept as simple as possible.

This presentation provides an overview of different analytical methods for various food matrices, extraction and migration approaches for diverse food contact materials with different fields of application and achievable LOQs. Furthermore, we present observations made during our method development and screening phases for food and food contact materials in the past two and a half years. These findings could provide first hints on the extent of contamination of certain product groups, the distribution of bisphenols in the various components of finished products, possible sources of contamination and the influence of the applied food packaging (type of packaging, storage conditions).

**Keywords:** bisphenol, BPA, FCM, packaging

### Q7

#### SENSITIVE QUECHERS-BASED METHOD FOR THE ANALYSIS OF STYRENE IN FOOD

**Florian Peltier**\*<sup>(1)</sup>, Iris Wyns<sup>(1)</sup>, Els Van Hoeck<sup>(1)</sup>, Mélanie Di Mario<sup>(1)</sup>

<sup>1)</sup> *Sciensano, Belgium*

\*Corresponding author - E-mail: [florian.peltier@sciensano.be](mailto:florian.peltier@sciensano.be)

Polystyrene is widely used as a food contact material (FCM), primarily for dairy products. However, its use is raising concerns about consumer safety. Indeed, due to incomplete polymerisation reactions or packaging degradation, styrene monomers are likely to migrate into consumed food, which might pose a hazard to consumers. The World Health Organization (WHO) lists adverse health effects from short-term and long-term exposure to the substance, such as respiratory tract irritation, organ damage (particularly liver and kidneys), reduced fertility, DNA mutations, etc. The European Chemicals Agency (ECHA) also classifies the substance as a CMR (carcinogenic, mutagenic, or toxic to reproduction) and highlights its harmful environmental effects. The substance is under re-evaluation by the European Food Safety Authority (EFSA).

Although plastic FCM are regulated at the European level (Regulation (EU) No 10/2011), no specific migration limit for styrene in food is included. However, all FCM should comply with the general provisions of Regulation (EU) No 1935/2004, meaning that no organoleptic changes should occur. Therefore, the acceptable migration of styrene varies depending on the nature of the food. Based on the WHO guidelines, which suggest a concentration limit of 20 ppb for drinking water (based on the tolerable daily intake and different exposure ways), the European Union authorities are discussing a preliminary migration limit of 40 µg kg<sup>-1</sup> in foodstuffs.

More data on potential consumer dietary exposure is needed to anticipate a specific migration limit in the future, and thus, precise and robust analytical methods should be developed to quantify styrene in various relevant foodstuffs. In this study, an inexpensive, highly versatile method was developed based on the QuEChERS methodology in combination with liquid chromatography-fluorescence detection. As a result, minor protocol modifications allow its application to foodstuffs of very different natures like dairy products, composite food and raw meat and fish. The method was validated in-house in terms of recovery, repeatability, linearity, selectivity, specificity, expanded measurement uncertainty and, most importantly, the sensitivity of the method allowed for a quantification limit (LOQ) of 20 µg kg<sup>-1</sup>, which is half of the potential future limit. The method has demonstrated its reliability by achieving excellent z and zeta scores in a proficiency test organised by the European Reference Laboratory (EURL) for FCM (i.e. FCM-PT 23-02).

Finally, the validated method was used to analyse foodstuffs from the Belgian market, showing that styrene is present in some yoghurts and condensed milk, sometimes with concentrations exceeding 200 µg kg<sup>-1</sup>.

## Q8

### TOWARDS SUSTAINABLE FOOD PACKAGING: ENSURING FOOD SAFETY IN POST-CONSUMER RECYCLED PLASTIC MATERIALS

**Andrea Hochegger**\*<sup>(1)</sup>, **Erich Leitner**<sup>(1)</sup>

<sup>1)</sup> Institute of Analytical Chemistry and Food Chemistry, University of Technology Graz, Austria

\*Corresponding author - E-mail: andrea.hochegger@tugraz.at

To address the issue of rising packaging waste and its considerable impact on the environment, the European Parliament approved the new Regulation on Packaging and Packaging Waste in April this year. It aims to reduce packaging waste and resource consumption, thereby promoting a circular economy. The measures taken include a reduction of the total amount of packaging used by 5% by 2030, an increase in the use of reusable packaging and the setting of targets for the recycling of packaging waste. Of particular significance in terms of food safety is the mandatory content of post-consumer recycled (PCR) material in all plastic-based packaging [1]. While the use of recycled PET has been demonstrated to be safe, there is currently no clear evidence whether PCR polyolefins are [2]. To stimulate research and development, Regulation 2022/1616 defines "novel technologies", which are recycling technologies allowed to place products on the market without being approved by the European Food Safety Authority [3]. To demonstrate the capability of the technologies to produce safe materials, "appropriate methods" shall be used to collect comprehensive data. This data includes detailed information on the contaminants present in the input material and on the residual contaminants in the final recycled product, in particular genotoxic substances and endocrine disruptors, as well as the most likely sources of these contaminants and the resulting decontamination efficiency. An assessment of the migration of remaining contaminants into a packed food is also required. It has recently been demonstrated that, at the present time, no single analytical method is available being capable of answering all of the aforementioned questions. The discrepancy in the quality and quantity of information generated on two PCR polypropylene samples using selected one-dimensional and two-dimensional gas chromatography-based techniques for the analysis of volatile substances in the materials is already huge [4]. This approach, however, fails to address the numerous other substance classes or contaminants that may also be present (non-volatile, inorganic, microbiological activity, etc.), and it lacks information on genotoxic substances and endocrine disruptors.

This work aims to close the current knowledge gaps, identify the most suitable analytical techniques for the monitoring of recycling processes and to develop a fully automated safety assessment strategy. The data presented offer insights into the complex task, comparing the contamination levels of PCR plastic samples. The focus is on the advantages and disadvantages of comprehensive GC×GC-ToFMS for the characterisation of the respective materials, thereby outlining the challenges posed by Regulation 2022/1616.

[1] [https://www.europarl.europa.eu/doceo/document/TA-9-2024-0318\\_DE.pdf](https://www.europarl.europa.eu/doceo/document/TA-9-2024-0318_DE.pdf).

[2] <https://doi.org/10.3390/recycling8060087>.

[3] <http://data.europa.eu/eli/reg/2022/1616/oj>.

[4] <https://doi.org/10.1007/s00216-023-04599-6>.

**Keywords:** PCR plastics, food contact material, gas chromatography

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# MIGRANTS FROM FOOD CONTACT MATERIALS

## Q9

### ELEMENT RELEASE FROM LEAD CRYSTAL WARE AND METALLIC HIP FLASKS

**Oliver Kappenstein<sup>(1)</sup>, Sarah von Leliwa<sup>\* (1)</sup>, Thomas Tietz<sup>(1)</sup>, Stefan Merkel<sup>(1)</sup>, Andreas Luch<sup>(1)</sup>, Roman Schmidt<sup>(1)</sup>**

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

<sup>\*</sup>Corresponding author - E-mail: Sarah.vonLeliwa@bfr.bund.de

Element release is a major concern in the use of food contact materials such as tableware, cutlery and bottles. For instance, from 2012 to 2017, 32.9% of all alerts registered with the European Rapid Alert System for Food and Feed concerned the release of metal ions from food contact materials [1]. This study investigates the release of 21 elemental ions from two specific food contact materials (i) lead crystal ware and (ii) metallic hip flasks into different food simulants as well as alcoholic beverages. For this purpose, an ICP-MS method including a sample pre-treatment based on microwave-assisted digestion was developed and validated. Elemental ion release from lead crystal glasses into artificial tap water, 0.5% citric acid solution and white wine, respectively, was only detected for Pb. Within 24 h, Pb release from crystal glass was shown to increase with time. To account for repeated use, at least three consecutive release experiments were performed, which showed - with one remarkable exception - constant or decreasing levels of element ion release. However, after a four months resting period, Pb release from crystal glass was higher than before. In contrast, all 21 elemental ions were detected to be released from the hip flasks into 0.5% citric acid solution, apple liqueur and herb liqueur, respectively. Release of Cd, Cr, Ni, As, Tl, Sn and most prominently Pb from hip flasks was in the range of and above the respective release limit (SRL) as set by the Council of Europe (CoE). When focussing on the third repetition, only one out of six hip flasks met the suggested SRL for all determined elements in all test solutions. This demonstrates both, that the SRLs of the CoE can be met and that producers of hip flasks may have to review their manufacturing processes.

[1] Kuchheuser P, Dünnebier, K, Hoffbauer J, Butschke A, Birringer M. 2018. Alert notifications concerning food contact material published in the Rapid Alert System for Food and Feed between 2012 and 2017. *Journal of Consumer Protection and Food Safety*. 13:347-357.

**Keywords:** food contact material, lead, crystal ware, hip flasks

**Acknowledgement:** The project was funded by the German Federal Institute for Risk Assessment (BfR) under grant number BfR-CPS-08-1322-513.

## Q10

### FRENCH REGULATION FOR PRINTING INKS. IS THE 1 PPM LIMIT FOR POLYAROMATIC MOAH ANALYTICALLY FEASIBLE? A CASE STUDY

Michael Koch<sup>(1)</sup>, Eileen Schulz<sup>\* (1)</sup>, Agnes Buczkowski<sup>(1)</sup>, Thomas Behnke<sup>(1)</sup>, Erik Becker<sup>(1)</sup>

<sup>1)</sup> Institut Kirchhoff Berlin GmbH a Mérieux NutriSciences Company, Germany

<sup>\*</sup>Corresponding author - E-mail: [agnes.buczkowski@mxns.com](mailto:agnes.buczkowski@mxns.com)

The French regulation on mineral oil content for printing inks will come into full force from 2025 [1]. It contains strict limits on MOSH (mineral oil saturated hydrocarbons) and MOAH (mineral oil aromatic hydrocarbons). According to this legal norm, MOSH shall not exceed 0.1% and the content of MOAH with 1- to 7-rings as a sum is limited as well with 0.1%. An additional requirement limits the content of MOAH compounds with 3- to 7-rings to 1 mg/kg. Mineral oil containing printing inks, especially if used for food contact materials, could be of toxicological concern in case of migration into food because of MOAH containing 3- to 7-rings as stated by EFSA [2]. Therefore, the mentioned limits are understandable but is there an analytical method suitable for reliable determination of the required value of 1 mg/kg?

The variety of printing inks with regard to the formulation is quite high. This depends mainly on the intended application of certain inks and substrates used. Usually the expected ingredients are dyes or pigments, binding agents (e.g. various resins), diluents (e.g. organic solvents) and additives (eg .defoamers) in different proportions [3]. Fractions of mineral oil (in certain boiling range) could also be used as ingredients, however recently the majority of manufacturers in Europe try to avoid its use. In some cases, printing inks could contain a large amount of vegetable oils and/or fatty acid esters as binding agents/diluents, which could be contaminated with mineral oil. Furthermore, mineral oil-based lubricants can be carried over during the manufacturing and transport processes.

To carry out a feasibility study on reaching the required limit of 1 mg/kg for 3- to 7-ring MOAH, a set of 5 printing inks used for offset printing (mostly for packaging) was selected. The analytical activities comprised optimization of sample preparation, screening with the standard LC-GC-FID method for MOSH/MOAH and use of GCxGC-TOF (two-dimensional gas chromatography with time of flight detection) for MOAH identification. For separation of interfering resin compounds and isolation of TPAF (tri- and polyaromatic fraction) DACC (donor-acceptor complex chromatography) was performed [4]. Finally, GCxGC-FID (two dimensional GC with flame ionization detection) measurements ensured the quantification of a spiked polyaromatic fraction in printing ink.

[1] Arrêté du 13 avril 2022 précisant les substances contenues dans les huiles minérales dont l'utilisation est interdite sur les emballages et pour les impressions à destination du public.

[2] EFSA Update of the risk assessment of mineral oil hydrocarbons in food, 12th. July 2023.

[3] R.H. Leach, R.J. Pierce: *The Printing Ink Manual*. 5. Edition 2007.

[4] M. Koch et al. *J. Sep. Sci.* 43 (6) (2020) 1089-1099.

**Keywords:** mineral oil contaminants, MOSH/MOAH, migration, printing Inks, FCM

## Q11

### A COMPREHENSIVE ANALYTICAL WORKFLOW FOR SAFETY ASSESSMENT: IDENTIFICATION OF MIGRATING SUBSTANCES FROM PBS/PLA BIODEGRADABLE PACKAGING FOR FRESH-CUT PRODUCE

Jahan Zaib Ashraf<sup>(1)</sup>, Emmanouil Tsochatzis<sup>(1)</sup>, Sandra Pati<sup>(2)</sup>, Maria Luisa Amodio<sup>(2)</sup>, Annalisa Apicella<sup>(3)</sup>, Loredana Incarnato<sup>(3)</sup>, Mario M. Martinez<sup>\*(1)</sup>

<sup>1)</sup> Department of Food Science, Aarhus University, Denmark

<sup>2)</sup> Department of Agriculture, Food, Natural Resources and Engineering (DAFNE) University of Foggia, Italy

<sup>3)</sup> Department of Industrial Engineering, University of Salerno, Italy

\*Corresponding author - E-mail: mm@food.au.dk

The migration of intentionally added substances (IAS) and non-intentionally added substances (NIAS) from bio-based and biodegradable food contact material (FCM) is of growing concern. The present study investigated the migration of IAS or NIAS from two types of biodegradable FCM polymers: polybutylene succinate (PBS) and polylactic acid (PLA) as well as their respective 70:30 and 50:50 (w/w) PBS: PLA blends. A migration testing study was performed according to Commission Regulation (EU) No.10/2011, using three different food simulants, representing hydrophilic (simulant A; 10% v/v ethanol), relatively lipophilic and dairy foods (simulant D1; 50% v/v ethanol), and acidic foods (simulant B; 3% w/v acetic acid). The tests were performed at 20°C, 40°C, and 70°C. A comprehensive analytical workflow was applied, employing ultra-high performance liquid chromatography quadruple time-of-flight mass spectrometry (UHPLC-QTOF-MS) and gas chromatography quadruple time-of-flight mass spectrometry (GC-QTOF-MS) to examine migration of both semi-volatile and non-volatile compounds as well as volatile organic compounds (VOCs), respectively. The UHPLC-QTOF-MS analysis showed that temperature has a significant impact on the migration of NIAS, and particularly oligomers. Especially in case of PLA, at temperatures above 40°C, considerably greater migration than in the other materials was observed, irrespective of the food simulant. On the other hand, the PBS: PLA blend, particularly the 70:30 w/w, exhibited higher stability based on lower migration of oligomers, even when exposed to higher temperature (70°C). The most abundant non-volatile oligomers that were identified and structurally elucidated was the linear lactic acid oligomers HO-[LA]7-H (heptamer) and HO-[LA]8-H (octamer), along with bis (2-ethoxy ethyl) adipate coming also from the PLA. Additionally, oligomers of butylene succinate from the PBS and PBS: PLA blend packaging were also identified, including dimers, trimers, tetramers, and pentamers, as well as butylene succinate and succinic acid complex oligomers. The GC analysis of VOCs revealed the presence of aldehydes (2-methyl pentanal, hexanal, 5,5-dimethyl-hexanal, and nonanal), carboxylic acids (butanoic acid, pentanoic acid, 2-methyl propanoic acid), a ketone (6-methyl-5-heptene-2-one), an ester (3-methylheptyl acetate), an alkyne (1-heptadecyne), two aliphatic alcohols (2-ethyl-1-hexanol and 1-octanol) and an aromatic alcohol (2-phenoxy ethanol). The applied analytical workflow showed that a comprehensive analysis of IAS and NIAS is very important for the in-depth risk assessment of biodegradable polymers. Moreover, it provided useful insights on the diverse nature of IAS and NIAS in biodegradable polymers, especially oligomers. Finally, it was demonstrated that blending biodegradable polymers reduced the migration of PLA oligomers, whilst PBS oligomers content remained identical.

**Keywords:** oligomers, biopolymers, migration, UHPLC- QTOF-MS, GC-QTOF-MS

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## Q12

### MICROWAVE-ASSISTED EXTRACTION AND UHPLC-QTRAP®-MS/MS ANALYSIS OF PLASTICISERS IN VARIOUS CHEESES

**Aurelie Bugey**\*<sup>(1)</sup>, **Doriane Santimaria**<sup>(1)</sup>, **Fabrice Gillerat**<sup>(1)</sup>, **Ngoc-Huy Ho**<sup>(1)</sup>, **Didier Ortellì**<sup>(1)</sup>, **Patrick Edder**<sup>(1)</sup>

<sup>1)</sup> Official food control authority of the State of Geneva, Switzerland

\*Corresponding author - E-mail: aurelie.bugey@etat.ge.ch

The contamination of food by packaging materials has become a significant issue due to growing concerns about food safety. Amongst potential contaminants, plasticisers are in the forefront because they are widely used and can easily migrate directly from packaging into food products. They are mainly added directly in the plastic layer to improve flexibility and durability, but they also be found in adhesives, lubricants or sealants. Notably, some plasticisers, especially phthalates, are known to have reprotoxic effects and act as endocrine disruptors. Consequently, European and Swiss authorities [1, 2] have approved only five phthalates for use in plastic food contact materials, each subject to group restrictions and specific migration limits. Additionally, there are usage restrictions, such as prohibiting their contact with fatty foods.

To address this issue, an analytical method was developed and validated using UHPLC-Qtrap®-MS/MS for the targeted screening of 17 phthalates and one adipate in cheese.

The sample preparation involved a microwave-assisted extraction using acetonitrile, which enhanced analyte solubility and desorption kinetics while minimising sample preparation time and handling. After centrifugation, the extracted sample was directly injected into an UHPLC-Qtrap®-MS/MS system, where its selectivity and sensitivity allowed for the injection of just 0.2 µL. The isobaric phthalate pairs were effectively separated on a polar chromatographic support, enabling clear and precise identification of each compound. Additionally, specific transitions were included to confirm identification of each isomer. Finally, to prevent cross-contamination from these ubiquitous compounds, a carbon-based pre-column was placed before the injector to trap any interfering substances.

Unlike some previously published methods, this procedure is both rapid and suitable for a wide range of cheese types, including fresh, hard, semi-hard and soft cheeses, made from both cow's and goat's milk.

In-house validation, conducted according international criteria using a cheese pool, demonstrated satisfactory performances for each compound in terms of linearity, precision and trueness.

Furthermore, a survey of retail cheeses packaged in plastic and sold on the Swiss market revealed several instances of non-compliance.

[1] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.

[2] Swiss Ordinance RS 817.023.21 of the Food Department of Home Affairs (FDHA) on articles and materials 23 November 2005 - Annex 2.

**Keywords:** food contact material, plasticisers, phthalates, microwave-assisted extraction, UHPLC-QTrap®-MS/MS

## Q13

### TRACE ANALYSIS OF BISPHENOL A IN CANNED FOOD BY DI-SPME-ARROW-GC-MS/MS

**Deborah Bertuola**<sup>\*(1)</sup>, **Anna Romanotto**<sup>(1)</sup>

<sup>1)</sup> PiCA Prüfinstitut Chemische Analytik GmbH a Tentamus Group Company, Germany

\*Corresponding author - E-mail: [deborah.bertuola@pica-berlin.de](mailto:deborah.bertuola@pica-berlin.de)

Bisphenol A (BPA) is a co-monomer used as additive in the production of various materials, some of which are intended for the food industry: packaging, food storage containers, but also epoxy resins used for the cans metal coating. The human exposure to this substance can also occur through its migration in foods, that are in contact with the aforementioned materials [1]. The dangerous effects of BPA on human health, widely discussed over the years [2,3], were reconsidered again in 2023 by EFSA, that set a new extremely low tolerable daily intake (TDI) for this compound [4]. The improvement of the BPA quantification in term of sensitivity has therefore become an incontrovertible requirement of the food analysis; for this reason, we developed an analytical method for the quantification of BPA in canned food, exploiting the sensitivity and selectivity of the direct immersion SPME technic associated with the use of the multiple reaction monitoring spectrometry (MRM). The main accomplished focuses of the method development were the achievement of a low detection limit (at least 1 ppb), the decrease of external contamination and the reduction of the SPME background phenomena, through ad hoc sample processing and cleaning procedures of the solid phase after the sample extraction. Possible overestimates or underestimates of the analyte concentration, caused by the matrix effect were eliminated through the use of the Bisphenol-A-(diphenyl-13C12) as internal standard. In all analysed matrices until now, it was possible to successfully detect both traces of BPA and the addition of BPA-13C at the set limit of 1 ppb, indicating a good efficiency of the used phase to adsorb and desorb this analyte and its isotope. The advantages of the method are certainly the complete elimination of plastic equipment, the minimal sample preparation steps, the selectivity of the SPME-MRM coupling and the convenience of the BPA concentration calculation through the use of its carbon-13 isotope. We have therefore decided that, given these promising aspects, the method will be now optimized and validated, in order to monitor low levels of BPA in canned food samples.

[1] EFSA. An official website of the European Union. 2023. <https://www.efsa.europa.eu/de/topics/topic/bisphenol>.

[2] World Health Organization & Food and Agriculture Organization of the United Nations. (2011). Joint FAO/WHO expert meeting to review toxicological and health aspects of bisphenol A : final report, including report of stakeholder meeting on bisphenol A, November 2010, Ottawa, Canada.

[3] Hengstler JG, Foth H, Gebel T, Kramer PJ, Lilienblum W, Schweinfurth H, et al. "Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A". April 2011. *Critical Reviews in Toxicology*. 41 (4): 263-291.

[4] EFSA. Re-evaluation of the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. April 2023. *EFSA Journal* Volume 21, Issue 4.

**Keywords:** bisphenol A, DI-SPME-ARROW, GC-MS/MS, low LOD, canned food

## Q14

### COMBINED QUALITATIVE AND QUANTITATIVE ANALYSIS OF FOOD PACKAGING MATERIALS USING QTOF MASS SPECTROMETRY

Daqiang Pan<sup>(1)</sup>, Clemens Bidmon<sup>(2)</sup>, Jianru Stahl-Zeng<sup>(1)</sup>, Andre Schreiber<sup>(1)</sup>, Gitte Barknowitz<sup>(1)</sup>

<sup>1)</sup> SCIEX, Germany

<sup>2)</sup> TÜV Rheinland, Germany

\*Corresponding author - E-mail: Daqiang.Pan@SCIEX.com

Introduction: E&L compounds have long been a safety concern, with EU commission regulation 10/2011 being in place for over a decade to regulate food contact materials, ensuring their safety. Within this, numerous compounds are stated, with respective limits or prohibition given. Therefore, these components need to be managed to ensure safety. However, this list isn't exhaustive and so, analysis of unknown compounds within the material must be investigated. These components are deemed to be non-intentionally added substances (NIAS) and need to be identified to ensure they are controlled to an acceptable level. Here, we have explored the use of QTOF mass spectrometry for the analysis of food packaging materials. Three methods of analysis were utilized: suspect screening, non-targeted screening and quantitation.

Results: For E&L applications the importance of a suspect list or library cannot be understated due to the vast number of compounds of relevance. Initially, a suspect list of 369 compounds was provided by TÜV Rheinland. From those 369 compounds, 102 were identified in positive ion mode, 69 in negative, all with 70 in positive ion mode and 7 in negative.

In addition to suspect screening, it is paramount to understand compounds which sit outside of the target list as NIAs and perform non-targeted screening. The same data was utilized however, the data was processed differently. Initially, the same libraries were used to assess the possible matches, with 44 identified in the positive ion, meaning that 17 additional compounds were identified. Subsequently to this, both the Formula Finder and non-targeted peaks functionality in the software was utilized to assess compounds with no library hit. The data was reviewed to determine whether the unknown component is genuine prior to using ChemSpider. The ChemSpider tool is built into the software and provides a tentative identification of the unknown compound so that a standard can then be analyzed to confirm its identity. Once this has been achieved, this new compound can be added to the library to improve its efficacy, reducing the time taken and therefore improving efficiency of the analysis.

Finally, quantitation of components which are of interest or at risk of being toxic was performed. This is possible when using DDA acquisition (MS1 only) however, for the maximum sensitivity and specificity, MRM<sup>HR</sup> was utilized. A small subset of compounds was analyzed, with LOQs down to 0.1 µg/L in solvent and a linear range between 0.1 and 100 µg/L.

Novel aspect: Utilizing three methods of analysis to provide comprehensive characterization of food contact materials for extractable and leachable compounds.

**Keywords:** NIAS, food contact material, extractables & leachables, food packaging

**Acknowledgement:** The authors would like to thank TÜV Rheinland for their collaboration.

### Q15

#### BIO-BASED DISHES: SUSTAINABLE SOLUTION OR HIDDEN HEALTH RISK?

**Kamila Bechynska<sup>(1)</sup>, Vit Kosek<sup>(1)</sup>, Leos Uttl<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>, Emma Bradley<sup>(2)</sup>, Claire McKillen<sup>(2)</sup>, Monika Tomaniova<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>**

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> Fera Science Ltd, United Kingdom

\*Corresponding author - E-mail: hajslovj@vscht.cz

Bio-based tableware is currently used as a green alternative to traditional plastic tableware. Unlike traditional fossil-based polymers, biopolymers are biodegradable or compostable. Among wheat, coconut or corn starch, bamboo has become the most popular raw material due to its short life cycle and good mechanical properties. Although biomaterial tableware is often presented as a 100% natural, the main ingredient is actually a melamine-formaldehyde resin, with bamboo fibre used only as a filler. Since November 2018, the Rapid Alert System for Food and Feed has reported a number of notifications on the occurrence of undeclared additives and non-compliance with EU legislation, and although their number has been decreasing, the problem with this type of product persists. In the presented study, 33 samples of bamboo and other bio-based dishes, including coconut, corn starch, wheat bran, wheat or rice husks, were purchased from stores in the Czech Republic, the United Kingdom, and China. As a first step, non-targeted screening was performed on the prepared 3% aqueous acetic acid, ethanol and isooctane extracts using ultra-high performance liquid chromatography coupled to high-resolution tandem mass spectrometry (UHPLC-HRMS/MS). In addition to many secondary plant metabolites, a number of various contaminants were identified in the extracts. The most frequent was melamine, which was detected in 32% of the samples tested. Other notable contaminants included pesticide residues, phthalates and stabilisers or modifiers used in the production of polymer resins. In subsequent migration tests performed according to the EURL guideline, six non-compliant bamboo products were found to contain melamine exceeding the limits set by Commission Regulation (EC) 2011/10/EU. Melamine was also found to migrate in low concentrations into hot lemon tea and orange juice. Targeted screening of 443 pesticides showed the highest number of residues in cereal-based dishes, when only disinfectants were found in bamboo-based dishes.

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### Q16

#### MIGRATION TESTING OF CROTONIC ACID FROM POLYHYDROXYBUTYRATE BASED FOOD CONTACT MATERIALS BY GC-MS/MS

Uwe Oppermann<sup>(1)</sup>, Franky Puype<sup>\*(2)</sup>

<sup>1)</sup> Shimadzu Europe GmbH, Germany

<sup>2)</sup> ITC, Czech Republic

\*Corresponding author - E-mail: frankypuype@seznam.cz

Recently polymeric food contact articles based on poly(hydroxybutyrate-co-valerate) (PHBV) and polyhydroxybutyrate (PHB) in combination with starch/polylactate appear on the European market. PHB and PHVB polymers are produced by microorganisms/genetically modified bacteria and are basically easy-biodegradable polyesters. In order to check the EU compliance of such products with the Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food one parameter might be quite critical namely the crotonic acid migration. Crotonic acid (FCM substance No. 467, CAS No. 107-93-7) should not migrate into food at a concentration level higher than 0,05 mg/kg (for 6 dm<sup>2</sup>/kg food simulant).

Crotonic acid has been formed during the chain scission reaction of PHBV and PHB and might bring with the direct quantification in migration media broad analytical challenges. Therefore, an analytical derivatization procedure has been developed to stabilize and change the polarity of this target analyte. Migration media like 3% acetic acid, 10% and 95% ethanol were used within this study and crotonic acid has been derivatized to the ethylester using the already presented ethanol (for 10% and 95% ethanol food simulants), or after ethanol addition (3% acetic acid food simulant).

The method required the optimized addition of concentrated BF<sub>3</sub> to the migration solutions and optimized derivatization conditions: 24h static at 60°C. After the derivatization step, water has been added and the target analytes were extracted by n-hexane. A similar internal standard has been chosen to cover the derivatization and extraction efficiencies. The ethyl-derivates were measured by GC-MSMS (Shimadzu TQ8050 NX) achieving compliance evaluation.

**Keywords:** mass spectroscopy, food contact materials



### Q17

#### **ADVANCED SEPARATION OF MINERAL OIL AROMATIC HYDROCARBONS BY NUMBER OF AROMATIC RINGS USING DONOR-ACCEPTOR-COMPLEX CHROMATOGRAPHY TO EXTEND ON-LINE COUPLED LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY**

**Martin Eckardt**<sup>(1)</sup>, **Martin Lommatzsch**<sup>(1)</sup>, **Johanna Holzapfel**<sup>(2)</sup>, **Sebastian Säger**<sup>(1)</sup>, **Thomas Simat**<sup>(2)</sup>

<sup>1)</sup> *Laboratory Lommatzsch & Säger GmbH, Germany*

<sup>2)</sup> *Technical University Dresden, Germany*

*\*Corresponding author - E-mail: eckardt@mosh-moah.de*

An automated implementation for a subfractionation of mineral oil aromatic hydrocarbons (MOAH) into a mono-/di-aromatic fraction (MDAF) and a tri-/poly-aromatic fraction (TPAF) is presented, which is highly demanded by the European Food Safety Authority (EFSA) respecting the genotoxic and carcinogenic potential of MOAH. For this, donor-acceptor-complex chromatography (DACC) was used as a selective stationary phase to extend the conventional instrumental setup for the analysis of mineral oil hydrocarbons via on-line coupled liquid chromatography-gas chromatography-flame ionization detection (LC-GC-FID). A set of six new internal standards was introduced for the verification of the MOAH fractionation and a quantification of MDAF and TPAF, respectively. The automated DACC approach was applied to representative petrochemical references as well as to food samples, such as rice and infant formula, generally showing well conformity with results obtained by state-of-the-art analysis using two-dimensional GC (GCxGC).

The presented approach can be implemented easily in existing LC-GC-FID setup for an automated and advanced screening of MOAH to lower the need for elaborate GCxGC analysis also in routine environments.

**Keywords:** MOSH/MOAH, HPLC-GC-FID, aromatic rings, chromatography

## Q18

### MIGRATION OF CYCLIC SILOXANE OLIGOMERS INTO FOOD - DETERMINATION OF D4 TO D25 VIA ONLINE COUPLED HPLC-GC-FID

**Martin Eckardt**<sup>(1)</sup>, **Sebastian Säger**<sup>(1)</sup>, **Ann-Kathrin Seidel**<sup>(2)</sup>, **Thomas Simat**<sup>(2)</sup>

<sup>1)</sup> *Laboratory Lommatzsch & Säger GmbH, Germany*

<sup>2)</sup> *Technical University Dresden, Germany*

*\*Corresponding author - E-mail: eckardt@mosh-moah.de*

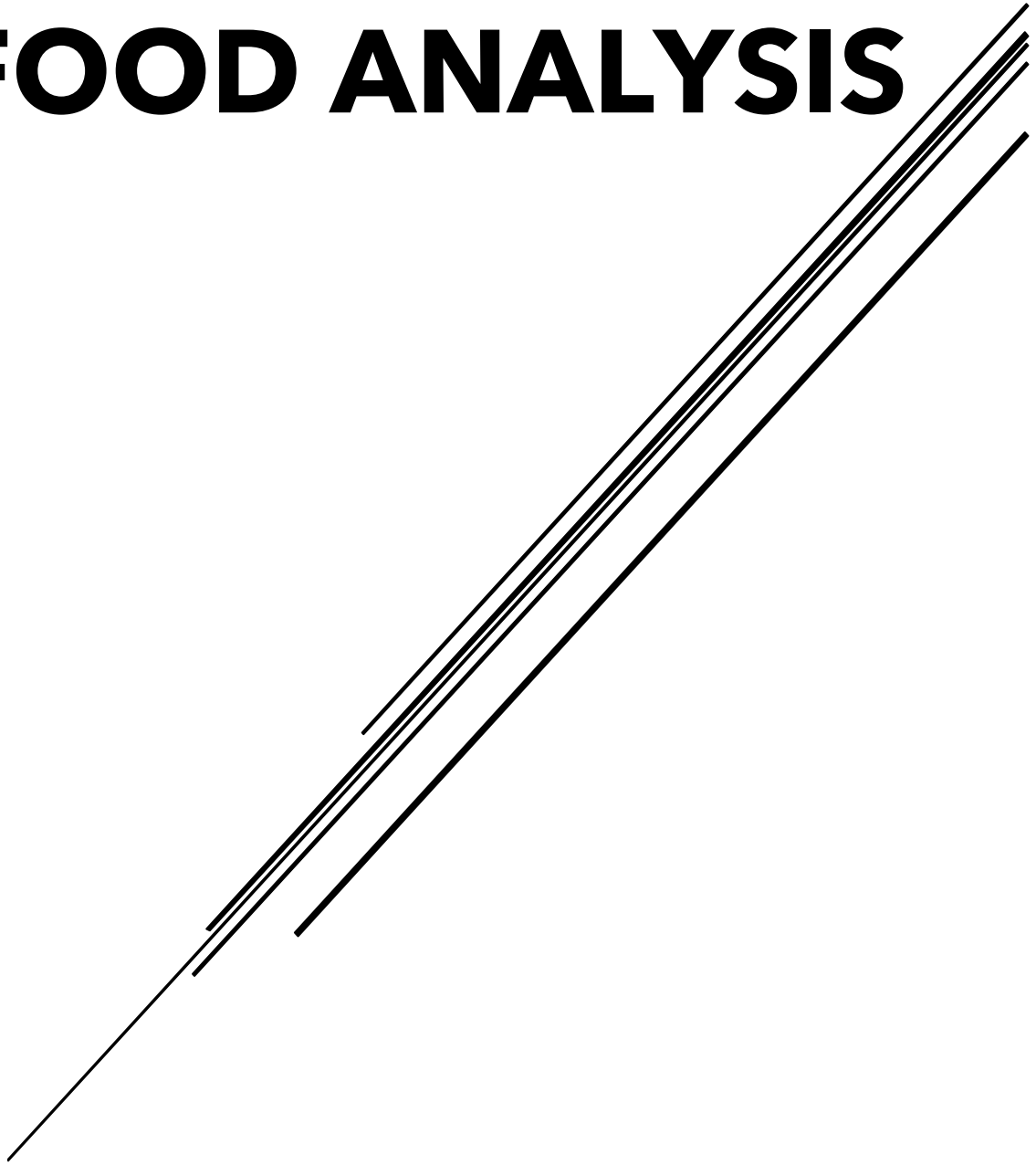
Silicone elastomers made from polydimethylsiloxane are widely used in food contact application, e.g., as multiple-use kitchen utensils. Silicone contains low molecular weight cyclic siloxane oligomers (D<sub>n</sub>), that can migrate into food and potentially endanger human health. To date, little is known about the migration quantity of cyclic siloxanes into real foodstuffs, since suitable analytical techniques have not been available.

This work first time presents an analytical method for a reliable determination of cyclic siloxanes from D4 to D25 in food via on-line coupled liquid chromatography-gas chromatography-flame ionization detection (LC-GC-FID) after solvent extraction from food matrix. Migration examples are provided from high-temperature (baking in muffin molds) as well as moderate-temperature contact experiments (chocolate pouring). The results show, that migration of cyclic siloxanes can occur in relevant amounts up to ~ 360 mg/kg food significantly exceeding overall migration limits of 60 mg/kg food, that are defined as a general criterion of inertness.

The presented method is an essential step forward in terms of exposure evaluation of humans to cyclic siloxanes. It can be a basis for the determination of harmonized specific migration limits for cyclic siloxanes in the future.

**Keywords:** siloxanes, oligomers, HPLC-GC-FID, volatile organic compounds, migration

# **(MULTI)OMICS IN FOOD ANALYSIS**



## R1

### VOLATOLOMICS TO INVESTIGATE THE RESPONSE OF LISTERIA MONOCYTOGENES TO PH CHANGES IN ITS CULTURE MEDIUM

**Aya Fakh\***<sup>(1)</sup>, **Jeremy Rate**<sup>(1)</sup>, **Dimitra Tsourek**<sup>(2)</sup>, **Frederic Mercier**<sup>(3)</sup>, **Nathalie Kondjoyan**<sup>(1)</sup>, **Nelly Caccia**<sup>(4)</sup>, **Cristian Botta**<sup>(2)</sup>, **Kalliopi Rantsiou**<sup>(2)</sup>, **Erwan Engel**<sup>(1)</sup>

<sup>1)</sup> French National Research Institute for Agriculture, Food and Environment (INRAE), UR QuaPA, MASS Group, Saint-Genès-Champanelle, France, France

<sup>2)</sup> Department of Agricultural, Forest and Food Sciences, University of Turin, Turin, Italy

<sup>3)</sup> French National Research Institute for Agriculture, Food and Environment (INRAE), UR QuaPA, MASS Group, France

<sup>4)</sup> INRAE, Université Clermont Auvergne, UMR454 MEDiS, 63000 Clermont-Ferrand, France., France

\*Corresponding author - E-mail: aya.fakh@inrae.fr

Applied to the microbial safety of foods, volatolomics (branch of metabolomics focusing on volatile organic metabolites) might provide key information for characterizing foodborne pathogens and their behaviour in response to stresses whether in pure culture or in ecosystems. Hence, the aim of the present work was to assess its relevance to characterize the response of foodborne pathogen when grown at different pH. Candidate markers of adaptation will be useful in predicting the behaviour of microorganisms under conditions encountered throughout the food chain.

Taking *Listeria monocytogenes* (*L. monocytogenes*) - grown on Tryptic Soy Broth Yeast Extract (TSBYE) - as a model pathogen culture, we implemented a two-step strategy.

The first step aimed to determine the most informative bacterial extract for further volatolomics. Based on case-control experiments (in vitro cultures on synthetic growth medium with *L. monocytogenes*), volatolomics was performed by headspace solid phase microextraction (HS-SPME) hyphenated with gas chromatography and high-resolution mass spectrometry (GC-Q Exactive-Orbitrap MS) on three types of samples: (i) the pellet collected after centrifugation of the whole culture which concentrates bacterial cells; (ii) the supernatant collected after centrifugation of the whole culture, which includes what the bacteria released into the growth medium; (iii) the whole culture, which contains global information diluted by the growth medium, but avoids handling of the bacteria samples required by the centrifugation step. The non-targeted analysis of the volatolome using Compound Discoverer™ software shows different and complementary information among the three types of samples, with 83% of the total information covered only by the whole culture and the pellet. Identification of these markers was then carried out, which revealed several chemical groups present in the volatolome, such as hydrocarbons, aldehydes, alcohols, carboxylic acids, and ketones and as well as other groups that will all be discussed according to the literature.

In a second step, the response of *L. monocytogenes* to different pH of the TSBYE growth medium was studied through volatolomics analysis of pellet and whole culture samples, collected after bacterial culture. Pre-adaptation was performed in a broth model containing citric acid and two low pH values (5.5 and 5.2), and a pH control (7.2). Volatolomics highlighted markers under- or overexpressed by *L. monocytogenes* in response to the different pH conditions. The discussion will focus on the origin of these markers with regard to the literature and on their fate depending on the pH conditions.

**Keywords:** volatolomics, listeria monocytogenes, GC-HRMS, untargeted analysis, stress markers

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## R2

### APPLICATION OF NMR SPECTROSCOPY TO STUDY THE EFFECT OF MALTING PROCESS ON LEGUME SEEDS

**Mattia Spano**<sup>\*(1)</sup>, **Alessio Cimini**<sup>(2)</sup>, **Anna Maria Giusti**<sup>(3)</sup>, **Lorenzo Maria Donini**<sup>(3)</sup>, **Mauro Moresi**<sup>(2)</sup>, **Luisa Mannina**<sup>(1)</sup>

<sup>1)</sup> *Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Italy*

<sup>2)</sup> *Department for Innovation in the Biological, Agrofood and Forestry Systems, University of Tuscia, Italy*

<sup>3)</sup> *Department of Experimental Medicine, Sapienza University of Rome, Italy*

*\*Corresponding author - E-mail: mattia.spano@uniroma1.it*

Malting is a multistep process used to produce barley malt, mainly applies in brewing technology. However, basic aspects of malting can be applied to a wide range of plant seeds, such as legumes. Legume seeds are one of the world's most important sources of nutrients. However, legume seeds such as chickpea, lentil, beans, peas, and soybeans are difficult to process since the long boiling times required, the resistance to mechanical damage, and the high levels of anti-nutritional factors namely phytic acid, tannins, oligosaccharides, enzyme inhibitors. Among the several nutritional and technological advantages of malting process, the reduction of anti-nutritional compounds in favor of nutritional ones is emerging as potentially effective.

In the present work, the effect of malting process on legume seeds produced in Lazio region was observed by both NMR-based untargeted and targeted approaches. Several metabolites belonging to different chemical classes were identified in both hydroalcoholic and organic Bligh-Dyer extracts. Moreover, <sup>31</sup>P NMR experiments were used to identify and quantify phytic acid. The observed reduction of anti-nutritional factors confirmed the efficiency of malting process in improving legume seed nutritional properties.

**Keywords:** legume seeds, NMR metabolomics, malting process

## R3

### NONTARGETED DIRECT HEADSPACE GC-IMS OF MAMMALIAN CELL CULTURES

**Richard Frey**<sup>\*(1)</sup>, **Sarah Winkler**<sup>(1)</sup>, **Rüdiger Rufolf**<sup>(2)</sup>, **Philipp Weller**<sup>(3)</sup>

<sup>1)</sup> University of Applied Sciences Mannheim, Institute for Instrumental Analytics and Bioanalytics, Germany

<sup>2)</sup> University of Applied Sciences Mannheim; Institute for Molecular and Cell Biology, Germany

<sup>3)</sup> University of Applied Sciences Mannheim; Institute for Instrumental Analytics and Bioanalytics, Germany

\*Corresponding author - E-mail: [r.frey@hs-mannheim.de](mailto:r.frey@hs-mannheim.de)

The common approach for volatile profile analysis of cell cultures today is typically based on enrichment by either thermal desorption [1] or solid phase micro extraction (SPME) [2], followed by gas chromatography-mass spectrometry (GC-MS). Although some publications refer to the use of gas chromatography-ion mobility spectrometry (GC-IMS) for volatile organic compound (VOC) analysis, in bacterial cultures [3] or human breath [4], applications of GC-IMS for the characterization of VOCs in mammalian cell cultures are scarce. Therefore, this study presents a novel, enrichment-free method for the analysis of VOCs released from mammalian cell cultures by GC-IMS. The method involves direct headspace sampling from the cell culture flasks, eliminating the need for additional sample preparation steps. This method was applied in a non-target based approach followed by chemometric analysis to discriminate between different confluency levels of CCD-1137Sk and HT-29 cells. The results allowed for a differentiation based on their respective metabolite profiles, that were then correlated with the confluency levels. The prototype system consists of a sample oven, a mass flow controller and a GC-IMS system that samples the headspace portion in the cell culture flask which is subjected to a laminar flow by a constant gas stream and subsequently sampling this gas stream into the sample loop for rapid analysis via GC-IMS. The resulting dataset features a retention time coordinate, a drift-time coordinate and a batch time coordinate. The three-dimensional dataset was pre-processed and subsequent dimensionality reduction and visualization was carried out in Python with the "gc-ims-tools" toolbox [5].

The setup described in this work combined with multivariate analysis could discriminate two cell-cultures at four different confluency levels based upon differences in their volatile profile.

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**Keywords:** GC-IMS, non-target, online headspace monitoring, mammalian cell culture, volatilomics

**Acknowledgement:** This project is funded by DFG (German Research Foundation).

### R4

#### CHEMICAL CHARACTERIZATION OF HEMP SEED (*CANNABIS SATIVA* L.) POLAR METABOLITES THROUGH LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY

**Lorenzo Del Vecchio**<sup>(1)</sup>, **Guillem Campmajo**<sup>(2)</sup>, **Vita Maria Cristiana Moliterni**<sup>(3)</sup>, **Chiara Dall'Asta**<sup>(2)</sup>, **Gianni Galaverna**<sup>(1)</sup>, **Martina Cirlini**<sup>(1)</sup>

<sup>1)</sup> University of Parma, Department of Food and Drug, Parco Area delle Scienze 49/A, Parma 43124, Italy, Italy

<sup>2)</sup> Department of Food and Drug, University of Parma, Italy

<sup>3)</sup> Council for Agricultural Research and Economics Genomics and Bioinformatics Research Centre, Fiorenzuola d'Arda, Italy, Italy

\*Corresponding author - E-mail: [lorenzo.delvecchio@unipr.it](mailto:lorenzo.delvecchio@unipr.it)

*Cannabis sativa*, *sativa* L., also known as "industrial hemp", is considered one of the most versatile crops, and its cultivation has significantly increased worldwide, mainly due to its use in the food, feed, pharmaceutical, and cosmetic sectors, among others.

In comparison to recreational hemp industrial hemp produces lower levels of  $\Delta$ -9 tetrahydrocannabinol ( $\Delta$ -9 THC), with concentrations below 0.3% on a dry weight basis. In recent years, industrial hemp has attracted special interest by a nutraceutical and commercial perspective due to its peculiar composition in several phytochemicals known to be related to human health benefits.

In this line, hemp seeds are rich in several chemical classes, encompassing unsaturated fatty acids, phenylpropionamides (such as lignanamides and cinnamic acid amides), and phenolic compounds, among others, whereas their content in cannabinoids is usually very low and attributed to flower bracts contamination during post-harvesting processing. Therefore, for instance, hemp seeds are used for oil extraction, while the residual seed cake can also be included in foodstuffs in the form of flour.

This study aimed to exploit the potential of liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS) to characterize the metabolomic profile of hemp seed samples. A collection of 24 hemp seed samples (Chemotype III) at different ripening stages (mature and immature) belonging to 18 different EU-registered varieties, have been analyzed.

Polar metabolites were extracted by a solid-liquid extraction (SLE) based on a two-phase separation system, and subsequently detected through LC-TWIMS-HRMS. Then, data evaluation was carried out by both suspect and non-targeted analysis. In the former case, an in-house database containing HRMS and IMS data of metabolites previously reported in *Cannabis sativa* L. plant was used. In the latter case, non-targeted analysis was carried out by the Progenesis Q1 software.

The proposed method allowed a comprehensive characterization of hemp seed samples, due to the tentative annotation and the identification of more than 35 metabolites, mainly covering cannabinoids, phenolic compounds, and phenylpropionamides. Moreover, the metabolomic profile of the immature hemp seeds – lacking an optimal endosperm development with a putative reduction in germinability and/or nutritional value – showed clear differences with the mature ones. These results give an overview of the metabolome composition of different hemp seed samples with phytochemicals variability depending on the variety and ripening stage.

**Keywords:** industrial hemp, hemp seeds, lignanamides, phenolic compounds, high-resolution mass spectrometry

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### R5

#### CHEMICAL CHARACTERIZATION BY LC-HR-MS/MS OF AN INNOVATIVE AND ENRICHED TARALLO PUGLIESE FORTIFIED WITH LEGUME END-PRODUCTS

Antonella Lamonaca<sup>(1)</sup>, Federica De Bellis<sup>(1)</sup>, Anna Luparelli<sup>(1)</sup>, Leonardo Caputo<sup>(1)</sup>, Laura Quintieri<sup>(1)</sup>, Rosa Pilolli<sup>(1)</sup>, Elisabetta De Angelis<sup>(1)</sup>, Linda Monaci\*<sup>(1)</sup>

<sup>1)</sup> Institute of Sciences of Food Production, ISPA- CNR, Italy

\*Corresponding author - E-mail: linda.monaci@ispa.cnr.it

The market of innovative and functional foods has progressively expanded in the last decades with a multitude of innovative and nutritionally valued food products launched on the market. These health promoting foods contain, in addition to nutrients, other components that may be beneficial to the health; they encompass most nutrient-dense foods such as fish, beans, whole grains, and nuts, as well as fortified, enriched foods [1].

Pulses and legumes represent important sources of plant protein, excellent reservoirs of dietary fiber, complex carbohydrates resulting in low GI (glycemic index) and are rich in bioactive compounds, phenolic acids, flavonoids, and tannins [2]. Food processes of pulses often lead to a loss of valuable by-products rich in phenolics and fibres therefore, the current concerns of recycling bio-products disposed from food industries play an important role in the circular economy contributing to valorise and re-utilize end-products that otherwise would increase the production of organic wastes.

In this note the chemical characterization of lentil hulls powder and the production and chemical profiling of the Italian Traditional bakery snack product "Tarallo Pugliese", fortified with lentils hull flour at different inclusion levels, will be presented. In order to identify the antioxidant and beneficial compounds characterizing lentil hull flour also detected in Tarallo Pugliese thus contributing to accrue the overall beneficial properties of this innovative snack, different types of Tarallo Pugliese (prepared using wheat flour type 00 and lentils hull flours at different ratios) were produced upon slight modifications of the traditional Italian Tarallo recipe.

Taralli samples were grinded, extracted and analysed by High Resolution Mass Spectrometry in Full-MS/data dependent-MS MS mode combined with software assisted treatment of final data for correct peak assignment and compound identification. Specific compounds belonging to the polyphenol category known for their beneficial properties on humans including catechin, gallic acid and procyanidin proved to characterize lentils hulls have been detected in Taralli fortified with legume hulls at 20% inclusion level, showing the highest intensity by Mass Spectrometry compared to samples without addition. Further investigations will be directed to assess the sensorial properties of the produced prototype samples.

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**Keywords:** chemical analysis, high Resolution mass spectrometry, bakery products, health promoting foods, legumes bioproducts

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## R6

### COMPUTER VISION HIGHLIGHTS QUALITY TRAITS IN ARTISANAL CHEESE: INVESTIGATING VALCASOTTO CHEESE BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY AND QUANTITATIVE VOLATILOMICS

**Fulvia Trapani<sup>(1)</sup>, Andrea Caratti<sup>(1)</sup>, Angelica Fina<sup>(1)</sup>, Erica Liberto<sup>(1)</sup>, Francesco Ferrero<sup>(2)</sup>, Giorgio Borreani<sup>(2)</sup>, Fabio Bruno<sup>(3)</sup>, Daniele Giaccone<sup>(4)</sup>, Andrea Revello Chion<sup>(4)</sup>, Chiara Cordero<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>2)</sup> *Department of Agricultural, Forest and Food Sciences, University of Turin, Italy*

<sup>3)</sup> *Bepino Ocelli Agrinatura S.r.l, Italy*

<sup>4)</sup> *Associazione Regionale Allevatori Piemonte (ARAP), Italy*

*\*Corresponding author - E-mail: chiara.cordero@unito.it*

Today, assessing the quality of food products is vital for consumer satisfaction and safety. Food quality is a multifaceted concept, influenced by numerous factors, which makes its evaluation complex. Conventional analytical methods often lack the ability to fully provide a comprehensive and objective understanding of the molecular and biochemical complexities underlying food quality, especially in complex matrices like cheese. This study employs advanced analytical techniques combined to Artificial Intelligence tools (i.e., Computer Vision - CV) to highlight molecular interactions along with artisanal cheese ripening in cellars. The research emphasizes the benefits of using comprehensive two-dimensional gas chromatography coupled with parallel detection by mass spectrometry and flame ionization detector (GC×GC-MS/FID) over conventional one-dimensional GC. The superior chromatographic resolution and sensitivity enables accurate quantification of a large set of analytes by exploiting the FID predicted relative response factor concept. Moreover, by image pattern recognition algorithms that track and align features over many patterns, CV could be featured providing a prompt evidence of compositional differences among samples classes. Valcasotto cheese was taken as a reference for artisanal production within the concept of Prodotto Agroalimentare Tradizionale - Traditional Food Product. The sampling, covering the entire production chain, included milk from two farms and harvest seasons (early spring and summer), and corresponding curds further ripened in two different locations (i.e., controlled ambient at 4°C and Valcasotto caves) for 30, 90 and 120 days. By multiple headspace solid phase microextraction (MHS-SPME), we optimized the capture of a broad range of volatiles and semi-volatiles developed along the cheese making process. Moreover, by quantitative volatilomics marker volatiles and impactful odorants - including key-aroma compounds, were precisely tracked across samples facilitating the identification of markers qualifying the unique yet distinctive traits of the artisanal production of Valcasotto. Of the most informative analytes, highlighted by CV on image classes (milk vs. curd vs. T30 vs. T60 vs. T120 days ripened cheese), acetoin, phenylethylalcohol, 1,8-cineole; limonene,  $\gamma$ -terpinene, and terpineol characterize curd volatilome and concur in the aroma traits of this semi-finished product. 1-Octanol, 1-octen-3-ol,  $\delta$ -decalactone, ethyl-3-methyl butanoate, and ethyl hexanoate had meaningful variations at early ripening stages in traditional caves vs. controls. At the latest stage, when Valcasotto cheese is ready for marketing, sulfur derivatives (dimethyl sulfone, dimethyl sulfide, dimethyl sulfoxide), short chain fatty acids (butanoic acid, 2-methyl butanoic, and propionic acid) and methyl ketones formed by  $\beta$ -oxidases (2-nonanone, and 2-heptanone) dominate likely imparting characteristic aroma traits of musty, cabbage, and rancid notes.

**Keywords:** cheese, computer vision, food analysis, comprehensive two-dimensional gas chromatography, mass spectrometry

## R7

### ADVANCING FECAL VOLATILOME PROFILING BY TWO-DIMENSIONAL GAS CHROMATOGRAPHY-TIME OF FLIGHT MASS SPECTROMETRY (GC×GC-TOF MS) AND IMAGE PATTERN RECOGNITION

**Fulvia Trapani<sup>(1)</sup>, Andrea Caratti<sup>(1)</sup>, Erica Liberto<sup>(1)</sup>, Ilario Ferrocino<sup>(2)</sup>, Luca Coccolin<sup>(2)</sup>, Ilaria Goitre<sup>(2)</sup>, Valentina Ponzio<sup>(2)</sup>, Simona Bo<sup>(3)</sup>, Chiara Cordero<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>2)</sup> *Department of Agricultural, Forest and Food Sciences, University of Turin, Italy*

<sup>3)</sup> *Department of Medical Sciences, University of Turin, Italy*

*\*Corresponding author - E-mail: chiara.cordero@unito.it*

Comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOF MS) represents a significant advancement in analytical techniques, offering exceptional resolution, sensitivity and the ability to characterize complex samples with high chemical dimensionality. Unlike one-dimensional gas chromatography methods, GC×GC-TOF MS provides a more precise and detailed fingerprint of volatile organic compounds (VOCs) in a closed range of volatilities/polarities where multiple co-elution might occur. Moreover, thanks to the band-compression in space provided by thermal modulation, a very large dynamic range of concentrations can be covered. This study investigates the potential of GC×GC-TOF MS for the comprehensive analysis of fecal volatile metabolites, focusing on its application in profiling the fecal volatilome, an area that remains underexplored in metabolomics. Fifty individuals with suspected NCGS/WS were subjected to the double blind-placebo-controlled crossover gluten challenge test. Twenty-seven participants were gluten responsive (NCGS) and randomized into two arms: Experimental arm: 6 weeks with daily intake of commercial probiotics (*L. plantarum*, *L. paracasei* and *L. salivarius*) or Control arm: 6 weeks with daily intake of placebo. All participants were undergoing a gluten-free diet for 4 weeks (sampling point T1), after which gluten was reintroduced for 2 weeks (sampling point T2). By applying combined untargeted and targeted fingerprinting based on image pattern recognition on GC×GC-TOF MS data, diagnostic signatures of the diet intervention were highlighted. Over the 830 UT features (*i.e.*, detectable volatilome), consistently aligned across samples 2D patterns, about 200 were putatively identified and correlated to the study variables by both unsupervised and supervised chemometrics. Results confirmed meaningful changes in the fecal volatilome after 4 weeks (T1) of pro-biotic treatment (*vs.* placebo) accompanied by a gluten-free diet consolidated after further 2 weeks of treatment (T2) and normal diet. Partial-Least Squares Discriminant Analysis (PLS-DA) provided good classification models with 89% accuracy at T1 4 weeks and 90% at T2 6 weeks (considering both UT features % Response and Absolute Response). Targeted features with higher information potential resulted butanoic and propanoic acids esters, primary alcohols, aldehydes and several terpenoids. By Computer Vision (CV) image patterns were highlighted and confounding variables minimized between samples classes. Although based on a limited samples set, the comprehensive mapping of fecal volatilome by GC×GC, provides essential information on metabolic changes induced by diet intervention and pro-biotic supplementation supporting personalized nutrition. Moreover, by extending the knowledge on fecal volatilome composition, the complexities of biological systems can be better elucidated by connecting information with other omics (*e.g.*, metagenomics).

**Keywords:** fecal volatilome, GC×GC-TOF MS, computer vision, image patterns, PLS-DA

## R8

### FOOD METABOLOMICS SUPPORTS INDUSTRIAL QUALITY RESEARCH: UNREVEALING COMPOSITIONAL CHANGES IN GERMINATED PEANUTS BY MULTIDIMENSIONAL GAS CHROMATOGRAPHY PLATFORMS

Angelica Fina<sup>(1)</sup>, Andrea Caratti<sup>(2)</sup>, Fulvia Trapani<sup>(1)</sup>, Erica Liberto<sup>(2)</sup>, Irene Cincera<sup>(3)</sup>, Giuseppe Genova<sup>(3)</sup>, Cristina Casetta<sup>(3)</sup>, Carlo Bicchi<sup>(1)</sup>, Chiara Cordero<sup>\*(2)</sup>

<sup>1)</sup> *Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin, Italy*

<sup>2)</sup> *Department of Drug Science and Technology, University of Turin, Italy*

<sup>3)</sup> *Soremartec Italia srl, Italy*

*\*Corresponding author - E-mail: chiara.cordero@unito.it*

Peanuts (*Arachis hypogaea*) are among the most widely consumed legumes worldwide due to their nutritional value, taste, and affordability. The quality of peanuts is typically assessed based on their appearance, texture, flavor, and health benefits. Defining a quality standard for the peanut production chain is complex and requires understanding their chemical composition, stability, and sensory properties.

This study aims to investigate a germination defect known as splitting, where the two cotyledons of the peanut separate. Splitting is associated with early germination, leading to premature metabolic activation, which can negatively affect peanut quality by causing dull flavors or increasing the risk of over-roasting. To comprehensively map the chemical composition of peanuts and assess the impact of germination defects on roasted product quality, the study employs omics approaches.

Various peanut fractions were analyzed to understand the impact of germination defects. The study examined primary metabolites (*i.e.*, free amino acids, organic acids, and sugars) after defatting, extraction, derivatization, and subsequent comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOF MS) profiling [1]. The lipid fraction was analyzed for the presence of esterified and free fatty acids by dedicated lipid extraction, transesterification of the esterified fatty acid fraction, Fisher esterification for the free fatty acid fraction, and GC-FID analysis [2]. Lastly, the volatile fraction was explored using headspace solid-phase microextraction (HS-SPME) followed by GC×GC-TOFMS analysis.

Results indicate that metabolic activation in split seeds leads to higher concentrations of monosaccharides such as mannitol and glucitol, while aroma precursors like valine, threonine, and sucrose are present in lower amounts compared to whole peanuts. Additionally, meaningful differences were also evident in the amount and distribution of lipids depending on the kernel state (whole vs. split). The volatilome confirmed further differentiation between kernel states, with compounds such as 2-pentyl furan and dihydro-3-methyl 2(3H)-furanone showing higher responses in split peanuts.

In summary, this study highlights the significant impact of germination defects on peanut quality, providing valuable insights for defining high-quality peanuts based on chemical and sensory characteristics.

[1] Cialiè Rosso, M., *et al.* Adding extra-dimensions to hazelnuts primary metabolome fingerprinting by comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry featuring tandem ionization: Insights on the aroma potential. *J. Chromatogr. A* 2020, 1614, 1–11, doi:10.1016/j.chroma.2019.460739.

[2] Cialiè Rosso, M., *et al.* Shelf-Life Evolution of the Fatty Acid Fingerprint in High-Quality Hazelnuts (*Corylus avellana* L.) Harvested in Different Geographical Regions. *Foods* 2021, 10, 685, doi:10.3390/foods10030685.

**Keywords:** food metabolomics, high quality peanuts, comprehensive two-dimensional gas chromatography

### R9

#### NON-TARGETED FINGERPRINTING OF THE VOLATILE AND NON-VOLATILE FRACTION OF SICILIAN *COFFEA ARABICA* L. LEAVES

**Daide Rovelli\***<sup>(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Raquel Torrijos<sup>(2)</sup>, Martina Cirlini<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

<sup>2)</sup> University of Valencia, Spain

\*Corresponding author - E-mail: [davide.rovelli@unipr.it](mailto:davide.rovelli@unipr.it)

Climate change poses significant threats to coffee production, affecting the environment's temperature, humidity, and soil nutrients and leading to a projected 50% reduction in global coffee yield by 2050. This impacts small-scale farmers and major coffee-producing countries, necessitating adaptation strategies. Furthermore, climate change is altering the coffee belt, potentially benefiting regions historically unsuitable for coffee production. In this regard, Sicily's shift towards tropical crops, including coffee, highlights the possibility of new production locations and demonstrates how rising temperatures may alter agricultural adaptability. To better understand the chemical composition of Sicilian *Coffea arabica* leaves (cv. Caturra), an in-depth investigation of their non-volatile and volatile fractions was conducted to point out whether significant intra-age and inter-age differences exist. Studying this aspect is crucial to better understand how the plant adapts through a phytochemical response to these new environments. Their characterization can provide useful information for the valorization of coffee by-products, which were once considered waste but could offer economic value due to their content in bioactive compounds.

Therefore, in the present study, non-targeted metabolomics and lipidomics were carried out using liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (UHPLC-TWIMS-QTOF) to the polar and non-polar fraction of coffee leaves' extracts, respectively. Moreover, the evaluation of the volatile profile was performed using the technique of headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS). The metabolomics and lipidomics analyses allowed the tentative annotation of 335 compounds belonging to six main biochemical superclasses (i.e. lipid-like molecules, organic oxygen compounds, phenylpropanoids and polyketides, benzenoids, organic acids and organoheterocyclic compounds) through their proper spectral matching. Regarding the volatile fraction, 76 unique compounds were identified, and an aromatic note was also provided.

The obtained results evidenced a strong heterogeneity accumulation of secondary metabolites and the sample discrimination according to the age of the plant and the leaf. Moreover, the feasibility of a non-targeted approach to explore and deeply understand the phytochemical profile of a by-product that is yet to be fully characterized and comprehend the differences in trend and accumulation. These aspects are functional to highlight how plants and their interactions with external elements and agriculture practices can delineate a new phytochemical profile and unravel their systematic biochemical responses. These findings can also guide both the future supply of these by-products as new raw materials for producing infuses and the discovery of new valuable compounds fostering a circular economy.

**Keywords:** coffee arabica, coffee by-product, secondary metabolites, multiomics, novel food

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## R10

### EXPLORING THE PROTEIN PROFILE OF COFFEE LEAF INFUSIONS: A STUDY ON COFFEE ARABICA L. LEAVES

**Davide Rovelli\***<sup>(1)</sup>, Sara Cutroneo<sup>(1)</sup>, Tullia Tedeschi<sup>(1)</sup>, Chiara Dall'Asta<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

\*Corresponding author - E-mail: [davide.rovelli@unipr.it](mailto:davide.rovelli@unipr.it)

In the coffee sector, by-product recovery can enhance supply chain sustainability and provide additional revenue for farmers. Coffee leaves, for instance, are highly relevant given their known use in coffee-producing countries for hot infusions preparation, the latter of which have already been authorized as novel foods in the European Union (EU). According to EU Regulation, a Novel Food is defined as a food or food ingredient that was not significantly consumed by humans in the EU before May 1997. These innovative foods may access the market after consumer safety is established. Furthermore, an allergenicity risk assessment is required, which considers both the risk of sensitization, and the harm posed to the current allergic population. Specifically, the European Food Safety Authority (EFSA) reported that coffee leaf infuses do not raise safety concerns for the general population. However, current research conducted by the scientific community related to the bioactivity, applications and phytochemical composition of coffee leaves is very scarce compared to that conducted on coffee beans so far. To our knowledge, not much data about the proteomic profile of this coffee by-product and its infuses is available.

The study aimed to investigate the protein profile of the *Coffea arabica* leaves (cv. Geisha) collected from two different regions of the world: Laos and Nicaragua. Hot water extraction was performed following EFSA's reference procedure (55°C for 3 minutes) to obtain the coffee leaf infuses. Additionally, a longer time extraction (10 minutes) was evaluated to determine potential differences in protein profiles.

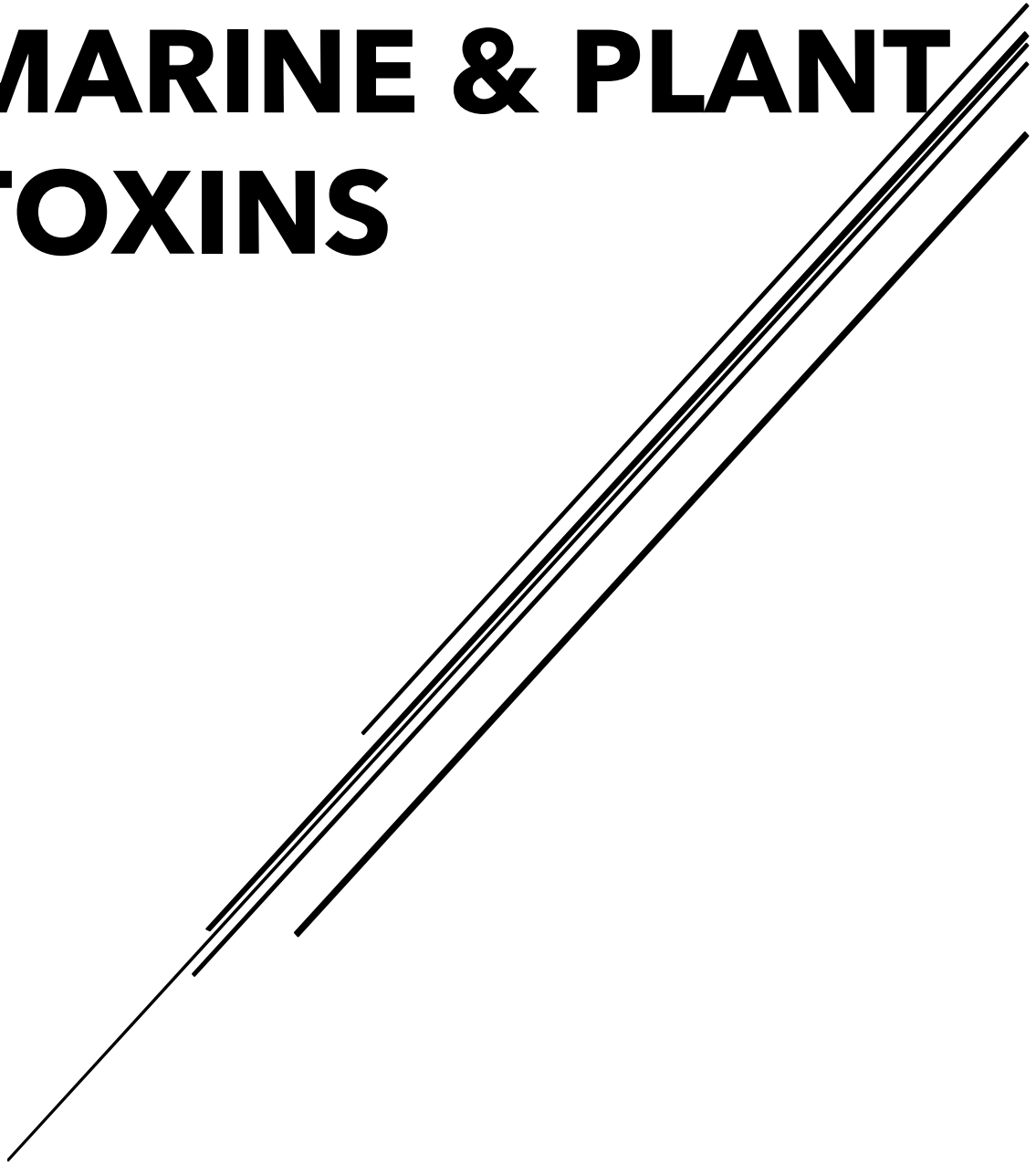
The qualitative protein profile of the samples was obtained by a polyacrylamide gel electrophoresis in reducing conditions (SDS-PAGE) revealing several bands at different molecular weights: 100, 75, 50, 35, 25, and 10 kDa. These bands underwent in-gel tryptic digestion, and the resulting peptides were then subjected to an in-depth investigation employing high resolution mass spectrometry (HRMS) analysis with an UHPLC-LTQ-Orbitrap. The proteins identified with the HRMS analysis were finally compared *in silico* with the known sequences of allergens already identified for green coffee beans, testing their homogeneity.

Preliminary results show the effectiveness of the *in silico* tools to provide a full characterization of the protein profile of coffee leaves. The existing knowledge on green coffee bean proteins was used as a starting point to investigate their presence in coffee leaves considering the strict interaction between leaves and beans during the latter's development. For this reason, the presence of these allergens in coffee leaves, and consequently in the infuses, cannot be completely ruled out. Furthermore, considering the limited data available on the proteomic profile of coffee leaves and their infusions, their evaluation is crucial for their potential use as food or as a botanical product.

**Keywords:** coffee arabica, coffee by-product, novel food, proteomic, leaf infusion

**Acknowledgement:** The authors would like to thank Luigi Lavazza S.p.A. for the supply of plant materials and the support. This work has been carried out in the frame of ALIFAR project, funded by the Italian Ministry of University through the program: Dipartimenti di Eccellenza 2023-2027. Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 1 Investment 3.3 - Call for tender No. 117 of 02/03/2023 of Italian Ministry of University and Research funded by the European Union - NextGenerationEU.

# **MYCOTOXINS, MARINE & PLANT TOXINS**



### S1

#### **DETECTION AND MOLECULAR CHARACTERIZATION OF AFLATOXIN AND OCHRATOXIN PRODUCE ASPERGILLUS SPECIES IN CAPSICUM SPICES IN SAUDI ARABIA**

**Abdulmohsen Aljardan**\*<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: aajardan@sFDA.gov.sa

Capsicum is a common spice used in food flavouring. However, they are prone to mycotoxin contamination. Mycotoxins are natural toxins produced by filamentous fungi and can pose serious risks to human health. Fungal contamination was assessed in 130 randomly collected samples following ISO 21527-2:2008 standards. Results revealed that 84.6% of the samples exceeded the acceptable fungal count limit (102 CFU/g) according to Gulf Cooperation Council (GCC) standards (GSO1016:2015). The predominant fungal isolates were *Aspergillus* (51.1%), notably *Aspergillus flavus* (38.8%) and *Aspergillus niger* (37.7%). Molecular characterization focused on crucial genes associated with aflatoxin (AF) and ochratoxin (OT) biosynthesis, 14.4% of the isolates exhibited all targeted AF genes. The mycotoxin analysis, conducted on 34.6% of samples via liquid chromatography-mass spectrometry (LC-MS), detected AFB1 in 28.8% (0.2-13.8 µg/kg) and OTA in 35.5% (6.87-59.00 µg/kg) of the tested samples. This study demonstrates the need of implementing rules governing the methods of storing, shipping, and packing spices in Saudi Arabia, which may help to minimize the prevalence of toxigenic fungus and mycotoxins. This was the first study in KSA that focused on *Aspergillus* in Capsicum products.

**Keywords:** mycotoxin, aflatoxin, ochratoxin

## S2

### ADVANCES IN MONITORING-CENTERED QUANTIFICATION OF MICROCYSTINS IN FISH MATRIX USING UHPLC-MS/MS

Wannes Hugo R. Van Hassel<sup>\*(1)</sup>, Camille Van Camp<sup>(1)</sup>, Jeroen Van Wichelen<sup>(2)</sup>, Julien Masquelier<sup>(1)</sup>

<sup>1)</sup> *Sciensano, Belgium*

<sup>2)</sup> *Research Institute for Nature and Forest, Belgium*

*\*Corresponding author - E-mail: wannes.vanhassel@sciensano.be*

Fish can potentially accumulate large amounts of cyanotoxins as toxic cyanobacterial blooms are commonly observed in aquatic ecosystems. Moreover, fish consumption has thus the potential to be a human health risk. Suitable and sufficiently robust analytical methods are required to quantify cyanotoxins in a plethora of different fish species. While reporting quantified cyanotoxins in multiple fish species, most studies do not take into account the potential differences of matrix for quantification in fish species during their validation. This study therefore aimed to optimize and validate an UHPLC-MS/MS method for the quantification of eight microcystin congeners and nodularin in the muscle tissue of five different fish species and cod liver. Moreover, the method was applied on multiple fish samples collected from Belgian waters.

Extensive optimizations of the extraction protocol compared to already published methods were necessary before validation of the method was possible. Moreover, elution gradients and the selection of the daughter ions had to be optimized to deal with the matrix effect propagated by the muscle tissues of different fish species.

Eventually, the validation of the method was achieved for the muscle tissue of all fish species and cod liver. A limit of quantification (LOQ) of 5 µg kg<sup>-1</sup> was achieved for MC-LY, MC-LR and MC-LF, while for the five MCs and NOD an LOQ of 10 µg kg<sup>-1</sup> was found.

Multiple microcystin congeners were detected in the liver of perch and sander, up to 88.3 µg/kg sum of analyzed microcystins. Moreover, 6 µg/kg of MC-LR was found in perch muscle tissue, while MC-RR was also detected.

**Keywords:** microcystin, fish, food safety, public health

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### S3

#### DETERMINATION OF ERGOT ALKALOIDS: COMPARISON OF EXTRACTION EFFICIENCIES IN SEVERAL CEREAL PRODUCTION CHAINS BY UPLC-MS/MS

**Eleonora Rollo**<sup>(1)</sup>, **Dante Cattellani**<sup>(2)</sup>, **Chiara Dall'Asta**<sup>(3)</sup>, **Nicola Dreolin**<sup>(4)</sup>, **Michele Suman**<sup>\*(5)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Viale delle Scienze 27/A-43124 Parma, Italy & Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Via Mantova 166-43121 Parma., Italy

<sup>2)</sup> Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Via Mantova 166-43122 Parma Italy

<sup>3)</sup> Department of Food and Drug, University of Parma, Italy

<sup>4)</sup> Waters Corporation, United Kingdom

<sup>5)</sup> Analytical Food Science, Barilla G. e R. Fratelli S.p.A., Via Mantova 166-43121 Parma, Italy & Department for Sustainable Food Process, Catholic University Sacred Heart, Piacenza., Italy

\*Corresponding author - E-mail: michele.suman@barilla.com

To date, there are more than 80 ergot alkaloids identified, their distribution depends on different factors (e.g. geographic regions, host plants etc.) and, dependent on the frequency and concentration ingested and the frequency of ingestion, they can cause acute and chronic toxic effect on human health. These toxins commonly infect cereal crops such as triticale and rye, and also wheat, barley and oats. In this regard, considering the growing consumption of plant-based foods compared to food of animal origin, the European Food Safety Authority, has highlighted the need to develop risk assessment strategies.

For this reason, the emphasis has been placed on the optimization of extraction efficiency, to quantify the main ergot alkaloids and their epimers, that are now available on the market (ergosine, ergocristine, ergocriptine, ergocornine, ergosinine, ergocristinine, ergocriptinine and ergocorninine).

The effectiveness of three different extraction conditions (acidic, alkaline and neutral) followed by a rapid clean-up using dispersive solid-phase extraction with C<sub>18</sub> sorbent was evaluated by ultra performance liquid chromatography tandem quadrupole mass spectrometry (UPLC-MS/MS), resulting in a short chromatographic run (16 min). The method was developed and validated in five different cereal production chains (rye, oat, wheat, wheat gluten and baby food). The efficiency and efficacy of extraction methods were evaluated and compared as well as obtained recoveries. The verified linear range was 0.5 - 500 µg kg<sup>-1</sup> for all the tested compounds, according to the legal limits. The limits of quantification were dependent on the analyte but almost independent from the matrices. Recovery values for the 8 ergot alkaloids spiked at levels of 2, 20 and 100 µg kg<sup>-1</sup> were calculated for each matrix extracted with its best extraction protocol and were completely satisfactory. The applicability and the trueness of the method were examined by analysing a set of 54 samples, including also other cereals like spelt, tritordeum and triticale, and evaluating some reference materials.

**Keywords:** ergot alkaloids, extraction procedures, liquid chromatography tandem quadrupole-mass spectrometry, cereals, baby food

**Acknowledgement:** NextGenerationEU - National Resistance and Resilience Plan (PNRR) - Mission 4 Component 2 Investment 1.3 - Notice No. 341 of 15 March 2022 of the Ministry of Universities and Research; Project Code PE00000003, MUR Directive Decree No. 1550 of 11 October 2022 granting of funding, CUP D93C22000890001, Project Title ON Foods - Research and innovation network on nutrition and nutrition Sustainability, Safety - Working ON Foods

## S4

### READY, SET, DETECT: SIMPLIFYING MYCOTOXIN ANALYSIS FOR THE FOOD INDUSTRY

Peter Carl<sup>\*(1)</sup>, Timm Schwaar<sup>(1)</sup>

<sup>1)</sup> SAFIA Technologies GmbH, Germany

\*Corresponding author - E-mail: carl@safia.tech

In the context of accelerating climate change, the risk of food contamination by dangerous mycotoxins—toxins produced by molds—increases significantly.[1] Traditionally, the detection of mycotoxins relies on chromatographic methods combined with mass spectrometry, which, while capable of detecting multiple toxins simultaneously, are cost-intensive and require highly qualified personnel. Rapid tests, in contrast, offer a cost-effective and user-friendly alternative but are typically limited to detecting only a single toxin.

The SAFIA Rapid Test System bridges this gap by integrating the advantages of both methods without their respective disadvantages. The SAFIA multiplex immunoassay can detect all regulated mycotoxins, including aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A, deoxynivalenol, T-2 toxin, and zearalenone at levels well below EU regulatory limits.

The SAFIA system includes an analysis kit, user-friendly software for data analysis, and a flow cytometer specifically designed for industrial applications. At its core is a particle-based competitive multiplexing immunoassay technology, initially adapted for environmental pollutant detection.[2] This system uses engineered microparticles featuring a fluorescent dye core for analyte coding, an inorganic protective shell, and surface modifications for antibody binding and anti-fouling properties. High-selectivity antibodies enable competitive binding to analytes, with dual fluorescence signals read by a flow cytometer.

Unique to the SAFIA system are its internal controls that mitigate false positives, a common issue in conventional immunoassays. Additionally, SAFIA operates in a wash-free mix-and-read manner, facilitating rapid and straightforward analysis in a 96-well plate format for high-throughput screening or a simplified tube format for on-site testing.

Validation against diverse food matrices demonstrates the robust performance of the SAFIA system, with minimal interference observed in most samples and excellent recovery rates (70-120%) and precision (mean CV < 13%) across all analytes. In summary, the SAFIA Mycotoxin System offers a streamlined solution for the rapid detection of mycotoxins in foodstuffs, promising to reduce product recalls, safeguard public health, and alleviate financial burdens on affected industries.

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[2] Carl, P.; Sarma, D.; Gregório, B. J. R.; Hoffmann, K.; Lehmann, A; Rurack, K.; Schneider, R. J., *Anal. Chem.* 2019, 91 (20), 12988-12996. doi: 10.1021/acs.analchem.9b03040.

**Keywords:** rapid test, multiplex immunoassay, internal control, mix-and-read assay, high-throughput screening

### S5

#### QUALITY ASSESSMENT AND REGULATORY IMPLICATIONS OF LIQUID MYCOTOXIN REFERENCE STANDARDS UNDER ISO 17034: ENSURING RELIABILITY AND CONSISTENCY

**David Steiner**<sup>\*<sup>(1)</sup></sup>, **Tibor Barók**<sup>(2)</sup>, **Michael Sulyok**<sup>(3)</sup>, **Andras Szekeres**<sup>(4)</sup>, **Monika Varga**<sup>(4)</sup>, **Levente Horvath**<sup>(2)</sup>, **Helmut Rost**<sup>(1)</sup>

<sup>1)</sup> LVA GmbH, Austria

<sup>2)</sup> Fumizol Ltd., Hungary

<sup>3)</sup> University of Natural Resources and Life Sciences, Vienna, Department of Agrobiotechnology IFA-Tulln, Institute of Bioanalytics and Agro-Metabolomics, Austria

<sup>4)</sup> University of Szeged, Department of Microbiology, Hungary

\*Corresponding author - E-mail: david.steiner@lva.at

Mycotoxins, toxic secondary metabolites produced by fungi, pose significant health risks to humans and animals through contaminated food and feed. Accurate measurement and monitoring of mycotoxins are crucial for food safety, which necessitates reliable reference standards. ISO 17034 provides guidelines for the production of reference materials, ensuring their quality and consistency. This study provides a comprehensive examination of liquid mycotoxin reference standards, focusing on the quality and consistency of these standards across various manufacturers. A total of 30 reference standards were tested, each comprising 10 samples of three key mycotoxins: Aflatoxin B1 (AFB1), Deoxynivalenol (DON), and Zearalenone (ZON). These standards were sourced from 10 leading global manufacturers. To ensure comparability, all standards were adjusted to the same concentration level.

The quality attributes of the standards were assessed using LC-MS/MS, HPLC-DAD, and LC-HRMS techniques. The study results indicated that 30% of the suppliers provided reference standards that fell outside the acceptable limits, as confirmed by both LC-MS/MS and HPLC-DAD methods. Additionally, impurities were detected across all standards.

The findings highlight the need for adjustments to the ISO 17034 standard, particularly regarding the determination of raw material purity. It is recommended that purity should be uniformly assessed using quantitative Nuclear Magnetic Resonance (q-NMR) analysis, as opposed to the currently used HPLC-UV or LC-MS/MS methods stated by most manufacturers. For liquid standards with a shelf life of  $\leq 1$  year, the total uncertainty should not exceed 3%, while those with a longer shelf life should maintain an uncertainty of no more than 5%.

Furthermore, the study underscores the importance of long-term stability monitoring for these standards. Without continuous monitoring, products may experience significant degradation, exemplified by an observed target value of only 80% in one instance. It is proposed that the results of q-NMR analysis be included in the certificates of every released batch to ensure transparency and reliability.

**Keywords:** stability, mycotoxins, impurity, certificate of analysis

### S6

#### TARGET AND SUSPECT SCREENING ANALYSIS, OCCURRENCE AND RISK ASSESSMENT OF PYRROLIZINE ALKALOIDS IN HONEY SAMPLES FROM DIFFERENT FLORAL ORIGINS

Laura Carbonell-Rozas<sup>\* (1)</sup>, Paula Soto-Rosina<sup>(1)</sup>, Jose Raul Belmonte-Sanchez<sup>(1)</sup>, Roberto Romero-González<sup>(1)</sup>, Antonia Garrido French<sup>(1)</sup>

<sup>1)</sup> University of Almería, Spain

<sup>\*</sup>Corresponding author - E-mail: lauracr@ual.es

Due to the harmful effects of pyrrolizidine alkaloids (PAs) on humans and animals, their control in food matrices is crucial for ensuring food safety. PAs occur in a vast number of plant species with approximately 660 PAs identified to date. Although many PA-containing plants are not directly consumed by humans, they can inadvertently contaminate food products through honeybees' activities, leading to the presence of PAs in honey and pollen. Given the potential transferability of PAs to these samples, investigating their occurrence in honey samples is essential.

In this study, 80 honey samples from different floral origins (lavender, heather, orange blossom, oak, eucalyptus, thyme and wildflower) were analysed to assess the occurrence of PAs. Two approaches were considered for this purpose. A target analysis of the 35 regulated PAs using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) by multiple reaction monitoring (MRM) mode, and a suspect screening for other structurally related PAs using LC-Orbitrap high-resolution mass spectrometry (HRMS), utilizing both public and in-house built libraries. In both cases, a liquid-liquid extraction (LLE) using acidified water followed by a selective solid-phase extraction (SPE) with cation-exchange cartridges (MCX) were used for the extraction and purification of PAs. From the target analysis, 61% samples were found positives with at least one PA. Indicine + lycopsamine were the most frequently detected PAs (appearing in a 40% of the analysed samples) while an eucalyptus honey presented the highest PA contamination, with 13.3 µg/kg (expressed as the sum of the 35 PAs). From the suspect screening, other PAs such as erucifoline were tentatively identified using the spectral information from the libraries and the filters applied in the analysis workflow. However, some false negatives were observed compared with the target analysis, and the identification of certain isomers was not unequivocal. Different chemometric analyses, such as Variable Influence on Projection (VIP) for Partial Least Squares Discriminant Analysis (PLS-DA) and Orthogonal PLS-DA (OPLS-DA), were employed to evaluate the relationship between the presence of certain PAs and the honey type. Finally, the exposure assessment and risk characterization were calculated as recommended by the European Food Safety Authority (EFSA) [1].

Overall, the combination of target and suspect screening methodologies provides a comprehensive and holistic determination of PAs in honey samples. Further research is still needed to evaluate the potential contamination of these samples and their harmful impact on both humans and honeybees, which may prompt policymakers to consider regulating PAs in honey.

[1] EFSA. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA Journal, 17 (3) (2019).

**Keywords:** pyrrolizidine alkaloids, honey samples, food safety, risk assessment

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### S7

#### EFFECT OF AFLASAFE® TZ01 IN AFLATOXINS REDUCTION AND ITS EMERGING CHALLENGES WITH FUSARIUM MYCOTOXINS IN MAIZE

**Sambwe Fundikira**<sup>(1)</sup>, Sarah De Saeger<sup>(1)</sup>, Rashid Suleiman<sup>(2)</sup>, Marthe De Boevre<sup>(1)</sup>, Kokeb Tesfamariam<sup>(1)</sup>, Martin Kimanya<sup>(3)</sup>

<sup>1)</sup> GHENT UNIVERSITY, Belgium

<sup>2)</sup> Sokoine University Of Agriculture, Tanzania

<sup>3)</sup> The Nelson Mandela African Institution of Science and Technology (NM-AIST), Tanzania

\*Corresponding author - E-mail: sambwe.fundikira@Ugent.be

Reducing aflatoxins contamination in crops is crucial for ensuring food safety and agricultural sustainability. Use of atoxigenic *Aspergillus flavus* strains have been used across the globe as a pre-harvest biocontrol strategy to outcompete toxigenic strains and reduce aflatoxin levels. This study comprehensively investigates the impact of Aflasafe® application in Tanzania on the concentrations of multiple mycotoxins in maize fields, contrasting treated and untreated conditions. The investigated mycotoxins include aflatoxins (AFB1, AFB2, AFG1, AFB2), nivalenol (NIV), deoxynivalenol (DON), neosolaniol (NEO), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON), fumonisins (FB1, FB2, FB3) and screening 31 *Aspergillus flavus* metabolites. A total of 158 (79 treated and 79 untreated) maize samples were collected from small holder maize farmers in Chemba and Kiteto districts in Tanzania. Multi mycotoxins analysis was performed using Liquid chromatography coupled with mass spectrometry (LC-MS/MS). Non-parametric paired t-test analysis was conducted to compare the mean difference in the concentration of mycotoxins between the control and treatment groups. Our findings have shown significant reductions in aflatoxin levels. The statistical significance of biocontrol effects was assessed through p-values. Our findings reveal significant reductions in aflatoxin levels due to Aflasafe® application, with mean reductions observed for AFB1 (60.71%, p=0.0276), AFB2 (95.91%, p=0.0111), AFG1 (91.56%, p=0.0140), and AFG2 (62.59%, p=0.0431). Additionally, significant decreases were noted for 3-AcDON (p=0.0451) and ROQ C (p=0.0001). Conversely, notable increases in certain *Fusarium* mycotoxins were observed, including DON (1143.18%, p=0.1921), NIV (55.40%, p=0.4214), and NEO (7180.49%, p=0.1315), suggesting potential ecological shifts favouring *Fusarium* species post-treatment. Amongst the *Aspergillus flavus* metabolites Aflatrem, Liporin C, Versiconical Hem AC and Paspalinine showed the occurrence in all samples. This underscores the efficacy of Aflasafe® in reducing aflatoxin contamination while highlighting the necessity for integrated management strategies to address the complex dynamics of *Fusarium* mycotoxins. Our study not only supports the strategic use of biocontrol agents like Aflasafe® but also emphasizes the need for continuous monitoring and adaptive management practices to ensure comprehensive food safety.

**Keywords:** Aflasafe®, atoxigenic *Aspergillus flavus*, *Fusarium*, food safety, *Aspergillus flavus* metabolites

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S8

### IN VITRO SYSTEM TO EXPLORE THE ABILITY OF NEW PERENNIAL GRAINS TO BIOTRANSFORM DEOXYNIVALENOL

**Marco Gozzi**<sup>(1)</sup>, **Maria Cavallero**<sup>(2)</sup>, **Enrico Rolli**<sup>(2)</sup>, **Chiara Dall'Asta**<sup>(1)</sup>, **Laura Gazza**<sup>(3)</sup>, **Gianni Galaverna**<sup>(1)</sup>

<sup>1)</sup> Department of Food and Drug, University of Parma, Italy

<sup>2)</sup> Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy

<sup>3)</sup> CREA Research Centre for Engineering and Agro-Food Processing, Italy

\*Corresponding author - E-mail: marco.gozzi@unipr.it

The impact of ongoing climate change and its associated events such as droughts, floods, and emerging pests, is altering biological and ecological dynamics strongly affecting agricultural systems. On one hand, the latter are required to become more sustainable, as a part of mitigation strategies within the framework of the European Green Deal, on the other hand, there is a need to find multiple approaches that allow agricultural systems to adapt to the challenges posed by climate change. Among the different strategies explored, perennial crops such as New Perennial Grains (NPGs), are emerging as a valuable tool because of their ability to regrow year after year, thus reducing the agricultural inputs required and the resulting environmental impact. NPG lines were created by crossing hexaploid or tetraploid wheat with *Thinopyrum* wheatgrasses to introduce perennial traits to bread and durum wheat. As NPGs are characterized by perennial and extensive root system, they contribute to soil health by increasing soil organic matter and biodiversity, reducing soil erosion as well. In addition, NPGs are able to access water and nutrients stored deeper into the soil compared to annual crops, making them potentially more resilient to abiotic stresses such as drought and heat waves. As climate change also impacts the safety and quality of cereal products influencing the development of plant diseases and the occurrence of mycotoxins as well, further investigations are needed to understand better the ability of NPGs to cope with these biotic stresses, against which plants have developed different defense strategies. Deoxynivalenol (DON) is one of the most common mycotoxins produced by *Fusarium* spp., and it is well-known to contaminate wheat and other cereals. The ability to biotransform DON into its glucoside form deoxynivalenol-3-glucoside (DON3Glc) was reported as a primary plant mechanism for resistance towards DON accumulation. Therefore, in order to explore this specific element of plant resistance, four different NPG lines (OK72, 20238, 235A and 11955) were cultured in *in vitro* controlled conditions and compared with an annual modern bread wheat variety (*Triticum aestivum* L. cv. Bologna) and the perennial wheatgrass species *Thinopyrum intermedium* (Host) Barkworth and D.R. Dewey. Preliminary results from UHPLC-MS/MS analysis showed differences in biotransformation capacity between the tested perennial wheat lines, even if, none of them appear to hold a stronger biotransformation ability compared to the annual variety tested.

**Keywords:** new perennial grains, deoxynivalenol, biotransformation

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## S9

### OPTIMIZATION AND VALIDATION OF A MULTI-MYCOTOXIN ANALYTICAL METHOD IN PLANT-BASED BEVERAGES APPLYING SALLE EXTRACTION COMBINED WITH UHPLC-MS/MS

**Raquel Torrijos**\*<sup>(1)</sup>, **Octavian Augustin Mihalache**<sup>(2)</sup>, **Chiara Dall'Asta**<sup>(2)</sup>

<sup>1)</sup> *Department of Food and Drug, University of Parma, & Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia., Italy*

<sup>2)</sup> *Department of Food and Drug, University of Parma, Italy*

\*Corresponding author - E-mail: Raquel.Torrijos@uv.es

The market for plant-based beverages (PBBs) has experienced significant growth in the past years due to a change in consumer habits associated with switching to alternative diets, ethical concerns, and health issues such as lactose intolerance and allergies to fresh milk. PBBs can be manufactured using different ingredients, mainly cereals, nuts, and legumes. However, the raw materials used can be contaminated with mycotoxins and, thus, transferred to the final product. In addition, a regulatory framework for mycotoxins in PBBs is currently missing, and no regulation has been established for legumes or their derived products. For this reason, new analytical procedures are necessary to address the incidence of mycotoxins in these commodities.

The objective of the work was to optimize an analytical method based on salting-out assisted liquid-liquid extraction (SALLE) followed by ultra-high-performance liquid chromatography coupled to mass spectrometry in tandem (UHPLC-MS/MS) for the simultaneous determination of regulated and emerging mycotoxins in PBBs. The extraction was optimized for the following analytes: aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>), ochratoxin A (OTA), fumonisins (FB<sub>1</sub>, FB<sub>2</sub>), HT-2, T-2, zearalenone (ZEN), deoxynivalenol (DON), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), enniatins (ENNB, ENNB<sub>1</sub>, ENNA, ENNA<sub>1</sub>), and beauvericin (BEA). Several parameters influencing the effectiveness of the extraction were investigated, such as the solvent used (type, pH, and volume) and the proportion of salts necessary to allow the salting-out effect. Better extraction efficiencies were found when 3 mL of acidified acetonitrile with 1.5% formic acid was used as extractant, and 2 g of magnesium sulfate (MgSO<sub>4</sub>) was added as salt. Then, the method proposed was extended to other PBBs, mainly almond-based, soy-based, and rice-based.

The method's fitness-for-purpose was evaluated in the four typologies of PBBs in terms of linearity, limits of detection (LOD), limits of quantification (LOQ), accuracy, precision, and matrix effect. Satisfactory performance characteristics were obtained, with recovery values >70.5% and intra-day/inter-day precision 80%, mainly emerging mycotoxins produced by *Fusarium* genera (ENNB, ENNB<sub>1</sub>, ENNA, ENNA<sub>1</sub>, BEA), and by *Alternaria* genera (AME, TEN). Moreover, co-occurrence of mycotoxins was frequently observed, suggesting that more monitoring studies should be performed to assess the potential risk of exposure for the consumers.

**Keywords:** plant-based beverage, mycotoxin, SALLE, UHPLC-MS/MS

**Acknowledgement:** *This work has been carried out in the frame of the ALIFAR project, funded by the Italian Ministry of University through the program 'Dipartimenti di Eccellenza 2023-2027'; the project "Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods (ONFOODS) (Project code PE0000003, Concession Decree No. 1550 of 11/10/2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001) and funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15/03/2022 of Italian Ministry of University and Research funded by the European Union - NextGenerationEU; and the FunShield4Med project (European Union's Horizon Europe Research and Innovation Program Gran Agreement No 202079173). R.T. would like to thank the Conselleria de Educaci3n, Universidades y Empleo of Generalitat Valenciana, for the APOSTD grant (CIAPOS/2022/015), funded by the European Social Fund.*

## S10

### SYNTHESIS AND APPLICATION OF STABLE ISOTOPICALLY LABELED ERGOT ALKALOIDS

**Sven-Oliver Herter**<sup>(1)</sup>, **Hajo Haase**<sup>(2)</sup>, **Matthias Koch**<sup>(1)</sup>

<sup>1)</sup> *Bundesanstalt für Materialforschung und -prüfung, Germany*

<sup>2)</sup> *Technische Universität Berlin, Germany*

\*Corresponding author - E-mail: [Sven-Oliver.Herter@bam.de](mailto:Sven-Oliver.Herter@bam.de)

Ergot alkaloids form a toxicologically relevant group of mold toxins (mycotoxins) that are among the most common contaminants of food and animal feed worldwide. Therefore, reliable controls are indispensable for the minimization of both health risks and economic damages. As a consequence of their toxicological significance, EU limit values for 12 priority ergot alkaloids were established for the first time in 2022. These limits range from 500 µg/kg in rye milling products down to 20 µg/kg in processed cereal-based foods for infants and young children [1]. Despite the use of high-performance liquid chromatography-mass spectrometry to quantify low concentrations of ergots in food, the current European standard procedure EN 17425:2021 [2] cannot be fully applied due to the lack of isotopically labeled reference standards.

The complex structure of the ergot alkaloids makes a total synthesis extremely challenging, expensive, and time-consuming. Consequently, we focused on a semi-synthetic approach to specifically demethylate the *N*<sup>6</sup>-atom of the lysergic acid moiety, a shared structural feature among all ergot alkaloids. This resulted in the formation of a norergot alkaloid, which was purified using preparative HPLC. The reaction of the norergot alkaloid with an isotopically labeled electrophilic methyl source, such as iodomethane or dimethyl sulfate, yielded the desired isotopically labeled ergot alkaloid. This methodology enabled the successful synthesis of all 12 stable isotopically labeled priority ergot alkaloids for the first time. Herein we present the problem of the current unavailability of these isotopically labeled standards and our approach to solve this urged demand. Moreover, we are able to present initial data on how these standards enhance the European standard procedure, EN 17425:2021.

[1] Commission Regulation (EC) No 2021/1399 of 24 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs. 2021: Official Journal of the European Union.

[2] Determination of ergot alkaloids in cereals and cereal products by dSPE clean-up and HPLC-MS/MS; EN 17425 2021.

**Keywords:** mycotoxin, ergot alkaloids, isotopic labeled standards, HPLC-MS/MS



## S11

### STUDY OF THE OPTIMIZED CHEMICAL HYDROLYSIS CONDITIONS TO CONVERT GC4 AND GC5 INTO THEIR DECARBAMOYL ANALOGUES DCGTX1 AND DCGTX4

Veronica Rey<sup>(1)</sup>, Ana Botana\*<sup>(1)</sup>, Luis Botana<sup>(1)</sup>

<sup>1)</sup> University of Santiago de Compostela, Spain

\*Corresponding author - E-mail: anamaria.botana@usc.es

PSTs are potent marine neurotoxins produced by dinoflagellates, including species from the genera Alexandrium, Gymnodinium, and Pyrodinium. Traditional detection methods like the mouse bioassay have been replaced in the EU by chemical methodologies, specifically ultrahigh-performance liquid chromatography with fluorescence detection (HPLC-FLD). However, detecting GC toxins remains challenging due to their retention during C18 solid-phase extraction (SPE) procedures, and the lack of commercially available standards complicates their quantification.

The study aims to establish an improved method for converting GC4 and GC5 to their decarbamoyl analogues, dcGTX1 and dcGTX4, under optimized hydrolysis conditions, considering factors such as pH, temperature, and reaction time.

Reaction temperature, time, and NaOH volume were crucial parameters.

Optimal conversion was achieved at 10°C for 15 hours with 200 µL NaOH.

Higher temperatures were less effective for dcGTX1 and dcGTX4 but beneficial for other analogues like dcGTX2 and dcGTX3.

The study confirmed the conversion of GC4 to dcGTX1 and GC5 to dcGTX4 with high yield under optimized conditions.

The fluorescence signal was used to measure the conversion rates, with higher signals indicating better conversion efficiency.

The chemical hydrolysis conditions to convert GC4 and GC5 into their decarbamoyl analogues dcGTX1 and dcGTX4 were successful. This method addresses the challenge of detecting GC toxins in shellfish and provides a reliable means for their quantification. The optimized conditions ensure high yields and could potentially facilitate the development of commercially available standards for these marine biotoxins.

**Keywords:** chemical hydrolysis, GC4 toxin, GC5 toxin

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## S12

### VALIDATION OF MULTI-ANALYTE METHODS FOR THE DETERMINATION OF QUINOLIZIDINE ALKALOIDS IN FOOD AND FEED WITH LC-MS/MS - RESULTS OF A COLLABORATIVE METHOD VALIDATION STUDY

**Carolin Hoyer**\*<sup>(1)</sup>, Yannick Seele<sup>(1)</sup>, Annika I. Ostermann<sup>(1)</sup>, Oliver Kappenstein<sup>(1)</sup>

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: carolin.hoyer@bfr.bund.de

Quinolizidine alkaloids (QA) are toxic secondary plant metabolites found in lupine species (*Lupinus* spp.) that offer protection against competing plants, herbivores, insects or pathogens. The QA containing seeds of certain lupine species (*L. albus*, *L. angustifolius*, *L. luteus*) are used for food and feed production (e. g. flour, grist) and are gaining increasing attention as ingredient in substitute food products (e. g. lupine-based coffee, lupine-based meat substitute, lupine drink). Furthermore, the transfer of QAs from lupine containing feed into cow milk was shown to occur. QAs are toxicologically relevant for animals and humans. Until now, maximum levels for QA in food and feed are not specified by European legislation. In order to protect health of animals and humans and to set maximum levels in the future, more comprehensive data about the occurrence of QA in food and feed is necessary. For this and for future monitoring, reliable and harmonized analytical methods are required.

Therefore, following the BfR Opinion on 'Risk assessment of the occurrence of alkaloids in lupin seeds' from 2017 and EFSA's 'Scientific opinion on the risk for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products' from 2019, the BfR developed two analytical methods to determine 11 QA in (1) food and feed with low water content and (2) food with higher water content. After solid-liquid extraction (1) or liquid-liquid extraction (2), protein precipitation as well as defatting, sample extracts are analysed by reversed-phase high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

In an interlaboratory study with 18 laboratories from different European states, the performance characteristics of both multi-analyte method protocols were assessed. The study was designed as a three-step approach: one training part for each of the analytical methods and one main validation part. The validation part was designed as a double-blind study with five ground samples of lupine seed-based feed and food with a low water content (lupine coffee, lupine meal, lupine slices, lupine mixture, lupine feed) and four samples of food with higher water content (milk, yogurt, lupine drink, cream). The assessed within-laboratory precision (relative standard deviation for repeatability, RSDr) was < 20 % for all analyte-matrix combinations. With a few exceptions, the assessed between-laboratory precision (relative standard deviation for reproducibility, RSDR) was < 25 %. The poster presentation will focus on the results of the collaborative method validation study.

**Keywords:** quinolizidine alkaloids, food and feed, LC-MS/MS, collaborative method validation study

**Acknowledgement:** All participating laboratories are kindly acknowledged for their contribution to the method validation study.

S13

### COMPREHENSIVE ANALYSIS AND TARGET QUANTIFICATION OF MYCOTOXINS IN FRUIT JUICE SAMPLES USING ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH HIGH-RESOLUTION MASS SPECTROMETRY

**Antonio Paolillo\***<sup>(1)</sup>, Ivana Ledenko<sup>(1)</sup>, Lidia Ciriaco<sup>(1)</sup>, Vincenzo Armini<sup>(1)</sup>, Sonia Lombardi<sup>(1)</sup>, Luana Izzo<sup>(1)</sup>

<sup>1)</sup> FoodLab, Department of Pharmacy, University of Naples "Federico II", Via Domenico Montesano, 49, 80131 Naples, Italy, Italy

\*Corresponding author - E-mail: antonio.paolillo@unina.it

Fruit juices are widely consumed around the world due to their pleasant taste and nutritional value. It is estimated that more than 30 billion liters of fruit juices are consumed annually. From a nutritional perspective, fruit juices serve as a significant source of essential micronutrients, including vitamin C and potassium. Additionally, their high water content contributes to body hydration. However, despite their numerous benefits, the consumption of fruit juices presents critical concerns, including potential mycotoxin contamination. Mycotoxins can contaminate fruit juices during the growth, harvest, and storage processes of fruits, posing health risks upon consumption. This study aimed to analyze 33 fruit juice samples obtained from Italian markets using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) method. This method allowed the detection of various mycotoxins ( $n=25$ ) through the use of ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC Q-Orbitrap HRMS). The analysis revealed the presence of up to 12 different mycotoxins, including alternariol monomethyl ether (AME), T2 toxin, and enniatin B (ENN B), which were the most prevalent. AME was detected in 18.2% of the samples, with an average concentration of 0.44  $\mu\text{g}/\text{kg}$ , while T2 toxin was found at an average concentration of 2.19  $\mu\text{g}/\text{kg}$ . The highest ENN B value detected was 1.81  $\mu\text{g}/\text{kg}$ . Moreover, up to six different mycotoxins were simultaneously detected in a single sample. These findings underscore the importance of continuous monitoring to ensure food safety and minimize the risk of exposure to harmful contaminants. In light of these results, consistent and rigorous oversight is crucial, particularly in safeguarding vulnerable groups such as children, who comprise a significant portion of fruit juice consumers.

**Keywords:** fruit juices, mycotoxins, food safety, QuEChERS method, UHPLC Q-Orbitrap HRMS

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## S14

### SEA TEMPERATURE INFLUENCES ACCUMULATION OF TETRODOTOXIN IN BRITISH BIVALVE SHELLFISH

**Monika Dhanji-Rapkova**<sup>\*(1)</sup>, **Mickael Teixeira Alves**<sup>(1)</sup>, **Joaquin Trinanés**<sup>(2)</sup>, **Jaime Martínez-Urtaza**<sup>(3)</sup>, **David Haverson**<sup>(1)</sup>, **Kirsty Bradley**<sup>(1)</sup>, **Craig Baker-Austin**<sup>(1)</sup>, **Jennifer Ritchie**<sup>(4)</sup>, **Andrew Turner**<sup>(1)</sup>

<sup>1)</sup> Centre for Environment, Fisheries and Aquaculture Science (Cefas), United Kingdom

<sup>2)</sup> Department of Electronics and Computer Sciences, Universidade de Santiago de Compostela, Spain; National Oceanic and Atmospheric Administration, Miami, USA; Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, USA, Spain

<sup>3)</sup> Department of Genetics and Microbiology, Facultat de Biociències, Universitat Autònoma de Barcelona, Spain

<sup>4)</sup> Faculty of Health and Medical Sciences, University of Surrey, United Kingdom

\*Corresponding author - E-mail: monika.dhanjirapkova@cefas.gov.uk

Tetrodotoxin (TTX), a potent and heat-stable neurotoxin mostly associated with pufferfish poisoning, has been found in bivalve shellfish from temperate seas. The first occurrences of TTX in European bivalves were reported from England and Greece in 2015. Since, TTX has been documented in a few, mainly estuarine, shellfish production areas in other European countries. A seasonal pattern has started to emerge, however the role of sea surface temperature (SST) on TTX has not been investigated in detail. We endeavoured to address the gap in knowledge during two major field studies conducted in Great Britain.

In the large screening study, TTX was analysed in > 3,500 bivalve samples collected throughout 2016 from around the British coast. Overall, we found that only 1.1 % of tested samples contained TTX above 2 µg/kg whole shellfish flesh and these samples all originated from ten shellfish production sites in southern England. For the first time, satellite-derived SST data were used to investigate temperature differences between sites with and without confirmed presence of TTX. Although average annual temperatures were similar in both groups, daily mean SST values were higher in summer and lower in winter at sites where TTX was found.

In the targeted study at the single site with history of TTX, continuous *in-situ* SST data and weekly TTX concentrations in shellfish samples were collected between May and August in multiple years. The start of the TTX accumulation period appeared to be linked to the warming of sea water from 16 °C, occurring annually in late spring of each year. During this transition period, daily mean SSTs around 16 °C had to be sustained for approximately two to three weeks for TTX to be detected.

Results from our studies support the hypothesis that temperature is potentially a key trigger of events leading to TTX accumulation in European bivalves. The impact of SST warming trends on spatial and seasonal TTX distribution has not been explored to date, mainly due to lack of systematic long-term TTX data. Beside temperature, other factors are also likely to play an important role in TTX occurrences, including the presence or absence of a biological source, which is yet to be confirmed.

**Keywords:** tetrodotoxin, bivalve shellfish, sea temperature, Great Britain

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### S15

#### ESTROGENIC IN VITRO EVALUATION OF SEVERAL PHASE II METABOLITES OF ISOFLAVONES

**Elisabeth Varga**<sup>\*<sup>(1)</sup></sup>, **Dino Grgic**<sup>(2)</sup>, **Dimitra Bella-Velidou**<sup>(2)</sup>, **Luca Dellafiora**<sup>(3)</sup>, **Doris Marko**<sup>(2)</sup>

<sup>1)</sup> *eUnit Food Hygiene and Technology, Centre for Food Science and Veterinary Public Health, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine, Vienna, Austria*

<sup>2)</sup> *Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Austria*

<sup>3)</sup> *Department of Food and Drug, University of Parma, Italy*

*\*Corresponding author - E-mail: elisabeth.varga@vetmeduni.ac.at*

The health benefits of the *Fabaceae* family are often associated with their content of isoflavones (ISFs). These are known phytoestrogens and despite their positive effects, the European Food Safety Authority (EFSA) concluded that there is insufficient scientific evidence to establish a significant cause-and-effect relationship between ISF consumption and the proposed health claims [1]. While phase II metabolites of ISF are considered detoxification products with less estrogenic properties, recent studies suggest that ISF metabolites may contribute to and even enhance the biological activity of ISF [2]. However, to date, there is little knowledge about phase II metabolites of ISFs, although they represent the major ISF metabolites in human plasma [3]. The present study aims to provide a more detailed insight into the role of phase II metabolism in the biological activity of ISFs focusing on estrogenicity and cytotoxicity in Ishikawa cells, an estrogen-sensitive cell line that expresses both estrogen receptors (ER $\alpha$  and ER $\beta$ ). Concentrations between 0.001 and 10  $\mu$ M were tested with intermediate dilution steps of 1:10. In addition, a possible cleavage during the incubation period to the parent compounds was investigated by high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Docking studies were performed to assess the binding capabilities of ISFs and metabolites with the ERs.

In these studies, certain glucuronides and sulfate metabolites of ISFs are capable of exhibiting estrogenic potential at a concentration of 10  $\mu$ M, while conjugation of lower concentrations appears to actually detoxify the estrogenic potential of ISF. In the docking studies, the sulfate conjugates showed the potential to bind to the ER. HPLC-MS/MS analysis revealed that the observed estrogenic potential of several metabolites is caused by the phase II metabolites itself and is not due to possible deconjugation processes.

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**Keywords:** mycotoxin, isoflavone, estrogenicity

**Acknowledgement:** *This research was funded by the Austrian Research Promotion Agency (FFG) and Biomin Holding GmbH through the Bridge project "ISOMYCOTOX - Combinatory endocrine activity of mycoestrogens and soy isoflavones in porcine feed" (No 880656), as well as the University of Vienna and the University of Parma.*

S16

### A LC-TWIMS-HRMS WORKFLOW TO INVESTIGATE FUNGAL METABOLITES INVOLVED IN *FUSARIUM PROLIFERATUM* MYCOTOXIN BIOSYNTHETIC PATHWAYS

Irene Picicci\*<sup>(1)</sup>, Guillem Campmajo<sup>(1)</sup>, Antonio Moretti<sup>(2)</sup>, Chiara Dall'Asta<sup>(1)</sup>

<sup>1)</sup> University of Parma, Italy

<sup>2)</sup> Institute of Sciences of Food Production, National Research Council, Italy

\*Corresponding author - E-mail: irene.picicci@unipr.it

Mycotoxins are toxic, low-molecular-weight secondary metabolites produced by certain genera of filamentous fungi. Their contamination can occur both pre- and post-harvest on most agricultural products; thus, it represents one of the major threats to food quality and safety. In fact, these compounds can not only act as fungal virulence factors on plants, causing significant economic losses, but also be a source of either chronic or acute toxicity to livestock and humans. For these reasons, in recent years, the collective effort has focused on developing advanced agricultural practices, targeted early detection methods in the field, and regulations establishing the maximum allowed levels of mycotoxins in various agri-food commodities. However, since toxic secondary metabolites production is variable and strictly dependent on the fungal genetic potential, environmental factors, and interaction with the host, the current climate change scenario is expected to reshape what is already known to this day. Therefore, it is crucial to develop accurate and advanced analytical approaches in order to get a proper understanding of the mycotoxin biosynthetic pathways and the potential shifts in metabolites and intermediates production, which could lead to variations in mycotoxin-commodity combinations and the related risks.

The present study aimed to establish an analytical workflow based on liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS) to map the variations in the *in vitro* mycotoxin production, as well as the differential accumulation of their intermediates and related fungal metabolites, in strains of *Fusarium proliferatum* isolated from date palm (*Phoenix dactylifera*) in Tunisia. Briefly, samples were inoculated on autoclaved rice and extracted by biphasic solid-liquid extraction (SLE). The obtained polar fraction was then analyzed by LC-TWIMS-HRMS and data analysis relied on both targeted and suspect analysis. In the latter case, an in-house database was built containing HRMS and TWIMS information of a large number of mycotoxins (mainly fumonisins) and other relevant metabolites associated with the *Fusarium* genus, as reported in online resources (*i.e.*, NP Atlas and MicotoXilico) or the literature.

The analytical workflow allowed the identification of the main toxic secondary metabolites related to the *Fusarium proliferatum* species. While the targeted approach revealed that the most abundant mycotoxins in the samples were Fumonisin B1, B2, and B3, along with Beauvericin; the suspect approach led to the identification of isomers of these compounds, their intermediates and other minor mycotoxins. Since *Fusarium proliferatum* is one of the most relevant maize pathogens and well represents the chemo-diversity within the *Fusarium* genus, obtained results could lay the groundwork for the creation of predictive models for the potential changes in mycotoxin patterns in adaptation to climate change.

**Keywords:** mycotoxins, LC-TWIMS-HRMS, fumonisins, climate change

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S17

### CAPILLARY ELECTROPHORESIS TANDEM MASS SPECTROMETRY AS EFFICIENT TECHNIQUE FOR MULTICLASS CYANOTOXIN ANALYSIS IN WATER AND VEGETABLE SAMPLES

Ana M. Garcia-Campana<sup>\*(1)</sup>, Rocio Carmona-Molero<sup>(1)</sup>, Maria del Mar Aparicio-Muriana<sup>(1)</sup>, Francisco J. Lara<sup>(1)</sup>, Monsalud Del Olmo-Iruela<sup>(1)</sup>

<sup>1)</sup> University of Granada, Spain

\*Corresponding author - E-mail: amgarcia@ugr.es

Cyanotoxins constitute a group of toxic secondary metabolites the presence of which in any water body poses a major health risk. Moreover, advanced organisms, such as edible plants exposed to these toxins, are a possible pathway for human exposure. Suitable analytical methods are needed to establish early-warning strategies for a better protection of human and ecosystems health. Hydrophilic interaction liquid chromatography (HILIC) coupled to mass spectrometry has been the preferred option to monitor these compounds avoiding a derivatization step, in order to achieve the separation of multiclass cyanotoxins with very different polarities. Nevertheless, green analytical chemistry is demanding more environmentally friendly analytical techniques.

In this sense, we propose the use of capillary electrophoresis coupled to tandem mass spectrometry (CE-MS/MS) to determine a mixture of eight cyanotoxins belonging to three different classes: cyclic peptides (microcystin-LR, microcystin-RR and nodularin), alkaloids (cylindrospermopsin and anatoxin-a) and three isomeric non-protein amino acids ( $\beta$ -methylamino-L-alanine, 2,4-diaminobutyric acid and N-(2-aminoethyl)glycine). Separation was achieved by using an acidic background electrolyte consisting of 2 M formic acid and 20 % acetonitrile in water. Parameters affecting MS/MS detection and the sheath-liquid interface were also studied. Finally, a combination of pH-junction, field-amplified sample stacking (FASS) and acid barrage as online preconcentration strategies, was employed to improve sensitivity and efficiency. The online preconcentration applied, in combination with a dual cartridge solid-phase extraction (SPE) system, allows to obtain limits of detection (LODs) in the very low range of  $\mu\text{g/L}$  for these multiclass cyanotoxins in reservoir water samples (from 0.005 to 0.10  $\mu\text{g/L}$ ). These results are comparable to those obtained by HILIC-MS/MS. Furthermore, for the first time cyanotoxins are analyzed in vegetable samples through CE-MS/MS using the same SPE procedure, following lyophilization and solid-liquid extraction with 6 mL 80% aqueous MeOH, obtaining LODs from 0.03 to 0.23  $\mu\text{g/kg}$  in spinach samples, for the studied cyanotoxins. Satisfactory precision was also obtained, with RSD% from 1.1 to 11.9, despite the complexity of the sample. In summary, the methods described here, environmentally friendly and sustainable, are great candidates for their application in the monitoring of the presence of the target analytes in these matrices, as alternative to HILIC-MS/MS.

**Keywords:** cyanotoxins, capillary electrophoresis, mass spectrometry, vegetable samples, waters

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S18

## DETERMINATION OF PHOMOPSINS IN FOOD - METHOD VALIDATION AND SURVEY

Sylvia Kalli<sup>(1)</sup>, Ilaria di Marco Pisciotano<sup>(1)</sup>, Patrick Mulder<sup>(1)</sup>, Josipa Grzetic Martens<sup>(1)</sup>, Martin Meijer<sup>(2)</sup>, Jos Houbraeken<sup>(2)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>2)</sup> Westerdijk Fungal Biodiversity Institute, The Netherlands

\*Corresponding author - E-mail: sylvia.kalli@wur.nl

Climate change and the European Union's resistance to genetically modified organisms (GMOs) have driven the need for alternative protein sources, such as lupins. However, lupins can be contaminated with phomopsins (PHOs), a group of mycotoxins produced by the fungus *Diaporthe toxica*, posing health risks to livestock and potentially humans. In 2012, the European Food Safety Authority (EFSA) published a Scientific Opinion on PHOs in lupins, highlighting the lack of European regulatory limits for these toxins, unlike Australia, which has set a strict limit of 5 µg/kg for PHOs in lupin seeds and lupin-containing foods [1,2]. Current studies and methods have predominantly focused on PHO A [3-6], despite EFSA highlighting the importance of addressing various PHO congeners, including PHO B, PHO D, and PHO amines A and B, for the safety of lupin-based products. In this study, an analytical method capable of detecting and quantifying multiple PHO congeners in lupin seeds and lupin-based foods was developed. Due to the scarcity of available standards and of naturally contaminated lupin materials, artificial contamination of lupin seeds was achieved by inoculating them with various *D. toxica* strains. We extended an existing in-house method for PHO A to include PHO amine A and other new PHOs. High-resolution mass spectrometry (HR-MS) was employed to identify new PHOs, for which no reference standards are available, in *D. toxica* incubations. HR-MS identified several known PHOs and a new congener, PHO I. The method demonstrated limits of quantification (LOQs) of 5.0 µg/kg for PHO A and 4.1 µg/kg for PHO amine A. Semi-quantitative validation yielded LOQs for the new PHOs ranging from 2.0 to 24 µg/kg PHO A equivalents. Screening of 45 lupin food and seeds samples revealed the presence of PHOs in one Australian lupin seed sample (12 µg/kg PHO A and 5.9 µg/kg PHO amine A). Artificially contaminated lupin seeds produced up to 9.2 mg/kg of total PHOs, including PHO E/F and PHO I. The artificial contamination approach shows promise for producing new PHOs, which could be used as reference standards for comprehensive method validation. Future work will focus on enhancing method sensitivity, and scaling up the production and purification of new PHOs to develop a fully validated method for all identified PHOs.

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**Keywords:** phomopsins, food, lupin, diaporthe toxica, high resolution mass spectrometry



S19

## TARGETED AND UNTARGETED LC-(HR)MS STRATEGIES FOR THE DETERMINATION OF CUCURBITACINS IN FOOD

Ilaria Di Marco Pisciotano<sup>\*(1)</sup>, Sylvia Kalli<sup>(1)</sup>, Patrick Mulder<sup>(1)</sup>, Elena De Vries<sup>(1)</sup>, Timo Jellema<sup>(1)</sup>, Josipa Grzetic Martens<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: [ilaria.dimarcopisciottano@wur.nl](mailto:ilaria.dimarcopisciottano@wur.nl)

Cucurbitacins (CBCs) are oxygenated triterpenoid compounds commonly produced by plants from the Cucurbitaceae family. These compounds serve as defense mechanism against herbivores because of their bitterness and toxicity. In recent decades, several human poisoning incidents potentially related to CBC ingestion have been reported. This has highlighted the need for a survey and ultimately risk assessment in food products. For this purpose, a targeted LC-MS/MS method has been developed for the determination of nine CBCs (A, B, C, D, E, E-2-glucoside, I, IIa, IIb) in pumpkin, zucchini and cucumber. The method's analytical performance parameters, such as trueness and precision, have been assessed at three different concentration levels. The sample preparation is fast and effective, involving a simple solid/liquid extraction with MeOH, and the method proved to be reliable and sensitive, allowing us to quantify the CBCs down to 10.0 µg/kg (LOQ) in the samples. The validated, targeted method was used to monitor CBC content in both supermarket and homegrown products, collected in the region between Wageningen and Ede (the Netherlands). Additionally, to detect any further CBCs in the positive samples, for which no reference standards were available, an exploratory untargeted high-resolution mass spectrometry (HRMS) workflow was also employed. The workflow leveraged the tendency of CBCs to undergo in-source fragmentation (ISF) when ionized in positive mode. This fragmentation behavior is characteristic of these compounds and can be used to identify unknown CBC analogs. The HRMS method was developed by optimizing the in-source collision energy on the available CBCs to maximize the ISF phenomenon and break the molecules down to their core structure. This approach enabled the classification of all known CBCs in the scientific literature in four major characteristic cores, facilitating the screening of unknown CBC analogs in the contaminated samples. When additional CBCs were detected, a straightforward full scan-data dependent MS<sup>2</sup> (FS-ddMS<sup>2</sup>) experiment was performed to obtain additional structural information on the unknown compounds. The FS-ddMS<sup>2</sup> experiment was run in negative ionization mode to prevent ISF, allowing for the acquisition of a more comprehensive MS<sup>2</sup> spectrum. A total of 71 samples were analyzed using the targeted LC-MS/MS method. Cucurbitacin B, D, E, E-2-glucoside and I were quantified in 5 pumpkin and 4 zucchini samples, whereas cucurbitacin C was found at LOQ level in one cucumber sample. Analysis with the HRMS workflow validated our strategy, revealing two putative CBC analogs in the most highly contaminated zucchini that was linked to an intoxication case in 2021.

**Keywords:** cucurbitacins, HRMS, survey, risk evaluation

### S20

#### MONITORING OF TOXIN-PRODUCING PHYTOPLANKTON IN THE ADRIATIC SEA (ITALY) IN 2023-2024 USING MICROSCOPIC AND MOLECULAR METHODS

**Barbara Bertasi**<sup>(1)</sup>, **Laura Ragni**<sup>(1)</sup>, **Sara Arnaboldi**<sup>(1)</sup>, **Enrico Pavoni**<sup>(1)</sup>, **Laura Pezzolesi**<sup>(2)</sup>, **Franca Guerrini**<sup>(2)</sup>, **Rossella Pistocchi**<sup>(2)</sup>, **Monica Cangini**<sup>(3)</sup>, **Irene Servadei**<sup>(3)</sup>, **Silva Rubini**<sup>(4)</sup>

<sup>1)</sup> Food Safety Department, IZSLER, Italy

<sup>2)</sup> Dep. of Biological, Geological and Environmental Science, Università di Bologna, Italy

<sup>3)</sup> Centro Ricerche Marine, Cesenatico, Italy

<sup>4)</sup> IZSLER, Italy

\*Corresponding author - E-mail: barbara.bertasi@izsler.it

In recent years, climate changes and the consequent presence of algae potentially producing toxins have become an increasing problem, especially in smaller basins, which are particularly affected by temperature increases. In northern Italy, the Adriatic Sea is constantly monitored because it hosts most of the Italian mussel farms, localized in the Emilia-Romagna Region. Currently, analyses for the presence of microalgae and toxins are performed using official methods (microscopy Utermöhl technique, and methods as HPLC and LC-MS), but many molecular methods are being studied for genera and species identification, including sequencing methods and PCRs. The aim of this work was to conduct a biennial monitoring by combining microscopic method and Real Time PCR for the detection of dinoflagellates and diatoms (*Alexandrium* spp., *Dinophysis* spp., *Pseudo-nitzschia* spp.). A total of 681 sea water samples were analysed to verify algae spread in Ferrara, Forlì-Cesena, Ravenna and Rimini provinces (Adriatic Sea), and 244 samples, mainly collected in 2023, were analysed by both methods. DNA was extracted using DNeasy®PowerWater® kit (Qiagen) after water filtration. Real Time PCRs were based on Sybr Green technology and performed on CFX96 instruments (Bio-Rad); In the provinces of Forlì-Cesena, Rimini and Ravenna, during 2023, *Alexandrium* spp. cells number was high in May and June, and decreased in the following months ( $10^2$ - $10^3$  cells/L); sporadically,  $10^2$  cells/L of *Dinophysis* spp. were detected in June, and *Pseudo-nitzschia* spp. was always present in the different periods of the year, with a high number of cells ( $10^5$ - $10^6$  cells/L) in specific areas. Unlike 2023, in 2024 a considerable number of cells of the three genera emerged from March. In the Ferrara Province, during 2023 *Pseudo-nitzschia* spp. was identified starting from March and in the following months with a decreasing trend; *Dinophysis* spp. was detected in June and maintained a sporadic presence in autumn. *Alexandrium* spp. was not detected except for a sporadic presence. During 2024, also in the Province of Ferrara the appearance in early spring of *Dinophysis* and *Pseudo-nitzschia* was observed. Many factors could cause of the recent early appearance of microalgae; for example, at the beginning of spring in the monitored areas a drop of one degree in temperature and an increase in humidity was measured from 2023 to 2024. Concerning molecular method, about *Alexandrium* and *Dinophysis* 12% more positive samples were detected by PCR than microscopy, except for *Pseudo-nitzschia* (a cycle threshold > 40 was selected to define negative samples); *Alexandrium* spp. melting curves seemed to confirm positives specificity. For *Dinophysis* and *Pseudo-nitzschia* it was difficult to verify result reliability due to the co-presence of many species. Despite the need for improvement for some species identification, PCRs could support microscopic investigation avoiding well-known difficulties in identifying toxic microalgae.

**Keywords:** algae toxins, Adriatic sea

## S21

### ROBUST, HIGHLY SENSITIVE AND FAST POLARITY SWITCHING QUANTITATION OF MYCOTOXINS IN DIFFERENT MATRICES

Carlos Bueno<sup>(1)</sup>, Jianru Stahl-Zeng<sup>\*(1)</sup>, Michael Scherer<sup>(1)</sup>

<sup>1)</sup> SCIEX, Spain

\*Corresponding author - E-mail: [jianru.stahl-zeng@sciex.com](mailto:jianru.stahl-zeng@sciex.com)

Mycotoxins are toxic fungal metabolites, which are derived from certain molds and fungi. The growth of mold can occur before crops are harvested or under inappropriate storage conditions such as warm and humid conditions. Consumption of food products containing mycotoxins can have serious health implications.

The most important classes of mycotoxins including the highly carcinogenic Aflatoxins (e.g. AFB1), trichothecenes (e.g. DON), Fumonisin (e.g. FB1), Ochratoxin (OTA) and Zearalenone (ZEN) and several others are regulated in many countries in the EU, mycotoxins in foodstuffs are regulated newly by the EC 2023/915, 2024/1038 and a recommendation document 2022/553 for alternaria toxins.

Mycotoxin analysis needs to be comprehensive and able to deliver accurate and consistent results across a wide range of matrices.

High sensitive instrument provides the possibility to minimize sample preparation steps, at the same time obtain high quality and reliable quantitative results to meet the requirements of different regulations.

In this poster, we will demonstrate a fast LC/MS method by using SCIEX 7500+ system to quantify EU regulated mycotoxin compounds in different matrices, including baby food. Positive/negative fast switching method is applied. LOQs in different matrices, which align with requirement of EU regulations will be present.

**Keywords:** EU regulations, robust, highly sensitive, baby food, sample prep

## S22

### DEVELOPMENT, VALIDATION AND APPLICATION OF DILUTE AND SHOOT TECHNIQUE FOR LC-MS/MS TO DETERMINE TROPANE ALKALOIDS IN SOYBEANS

Rufielyn Gravador<sup>(1)</sup>, Simon Haughey\*<sup>(1)</sup>, Brett Greer<sup>(1)</sup>, Chelsea Ugay<sup>(1)</sup>, Chris Elliott<sup>(2)</sup>

<sup>1)</sup> National Measurement Laboratory: Centre of Excellence in Agriculture and Food Integrity, Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast, N. Ireland BT9 5DL, United Kingdom

<sup>2)</sup> School of Food Science and Technology, Faculty of Science and Technology, Thammasat University, 99 Mhu 18, Pahonyothin Road, Khong Luang, Pathum Thani 12120, Thailand, Thailand

\*Corresponding author - E-mail: s.a.haughey@qub.ac.uk

Tropane alkaloids (TAs), particularly atropine and scopolamine, are plant toxins produced in considerable concentrations in an invasive weed, *Datura stramonium*. These TAs that contaminated cereals, grains or vegetables through co-harvesting or processing caused severe poisoning or death of consumers in many places, including Portugal, Uganda, Sudan, France and Turkey.

Consequently, this study aimed to develop and validate a simple, cost-effective method for rapidly detecting and quantifying them using LC tandem mass spectrometry.

A 1.00 g aliquot of ground soybean was extracted with 5.00 ml of acetonitrile: water (60:40) with 1% formic acid for 90 minutes in a multitube vortexer, sonicated for 30 minutes, centrifuged for 15 minutes, and the supernatant diluted, filtered, and injected into the LC system. Each sample was analysed within 8.5 minutes.

The validation parameters (recovery, limit of quantification (LOQ), linearity, precision, matrix effects) were within the acceptable limits set by the SANTE/11312/2021 or the Eurachem Guide.

With LOQ<sup>-1</sup>, the current method is suitable for quantifying atropine and scopolamine in various food commodities. The Commission Regulation (EU) 2023/915 set a maximum level of 1 to 15 µg kg<sup>-1</sup> for high-risk cereal crops and products. Of the 74 soybean samples collected from Asian countries (Cambodia, India and China), only one showed scopolamine content above the permitted maximum level, indicating a low probability of TA contamination in soybeans from these countries.

The method developed and validated here is simple and low-cost since no expensive clean-up step was required for the sensitive detection of atropine and scopolamine in soybeans. A low LOQ of <1 µg kg<sup>-1</sup> obtained by the current method shows that it can reliably quantify TAs at the EU-permitted maximum levels in various commodities.

**Keywords:** plant toxins, tropane alkaloids, liquid chromatography, solid-liquid extraction, cereals

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### S23

#### LEVERAGING SENSITIVITY AND SELECTIVITY OF THE SCIEX 7500 SYSTEM FOR TARGETED ANALYSIS OF MYCOTOXINS IN PLANT-BASED MEAT

Holly Lee<sup>\*(1)</sup>, Hitha PP<sup>(1)</sup>, Sujata Rajan<sup>(1)</sup>, Sashank Pillai<sup>(1)</sup>, Craig Butt<sup>(1)</sup>

<sup>1)</sup> SCIEX, Canada

\*Corresponding author - E-mail: holly.lee@sciex.com

Mycotoxins are low molecular weight compounds that are naturally produced as secondary metabolites by fungi (moulds). Contamination of food and feed by mycotoxins can cause disease and potentially death in humans and animals due to their reported cytotoxic, genotoxic, hepatotoxic and immunosuppressive effects. Although mycotoxins have been reported in animal- and plant-based foods, higher levels are often found in the latter due to direct exposure from spoiled agricultural commodities. This is concerning due to the recent shift in dietary preferences towards plant-based meat alternatives based on health and sustainability reasons. While the European Commission (EC) has established maximum residue levels (MRLs) for mycotoxins in some raw ingredients of plant-based foods, these final plant-based meat products are not yet regulated. As such, sensitive analytical methods are needed to ensure the safety of plant-based meats for consumption. Here, a sample preparation and LC-MS/MS method was developed for the quantitation of 10 mycotoxins in plant-based chicken and mutton products. The sensitivity of the SCIEX 7500 system enabled limits of quantitation (LOQs) as low as 10-25 pg/mL in the solvent-based calibration standards, with good quantitative performance (accuracy of 81-104%, %CV 2.0-12%). Good absolute recoveries (65-128%) and precision (%CV 1.0-16%) were achieved at 2 spiking levels of 1 and 10 ng/g in 3 plant-based meat matrices. Due to the absence of mass-labelled internal standards, standard addition was used to quantify some of the mycotoxins suffering from significant matrix effects. Using the QTRAP functionality on the SCIEX 7500 system, the MRM<sup>3</sup> workflow was also demonstrated to improve specificity for aflatoxin G2 (AFG2) in plant-based meat. The MS/MS/MS (MS<sup>3</sup>) fragmentation of an AFG2 precursor ion provided a dual mass filtering through monitoring a transition comprised of first- and second-generation product ions. This significantly enhanced the response of AFG2, which was not detected in the MRM acquisition due to coeluting interferences observed in a plant-based chicken extract. Removal of these interferences during the MRM<sup>3</sup> acquisition resulted in cleaner baselines and improved signal-to-noise (S/N), thereby potentially enabling lower limits of quantitation (LOQs) in the final method.

**Keywords:** mycotoxins, plant-based, MRM<sup>3</sup>, specificity, maximum residue limit

**S24**

### **HIGHLY EFFICIENT LC-MS/MS ANALYSIS OF MULTIPLE MYCOTOXINS UTILIZING BIPHENYL COLUMN SELECTIVITY WITH INERT COLUMN TECHNOLOGY**

**Tina Brandscher**<sup>\*(1)</sup>, Jan Pschierer<sup>(1)</sup>, Paul Connolly<sup>(2)</sup>, Diego Lopez<sup>(2)</sup>

<sup>1)</sup> Restek GmbH, Germany

<sup>2)</sup> Restek Corporation, United States of America

\*Corresponding author - E-mail: [tina.brandscher@restek.com](mailto:tina.brandscher@restek.com)

With an ever-growing list of mycotoxins to analyse, multi-analyte methods are an attractive alternative, affording time and cost savings to laboratories. However, with a range of chemistries across the broad group of mycotoxins this does prove a challenge. When analysed using a standard C18 column, high pH conditions are required for accurate analysis of Alternaria toxins and Ergot Alkaloids. These conditions typically are problematic for silica based HPLC columns, often causing significantly reduced column lifetime. Another factor at play with some groups of mycotoxins, are non-specific adsorption (NSA) or binding (NSB) with the metal surfaces of HPLC systems. These interactions further cause problems with peak shape, analyte sensitivity, and reproducibility from injection to injection.

In this work, Restek look to establish the benefits of coated column technologies by comparing methods developed on Inert and standard hardware to remove high pH requirements, along with matrix or chemical based passivation techniques for a wide panel of mycotoxin analytes.

**Keywords:** LC-MS, inert hardware, mycotoxins, biphenyl

S25

### BIOTRANSFORMATION OF HT-2 AND T-2 TOXINS USING PLANT APPARATUS AND ISOLATION OF THE RESULTING GLUCOSIDES

**Adam Behner**<sup>(1)</sup>, Martina Travnickova<sup>(2)</sup>, Zbynek Dzuman<sup>(1)</sup>, Jana Chrpova<sup>(2)</sup>, Milena Stranska<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

<sup>2)</sup> Crop Research Institute in Prague, Czech Republic

\*Corresponding author - E-mail: behnera@vscht.cz

Mycotoxins - known contaminants in a wide range of foods and feeds - can pose a serious health risk to humans and livestock due to their toxic effects. The mycotoxins HT-2 and T-2 toxin produced by *Fusarium* micromycetes are present in food and feed in addition to their free forms in conjugates with carbohydrates. These conjugated forms represent a potential "hidden" health risk due to hydrolysis and the release of free mycotoxins in the gastrointestinal tract of mammals. However, the unavailability of analytical standards for conjugated forms of HT-2 and T-2 toxin hampers routine analysis, which may lead to an underestimation of dietary intake. This study aims to exploit the natural ability of plants (*Triticum vulgare*) to biotransform free mycotoxins into their conjugated forms as part of their detoxification mechanisms and to isolate the resulting mycotoxin conjugates using modern preparative chromatography approaches. Subsequently, the purified fractions of conjugated mycotoxins will serve as analytical standards to enable their routine quantification and toxicity assessment.

**Keywords:** conjugated mycotoxins, HT-2 and T-2 toxin, biotransformation, high-resolution mass spectrometry, preparative HPLC

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S26

### VALIDATION OF AN LC-MS/MS BASED METHOD FOR MULTIPLE MYCOTOXINS AND PLANT TOXINS IN MEAT REPLACEMENT PRODUCTS

**Michael Sulyok**<sup>\*(1)</sup>, **Claudia Seeber**<sup>(1)</sup>, **Rudolf Krska**<sup>(1)</sup>

<sup>1)</sup> *University of Natural Resources and Life Sciences Vienna, Department of Agrobiotechnology (IFA-Tulln), Institute of Bioanalytics and Agro-Metabolomics, Austria*

*\*Corresponding author - E-mail: michael.sulyok@boku.ac.at*

In the recent years plant-based alternatives for meat products have become more and more popular, even among non-vegetarians and non-vegans, due to perceived health benefits, absence of veterinary drug residues and a decreased carbon footprint. However, these advantageous aspects might be outweighed by the fact that biotoxins produced by molds and plants are frequently present in raw materials such as soybeans, peas, chickpeas or seitan [1, 2].

We aim to expand the currently limited occurrence data on natural contaminants in plant based replacement products sold on the market. As a first step, an extended version of our multi-analyte method based on liquid chromatography coupled to tandem mass spectrometry [3] will be validated for finished products using different individual samples for spiking experiments. We expect that differences in the composition (protein source, presence of flavors, additives etc.) of individual samples belonging to the same product type (filet, mince, sausage, etc.) might result in significant dispersion of recoveries of the extraction and/or of matrix effects.

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[2] Mihalache, O. A.; Dellafiora, L.; Dall'Asta, C.. *Food Research International*, 2022. <https://doi.org/10.1016/j.foodres.2022.111490>.

[3] Sulyok, M.; Suman, M.; Krska, R. *npj Science of Food* 2024 8:49; <https://doi.org/10.1038/s41538-024-00294-7>.

**Keywords:** mycotoxins, plant toxins, LC-MS/MS, validation, alternative proteins



**S27**

## **2D-LIQUID CHROMATOGRAPHY - AN EFFICIENT ALTERNATIVE TO SPE AND IAC FOR THE DETERMINATION OF MYCOTOXINS IN FOOD**

**Sarah Schleicher**<sup>(1)</sup>, Susanne Sölter<sup>(1)</sup>, Mareike Rathke<sup>(2)</sup>, Clara John<sup>(2)</sup>, Sarah Kulas<sup>(2)</sup>, Mareike Reichel<sup>(2)</sup>

<sup>1)</sup> *Agilent Technologies Deutschland GmbH, Germany*

<sup>2)</sup> *Eurofins WEJ Contaminants GmbH, Germany*

*\*Corresponding author - E-mail: sarah.schleicher@agilent.com*

Mycotoxins are secondary metabolites formed by fungi and can enter the food chain through a primary fungal infestation of the harvested crop in the field or through a secondary infestation during storage after harvest. Grain, seeds, dry fruits, nuts and their processed products are particularly affected. Mycotoxins are known to have serious health implications, including carcinogenic, mutagenic and neurotoxic effects. To protect consumers, the EU has established maximum allowable levels for specific mycotoxins, such as aflatoxins and ochratoxin A, in various foods (Commission Regulation (EU) 2023/915).

Mycotoxins are usually analyzed using HPLC-FLD or HPLC-MS/MS. However, sample preparation is time-consuming and costly, as the sample has to be purified and enriched after extraction by solid phase extraction (SPE) or immunoaffinity chromatography (IAC). In addition, a balance must be found between effective matrix removal, high recovery, and reproducibility.

An alternative approach is the use of two-dimensional liquid chromatography (2D-LC). By chromatographic separation in a further dimension, matrix effects and interferences are reduced, and a direct measurement of the extract is possible. The potential of 2D-LC coupled with triple quadrupole MS for the determination of mycotoxins in various food matrices (grain, spices, coffee...) has been demonstrated. The sensitivity of the method meets the EU MRLs. Additionally, the method has been evaluated for linearity, reproducibility, and recovery for both a low and a high level-spike.

**Keywords:** 2D-LC, 2D-LC-MS, mycotoxins

S28

### A VALIDATED LC-MS/MS MULTI-METHOD FOR THE DETERMINATION OF 110 MYCOTOXINS AND PLANT TOXINS IN RAW MILK

**Ahmed El-Khatib<sup>(1)</sup>, Arnold Bahlmann<sup>(1)</sup>, Carolin Hoyer<sup>(1)</sup>, Christoph Hutzler<sup>(1)</sup>, Stefan Weigel<sup>(1)</sup>**

<sup>1)</sup> German Federal Institute for Risk Assessment (BfR), Germany

\*Corresponding author - E-mail: Ahmed.El-Khatib@bfr.bund.de

Milk is one of the most highly consumed food products in all age groups and forms the basis of dairy products. The quality and safety of milk are significantly influenced by the animal's diet, which is composed of a complex mix of individual feed components (e.g. forages and concentrates). Each raw feed component can carry different contaminants, leading to multi-toxin exposure in the overall animal diet. This multitude of toxins could finally find its way into the milk. The presence of contaminants such as mycotoxins and plant toxins can pose health risks to consumers.

Currently, only aflatoxin M1 is regulated by the European Union in raw milk, heat-treated milk and milk for the manufacture of milk-based products. However, there have been reports on the presence of beauvericin and enniatin B [1, 2], T-2 and HT-2 toxins [2], pyrrolizidine and tropane alkaloids [3] and quinolizidine alkaloids [4] in raw milk samples.

Within the framework of the EU project INTAQT, an LC-MS/MS method for the simultaneous determination of a wide range of mycotoxins and plant toxins (110 toxins) in raw milk has been developed and validated according to the SANTE 11312/2021 v2 guidelines in terms of recovery, precision and limit of quantification (LOQ). The method involved QuEChERS-based extraction and LC-MS/MS analysis using triple quadrupole mass spectrometry.

The toxins covered by the method include aflatoxins, trichothecenes (nivalenol and related compounds, T-2 toxin and related compounds, among others), fumonisins, zearalenone and related compounds, beauvericin, enniatins, ochratoxins, *Alternaria* toxins, ergot alkaloids as well as tropane alkaloids, pyrrolizidine alkaloids, and quinolizidine alkaloids.

The method has an LOQ for the regulated aflatoxin M1 of 0.025 µg/kg and showed an excellent recovery (80-110%) and precision ( $RSD_{WR} < 10\%$ ) for almost 80 toxins.

To demonstrate its applicability, the validated LC-MS/MS multi-method was applied to a number of samples from the market and local farms. The results revealed varying levels of mycotoxins and plant toxins.

The aim of this work was to investigate the occurrence of several mycotoxins and plant toxins and their co-occurrence in milk. The validated LC-MS/MS multi-method proved to be a reliable and efficient tool for the simultaneous determination of multiple mycotoxins and plant toxins in milk.

[1] Pietruszka K et al. (2023) J Vet Res 67:259-266.

[2] Leite M (2023) Toxins 15:605.

[3] Klein LM et al. (2024) Food Additives & Contaminants: Part A 41:629-647.

[4] Engel AM et al. (2022) J. Agric. Food Chem. 70:11749-11758.

S29

### A PROTEOMICS APPROACH FOR THE DETECTION AND IDENTIFICATION OF POISONING PLANTS - DATURA STRAMONIUM, DATURA METEL AND MANDRAGORA AUTUMNALIS - IN LEAFY PLANTS

**Giancarlo Biancotto**\*<sup>(1)</sup>, Sabina Noviello<sup>(1)</sup>, Roberta Masin<sup>(2)</sup>, Albino Gallina<sup>(1)</sup>, Lisa Ferrari<sup>(1)</sup>, Roberto Stella<sup>(1)</sup>

<sup>1)</sup> Istituto Zooprofilattico Sperimentale delle Venezie, Italy

<sup>2)</sup> Università Degli Studi Di Padova, Italy

\*Corresponding author - E-mail: gbiancotto@izsvenezie.it

In the last years, a significant increase of cases of poisoning caused by ingestion of poisonous plants present in packages of leafy plant foods or their preparations has been observed. News reports have documented serious episodes of alkaloid poisoning due to the ingestion of toxic plants, present in packages of spinach, chard, or in derived food preparations. In contrast with the determination of the toxic alkaloids, which is relatively simple, the identification of the toxic plant responsible of the toxic contamination is often very hard, if not impossible. In fact, due to the high content of alkaloids in leaves of toxic plants and their strong toxicological activity, even minimal fragments of leaves may cause serious cases of poisoning among consumers. In such cases, even operators with a strong botanical expertise can hardly recognize/identify such fragments when dispersed in fresh, cooked or processed vegetables.

For all these reasons, we are currently working at a project aimed at increasing the safety of leafy plant commodities, i.e. spinach and chard, by the improvement of a control system limiting the possible presence of toxic plants such as *Datura stramonium*, *Datura metel* and *Mandragora autumnalis*. Among other studies part of the same research project and in addition to the analysis of alkaloids responsible for the toxic effect, we applied a bottom-up non-targeted proteomics investigation -based on LC-HRMS/MS analysis- to identify marker peptides allowing for the detection and the identification of the considered toxic plants. To this end, we compared the proteomics profile of *Datura stramonium*, *Datura metel* and *Mandragora autumnalis* with that of *Spinacia oleracea*, *Cichorium intybus* and *Beta vulgaris* by using unsupervised PCA and PLS-DA to unveil peptide signals capable to identify uniquely each toxic plant (peptide markers). After identifying the sequence of such peptide markers (at least one specific peptide for each toxic plant), we tested their sensitivity and specificity by a targeted proteomics approach based on parallel reaction monitoring (PRM). Preliminary data demonstrate the capacity of the method to detect/identify the presence of the toxic plants down to 1% of contamination of the edible leaves.

Results obtained by the proteomics analysis indicate that this innovative approach can be used to detect and simultaneously identify toxic plants responsible of accidental poisoning and consequently favouring the tracking of the origin of the contamination.

**Keywords:** *Datura stramonium*, *Datura metel*, *Mandragora autumnalis*, proteomics, toxic plants

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S30

## NATURAL CONTAMINANTS IN PLANT-BASED MEAT ALTERNATIVES

**Nela Prusova<sup>(1)</sup>, Ema Vitova<sup>(1)</sup>, Tereza Dolezalova<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>, Milena Stranska<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: zacharim@vscht.cz*

Consumption of plant-based meat alternatives is currently increasing, mainly due to efforts to ensure sustainable food production for a rapidly growing population and for reasons related to individual health or personal preferences. The main sources of plant proteins are soybeans, peas, lentils, chickpeas and wheat. While these alternatives offer many health and environmental benefits compared to meat, they can also pose potential risks to human health due to the possible presence of natural toxins such as mycotoxins and plant alkaloids. These contaminants are not fully regulated in plant raw materials used for the production of meat alternatives and there is still insufficient information on their occurrence in these foods (Augustin Mihalache et al., 2022). Therefore, in this study, 58 mycotoxins, 16 tropane alkaloids and 32 pyrrolizidine alkaloids were determined in 77 samples of plant-based meat alternatives and other plant protein-rich foods. Separation of analytes was performed on an Acquity UPLC® HSS T3 reversed-phase column (100 mm x 2.1 mm, 1.8 µm; Waters), with subsequent detection assured by quadrupole-orbitrap mass spectrometer in full-spectral acquisition mode with systematic fragmentation of precursor ions. The most prevalent mycotoxins were tenuazonic acid, tentoxin, and enniatins, while atropine and scopolamine were the most common plant alkaloids. The findings suggest that the consumption of plant-based meat alternatives may contribute to dietary intake of these contaminants, which could potentially approach or exceed threshold exposure levels, especially in certain consumer groups. Analytical issues associated with matrix effects and potential quantification bias will also be discussed.

Augustin Mihalache, O., Dellafiora, L., & Dall'Asta, C. (2022). A systematic review of natural toxins occurrence in plant commodities used for plant-based meat alternatives production. *Food Research International*. 158, 111490. <https://doi.org/10.1016/j.foodres.2022.111490>.

**Keywords:** plant-based meat alternatives, mycotoxins, tropane alkaloids, pyrrolizidine alkaloids, high-resolution mass spectrometry

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### S31

#### QUINOLIZIDINE ALKALOIDS IN FOOD: DEVELOPMENT OF AN LC-MS/MS METHOD AND OBSERVATIONS FROM ROUTINE

Jörg Konetzki<sup>(1)</sup>, Juliane Böttcher<sup>(1)</sup>, Thomas Behnke<sup>(1)</sup>, Erik Becker<sup>(1)</sup>

<sup>1)</sup> Institut Kirchhoff Berlin GmbH a Mérieux NutriSciences Company, Germany

\*Corresponding author - E-mail: joerg.konetzki@mxns.com

Quinolizidine alkaloids (QA), of which more than 170 compounds have been identified so far, are used by plants for defense and protection against herbivores. They are synthesised mainly in lupins from the amino acid lysine and stored in all parts of the plant, including the seeds. Due to their high-quality protein, essential amino acids and unsaturated fatty acids, lupin seeds are a valuable component of vegetarian nutritional concepts. In addition to their use in food production, e.g. as flour, in meat and milk substitutes, spreads and pasta, lupins are also used as animal feed. Food products made from sources other than lupin seeds can also be contaminated. For example, it has been proven that QA passes into cow's milk and thus into dairy products of various kinds if feed with high QA concentrations is used [1].

A distinction is made between wild-growing "bitter lupins" (higher alkaloid content) and cultivated "sweet lupins" (lower alkaloid content). QA, which act as competitive acetylcholine inhibitors, can cause symptoms of poisoning in humans. These affect the nervous, circulatory and digestive systems. QA can cause respiratory paralysis.

A LC-MS/MS method for the determination of 10 QA in dry food and feed with a high lupin content and in moist food with a low or no lupin content (milk, dairy products, milk substitutes) was developed.

The main challenges during the development of the method were, on the one hand, the selection of a suitable calibration, as no isotope-labeled standards and no analyte-free lupin-based blank matrices were available. On the other hand, the chromatographic separation of the isomers lupanine and iso-lupanine is necessary for a correct quantification, especially if one of the two analytes is present in higher concentrations. Thirdly, during the extraction of certain matrices, a phase separation occurred with distribution of the analytes to both phases. We therefore had to change the extraction solvent.

Some observations from the routine complement the presentation of the method.

[1] J. Agric. Food Chem. 2022, 70, 11749-11758.

**Keywords:** quinolizidine alkaloids, lupins, plant toxins, secondary metabolites, LC-MS/MS

S32

### ALPHA-TOMATINE IN CHERRY TOMATOES: AN ITALIAN CASE OF GLYCOALKALOIDS INTOXICATION

Mariantonietta Peloso<sup>(1)</sup>, Ilaria Prizio<sup>(1)</sup>, Gaetan Minkoumba Sonfack<sup>(1)</sup>, Simona Pellicciotti<sup>(1)</sup>, Giorgio Fedrizzi<sup>(1)</sup>, Elisabetta Caprai\*<sup>(1)</sup>

<sup>1)</sup> Food Chemical Department, Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna, Italian Reference Laboratory for Plant Toxins in Food, Italy

\*Corresponding author - E-mail: [elisabetta.caprai@izsler.it](mailto:elisabetta.caprai@izsler.it)

In May 2024, a number of cases of tomato intoxication were reported in primary schools in Emilia-Romagna and other Italian regions. All episodes were associated with the consumption of cherry tomatoes distributed in schools through the "Fruit and Vegetables in Schools" project, which is supported by the European Union and the Italian Ministry of Agriculture. Symptoms reported included gastrointestinal disorders with nausea, vomiting and pharyngeal irritation. In general, the onset of symptoms was rapid (15-30 minutes) with an equally rapid resolution. In view of the widespread phenomenon of intoxication (affecting over 200 people), the competent local authorities commissioned extensive microbiological and chemical analyses to identify the cause. Preliminary analyses carried out on cherry tomatoes samples didn't highlight the presence of chemical contaminants and microbiological agents related to the observed poisoning symptoms. Consequently, the Reference Laboratory for Plant Toxins in Food, decided to investigate compounds naturally present in *Solanaceae* plants harmful to human health. Glycoalkaloids (GAs) are the common natural toxins, which are produced by plants as secondary metabolites, and contribute to plant resistance against pests and pathogens. EFSA's scientific opinion 2020 assessed the risks to human health associated with the presence of GAs in food chain, able to induce acute gastrointestinal symptoms. In tomatoes, the prevailing glycosylated product is  $\alpha$ -Tomatine ( $\alpha$ TM) and its tomatidine aglycone (TD). During tomato fruit ripening,  $\alpha$ TM is converted into other steroidal alkaloids. An integrated approach consisting of LC-HRMS and LC-MS/MS has been used for the identification and confirmation of these compounds from the cherry tomatoes extracts. The application of the LC-HRMS technique allowed to investigate and identify different compounds associated with different degrees of tomato ripeness (e.g.  $\alpha$ TM, dehydro-TM, acetoxy-TM and Esculeoside A). The LC-MS/MS confirmatory method developed for the GAs determination showed a content of  $\alpha$ TM ranging from 1.9 mg/kg to a maximum of 11.3 mg/kg in the 42 cherry tomato samples analyzed. The fruit exhibited a high degree of heterogeneity in terms of ripeness, with a notable decrease in  $\alpha$ TM content from green fruits to ripe tomatoes. It is important to highlight that tomatoes continue to ripen rapidly even after harvesting. However, some cherry tomatoes analyzed were observed to be unripe. It is therefore probable that the content of  $\alpha$ TM at the time of distribution and consumption were higher, considering approximately a time of 20 days from the harvesting to the analysis. This may have induced gastrointestinal symptoms. Nevertheless, further data on the occurrence of GAs and toxicity studies are necessary for all tomato cultivars available on the market.

**Keywords:** glycoalkaloids, cherry tomatoes, intoxication, LC-HRMS, LC-MS/MS

### S33

#### EXAMINATION ON THE EFFECT OF CHLORINE-CONTAINING LIQUID SUBSTANCE ON MYCOTOXIN PRODUCING FUSARIUM SPECIES

**Eniko Makkai**<sup>(1)</sup>, **Tibor Bartok**<sup>(2)</sup>, **Attila K. Horvath**<sup>(3)</sup>, **György Cseko**<sup>(3)</sup>, **Zoltan Gazdag**<sup>(4)</sup>, **Arpad Czeh**<sup>(5)</sup>

<sup>1)</sup> Soft Flow Ltd.; University of Pécs, Faculty of Sciences, Institute of Chemistry, External Department of Applied Molecular Sciences - Mycotoxin Research Group; University of Pécs, Faculty of Sciences, Institute of Biology, Department of Molecular Biology and Microbiology, Hungary

<sup>2)</sup> Fumizol Ltd., Hungary

<sup>3)</sup> University of Pécs, Faculty of Sciences, Institute of Chemistry, Department of General and Inorganic Chemistry, Hungary

<sup>4)</sup> University of Pécs, Faculty of Sciences, Institute of Biology, Department of Molecular Biology and Microbiology, Hungary

<sup>5)</sup> Soft Flow Ltd.; University of Pécs, Faculty of Sciences, Institute of Chemistry, External Department of Applied Molecular Sciences - Mycotoxin Research Group, Hungary

\*Corresponding author - E-mail: emakkai@foss.dk

Mycotoxins are hazardous to human and animal health and are extremely stable resulting difficulties in eliminating them during food processing procedures. The main mycotoxin producing fungi are among others the *Aspergillus*, *Fusarium* and *Penicillium* species.

It is almost impossible to completely remove all mycotoxins from contaminated crops as they enter the food chain. Therefore, targeting the toxin-producing fungi prior the appearance of the toxins might be a solution to eliminate the difficulties regarding mycotoxin management. Our research group focused on a chlorine-containing substance, a potential fungicide that could be a good alternative in prevention.

Based on the literature, different types of protocols are applied for minimum inhibitory concentration (MIC) determination on the filamentous fungi. Usually, in clinical studies CLSI (Clinical Laboratory Standard Institute protocol), while in agricultural studies an agar dilution method is applied.

Treating the fungi with a sub-inhibitory concentration could potentially induce toxin production, thus in our study the results of the two protocols were compared, to find the ideal method for assessing inhibitory concentrations. The protocols were compared by determining MIC on the fungi *F. verticillioides* on typical antifungals of the two fields: voriconazole (VOR) for clinical and metconazole (MET) for agricultural application. The methods were also compared based on the recommended evaluation time (48 hours and 8 days). We observed higher MIC values using the CLSI protocol compared to the agar dilution method. For VOR, MIC values were 4 µg/mL by CLSI and 1 µg/mL by agar dilution. For MET, MIC values were 1.25 µg/mL by CLSI and 0.313 µg/mL by agar dilution. Therefore, we continued our study applying the CLSI protocol.

We evaluated the chlorine-containing substance on mycotoxin-producing *Fusarium* species: the T2-producing *F. sporotrichioides* var. *minus* (DSM62425), the DON (deoxynivalenol)-producing *F. graminearum* (FZL Fg2022/17) and the fumonisin producer *F. verticillioides* (FZL Fv2022/1) from Fumizol Ltd. Szeged. For comparison, we included *A. flavus* ATCC 204304 as a reference strain. We observed inhibitory effect for all the species studied, therefore the chlorine-containing liquid substance is a promising compound for further research in application during mycotoxin management.

However, further studies are needed on other mycotoxin-producing filamentous fungi, and on the potential method of application for the chlorine-containing substance as well.

**Keywords:** mycotoxins, prevention, field and storage molds, agriculture, food safety

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S34

### ANALYSIS OF MULTI-MYCOTOXIN CONTAMINATION OF MAIZE SAMPLES IN HUNGARY, USING MYCOTOXIN DETECTION MYCOFOSSTM PLATFORM

Anna Orsolya Laczko<sup>(1)</sup>, Istvan Lakatos<sup>(2)</sup>, Petra Dorottya Tigyi<sup>(3)</sup>, Tamas Szörenyi<sup>(3)</sup>, Miklos Harasztia<sup>(3)</sup>, Andras Peter Vida<sup>(3)</sup>, Laszlo Robert Szemethy<sup>(4)</sup>, Arpad Czeh<sup>\*(5)</sup>

<sup>1)</sup> University of Pécs, Faculty of Sciences, External Department of Applied Molecular Sciences - Mycotoxin Research Group, Hungary

<sup>2)</sup> Department of Regional Game Management, Ministry of Agriculture, Hungary

<sup>3)</sup> Soft Flow Ltd., Hungary

<sup>4)</sup> Institute of Biology, University of Pécs, Hungary

<sup>5)</sup> Soft Flow Ltd.; University of Pécs, Faculty of Sciences, External Department of Applied Molecular Sciences - Mycotoxin Research Group, Hungary

\*Corresponding author - E-mail: [aczeh@foss.dk](mailto:aczeh@foss.dk)

Several factors influence toxigenic fungi growth and the production of mycotoxins as secondary metabolites. Environmental factors such as extreme humidity and temperature (recently occurring more often due to climate change) provide increasingly favorable conditions for mycotoxin producing fungi colonization. The microclimatic conditions in each area, and the cultivation or even storage conditions can influence the toxin levels in crops. About 10% of grain harvested globally is lost to natural mycotoxin contamination. This staggering loss underscores the urgent need to reduce global mycotoxin contamination in agricultural products.

Mycotoxins affect growth, disrupt hormonal balance, and are hepatotoxic, nephrotoxic, and carcinogenic. They are absorbed directly from plants and indirectly from meat. The toxins are absorbed in the small intestine; some are released into the bloodstream and excreted in milk, urine. Fungi can be divided based on their origin into fields and storage or their place of cultivation. *Fusarium* species are found in fields, while *Aspergillus* and *Penicillium* species are found primarily in storage. The *Fusarium* species produce DON, T-2 toxin, zearalenone, and fumonisins. In contrast, the *Aspergillus* and *Penicillium* species produce aflatoxin and ochratoxin A (OTA). Based on the results of a study covering the late 20<sup>th</sup> century, *Fusarium* toxins contaminate agricultural products at high levels in "epidemic" years when weather conditions were favorable for Fungal *Fusarium* infections of cereals. Another study's objective, covering the early 2010s, was to comprehensively evaluate the natural DON, total fumonisin, and aflatoxin contamination of corn samples collected from different geographical regions of Hungary. The results show significant spatial variations in toxin levels, providing a comprehensive understanding of the mycotoxin contamination landscape in Hungary.

Our objective was to compare mycotoxin contamination levels in 2023 based on samples collected in different agricultural zones of Hungary. The co-occurrence of the 6 essential mycotoxins was investigated using the MycoFoss™ platform. We determined both post- and pre-harvest toxin levels. The data shows that all 6 mycotoxins occur in some maize. At least 3 mycotoxins were present in all samples, and some of them even contained all 6 mycotoxins. The samples had the highest concentrations of OTA and fumonisin B1+B2+B3.

This study underscores the importance of continuous efforts in sampling and analyzing food and feed to ensure consumer safety in Hungary. Our results are consistent with those published by Alltech reporting on corn with multi-toxin infection. This study reports all 6 mycotoxins for all specimens. The data volume presented here is unfortunately insufficient for providing convincing evidence of how incorrect storage conditions threaten corn's suitability for consumption, however, should this study be extended/continued, the hypothesis may be reinforced.

**Keywords:** MycoFoss™ Platform, multi-mycotoxin contamination, mycotoxin analysis, toxigenic fungi



## S35

### DIVERSITY AND DETERMINATION OF PRYMNESIUM PARVUM TOXINS

Magdalena Pöchlhammer<sup>(1)</sup>, Alexander Conrad<sup>(1)</sup>, Doris Marko<sup>(2)</sup>, Elisabeth Varga<sup>\*(1)</sup>

<sup>1)</sup> Unit Food Hygiene and Technology, Centre for Food Science and Veterinary Public Health, Clinical Department for Farm Animals and Food System Science, University of Veterinary Medicine, Vienna, Austria

<sup>2)</sup> Department of Food Chemistry and Toxicology, Faculty of Chemistry, University of Vienna, Austria

\*Corresponding author - E-mail: elisabeth.varga@vetmeduni.ac.at

The microalga *Prymnesium parvum* is capable of forming harmful algal blooms that lead to devastating fish kills worldwide. For example, in July/August 2022 several hundred tons of fish were killed in the Oder River in Poland/Germany. The suspected causative agents are large ladder frame polyethers called prymnesins which are biomass associated. Currently, they are divided into three groups based on the number of carbon-atoms in the aglycon backbone (A-types: 91, B-types: 85 and C-types: 83). Whereas the backbone structure of the A-type [1] and B-type [2] prymnesins were elucidated by NMR; those of the C-type prymnesins is currently unknown. In a screening study the prymnesin profile of 26 different *P. parvum* strains were assessed and more than 50 different prymnesin-like molecular features could be tentatively identified by HRMS [3]. Each strain is only capable of producing one type, but several different congeners differing in the degree of saturation, chlorine content and attached sugar moieties. Furthermore, the importance of MS/HRMS investigations into the double chlorinated A-type revealed different fragmentation patterns in different strains. This further increased the structural complexity of prymnesins and showed the importance of MS/HRMS investigations.

Currently, due to the lack of analytical standards, an indirect quantification method using fluorescence tagging is necessary [4]. Further challenges include high potency of the toxins despite low production amounts, which require concentration steps, as well as the sticky properties of prymnesins and redissolving issues. Therefore, we are currently optimising an extraction method for the determination of prymnesins from water samples. Ideally, the method should be applicable for small- and large-scale extractions with a high extraction recovery. Both liquid-liquid extraction and solid-phase extraction were assessed.

In conclusion, the current state of knowledge about the diversity of prymnesins and the determination thereof in water samples will be presented.

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[3] Binzer S.B. and Svendsen S.K. *et al.*, A-, B- and C-type prymnesins are clade specific compounds and chemotaxonomic markers in *Prymnesium parvum* Harmful Algae 2019, 81, 10-17.

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**Keywords:** prymnesium parvum, prymnesin, phycotoxin

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S36

### QUANTUM GREEN: FAST AND INNOVATIVE LATERAL FLOW CASSETTES FOR QUANTIFICATION OF DEOXYNIVALENOL AND FUMONISIN IN CEREAL AND ANIMAL FEED, DETECTING TOXIN-FREE SAMPLES IN 45 SECONDS USING THE SAME EXTRACT

Sofia Iliopoulou<sup>(1)</sup>, Antonios Ntantasios<sup>(1)</sup>, Vasilios Meras<sup>(1)</sup>, Theodorakis Ntoupis<sup>(1)</sup>, Sevasti Laporda<sup>(1)</sup>, Alexandra Alevra<sup>(1)</sup>, Maura Begolli<sup>(1)</sup>, Georgios Papageorgiou<sup>(1)</sup>

<sup>1)</sup> ProGnosis Biotech, Greece

\*Corresponding author - E-mail: s.iliopoulou@prognosis-biotech.com

**Overview:** The aim of this study was to develop simple, rapid and highly sensitive methods, for the detection of Deoxynivalenol and Fumonisin in cereal and animal feed, and evaluate their recovery levels in spiked samples and reference materials. The method offers an eco-friendly, common extraction for both toxins.

**Introduction:** Deoxynivalenol (DON), also known as vomitoxin, and Fumonisin (FUMO) are members of the trichothecene mycotoxins produced by the filamentous fungi of *Fusarium* species. Grains are frequently infected by these fungi in the field or during storage. Deoxynivalenol, along with 3-acetyl- and 15-acetyl-DON, as well as Fumonisin, constitute highly toxic molecules. Due to their cytotoxicity, these toxins pose a significant risk to human and animal health. Most regulatory agencies worldwide have set limits on the allowable amount of DON, FB1 and FB2 in human and animal foodstuffs. Accurate and rapid determination of DON and Fumonisin presence in commodities is of paramount importance.

Quantum Green lateral flow cassettes are based on the competitive format immunoassay principle. A capture line of the toxin is placed below the control line. The detection system consists of specific antibodies against the toxin of interest, conjugated to colloidal-gold.

**Methods:** Deoxynivalenol/Fumonisin-free samples were chosen and spiked with a known amount of Deoxynivalenol and Fumonisin standard solution, according to the United States Department of Agriculture (USDA), Agricultural Marketing Service, GIPSA's Federal Grain Inspection Service (FGIS) protocol. The samples were extracted and then further diluted into diluent before being analyzed. Reference materials were also analyzed. Due to its fast scan technology, samples free of Deoxynivalenol/Fumonisin can be detected in 45 seconds. The total reaction time takes only 2 minutes to complete.

**Results:** The determination of the Deoxynivalenol and Fumonisin levels in the spiked samples of the matrices of interest showed that the recovery levels were acceptable. The results were also confirmed by analyzing the reference material. The Coefficient of Variation (CV) of all samples was within the acceptable range.

**Conclusions:** Prognosis Biotech S.A. demonstrates two innovative Lateral Flow methods, Quantum DON Green G40 and Quantum FUMO Green G70, for accurate detection and quantification of Deoxynivalenol (DON) and Fumonisin (FUMO) respectively, in grains, cereals and animal feed samples, using the same extract. They offer results in 45-120 seconds at levels ranging from 0,15ppm up to 5ppm and from 0.25ppm to 5ppm for DON and FUMO respectively. Samples free of DON or FUMO can be detected in 45 seconds. If the concentration of an analyte in samples is greater than the limit of quantification (LOQ), the analysis continues until 2 minutes are complete. This technology provides acceptable recovery and CV levels, high sensitivity and accuracy.

**Keywords:** quantum green, lateral flow test, green extraction, deoxynivalenol and fumonisin, ProGnosis Biotech

S37

### MULTI-MYCOTOXIN ANALYSES BY UPLC-MS/MS IN WHEAT: EMERGING MYCOTOXINS IN WALLONIA IN 2023 AND 2024

**Camille Jonard**<sup>(1)</sup>, **Anne Chandelier**<sup>(2)</sup>, **Damien Eylenbosch**<sup>(3)</sup>, **Anne-Michelle Faux**<sup>(3)</sup>, **Philippe Vermeulen**<sup>(1)</sup>, **Bruno Godin**<sup>(1)</sup>, **Sebastien Gofflot**<sup>(1)</sup>

<sup>1)</sup> Walloon Agricultural Research Centre, Knowledge and valorization of agricultural products Department, Belgium

<sup>2)</sup> Walloon Agricultural Research Centre, Life sciences Department, Belgium

<sup>3)</sup> Walloon Agricultural Research Centre, Productions in agriculture Department, Belgium

\*Corresponding author - E-mail: c.jonard@cra.wallonie.be

The present research is part of a Walloon project "ValCerWal" (funded by "Plan de relance de la Wallonie") conducted by the Walloon Agricultural Research Centre (CRA-W) in Belgium. This project aims to increase the valorisation of cereals such as wheat, spelt, etc. for local production to reach industrial and craft quality requirement. One of its specific goals is to do an overview of the mycotoxins observed on cereals in Wallonia.

Mycotoxins pose a significant threat to food safety and human health. This study focuses on the analysis of wheat aiming to detect and quantify mycotoxins, including both emerging and conventional ones. More specifically, this work proposes a review of the emerging mycotoxins detected in wheat in 2023 and 2024 harvests and highlight the link between the conventional and emerging mycotoxins contamination.

In summer 2023, a total of 114 samples of wheat flour coming from different locations from conventional and organic farming were analysed with a UPLC-MS/MS method able to detect 20 mycotoxins. For 2024 harvest, at least 80 samples were used from various locations in Wallonia.

In this study, the most commonly observed emerging mycotoxins in wheat in Wallonia are enniatins, with enniatin B being the most prevalent. In 2023, only a few samples were contaminated with enniatins, whereas in 2024, the majority of the samples contained varying levels of contamination. At this stage, enniatins detection does not appear to be related to the co-occurrence of deoxynivalenol or zearalenone, which are also frequently found in wheat, despite all being produced by *Fusarium* species.

In conclusion, this research provides a comprehensive analysis of mycotoxin co-contamination in wheat samples from various locations in Wallonia (Belgium) based on two 2 years. These data highlight the presence of enniatins in wheat. The need to define maximum contamination levels for these mycotoxins is still under discussion by the competent authorities, as there is still a lack of knowledge regarding the toxicity properties of these molecules. In addition, continuous monitoring and analysis of conventional and emerging mycotoxins is relevant as it enables the anticipation of risks, the warning of the sector, and the limitation of contaminated batches entering the processing chains.

**Keywords:** mycotoxins, UPLC-MS/MS, Wallonia, wheat, enniatins

S38

### MULTI-MYCOTOXIN ANALYSES BY UPLC-MS/MS: METHOD DEVELOPMENT AND APPLICATION TO BENINESE PEANUT PRODUCTS

**Camille Jonard**\*<sup>(1)</sup>, Sébastien Gofflot<sup>(1)</sup>, O. Herbert Iko Afe<sup>(2)</sup>, Christin Sogbossi Gbetokpanou<sup>(2)</sup>, Paulin Azokpota<sup>(2)</sup>, E. Yann Madode<sup>(2)</sup>, Caroline Douny<sup>(3)</sup>, Marie-Louise Scippo<sup>(3)</sup>

<sup>1)</sup> Walloon Agricultural Research Centre, Knowledge and valorization of agricultural products Department, Belgium

<sup>2)</sup> Laboratory of Food Sciences and Technology, Department of Nutrition, Food Sciences and Technology, Faculty of Agronomic Sciences, University of Abomey-Calavi, Benin

<sup>3)</sup> Université de Liège, Laboratoire d'Analyse des Denrées alimentaires, FARAH-SPV, Belgium

\*Corresponding author - E-mail: c.jonard@cra.wallonie.be

In a collaborative effort between the Walloon Agricultural Research Centre (CRA-W) and the Faculty of Veterinary Medicine at the University of Liège in Belgium, a multi-mycotoxin analytical method was developed using ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS). This method is capable of detecting and quantifying a wide range of mycotoxins, including deoxynivalenol and its derivatives, zearalenone, ochratoxins A and B, aflatoxin B1, enniatins, sterigmatocystin, fumonisin B1, and alternaria toxins. Initially, the method was developed and validated using wheat before being adapted for peanut products.

Following the development phase, a total of 20 peanut samples including raw peanuts (n=10), roasted peanuts (n=8), and peanut cake (n=2) collected from Beninese markets were analyzed. Eleven samples out of the 20 samples were found to contain mycotoxins at concentrations near or exceeding the maximum limits set by European regulations. To confirm these results, these 11 samples were then extracted and analyzed again in duplicate.

Upon confirmation, four mycotoxins were detected: aflatoxin B1, ochratoxins A and B, and sterigmatocystin. The most frequently detected mycotoxin was aflatoxin B1 with concentrations ranging between 1 and more than 200 µg/kg in nine peanut samples. Furthermore, three samples were found to be contaminated with ochratoxin A and ochratoxin B at levels more than ten times higher than the regulatory threshold of 5 µg/kg. Sterigmatocystin was found in three samples at levels below the limit of quantification.

In conclusion, the developed method effectively enables the detection and quantification of mycotoxins in peanut products. Important levels of mycotoxins were recorded in Beninese peanut samples. These findings highlight the critical need for ongoing monitoring and rigorous quality control within the peanut supply chain, particularly in regions susceptible to mycotoxin contamination.

**Keywords:** mycotoxins, UPLC-MS/MS, peanuts, Benin

S39

### OCCURRENCE OF MYCOTOXINS IN IRISH OATS OVER THE YEARS 2022-2023

Melissa Di Rocco<sup>\*1)</sup>, Diana Bucur<sup>2)</sup>, Steven Kildea<sup>2)</sup>, Martin Danaher<sup>1)</sup>

<sup>1)</sup> Teagasc Food Research Centre, Ireland

<sup>2)</sup> Teagasc Oak Park, Ireland

\*Corresponding author - E-mail: melissa.dirocco@teagasc.ie

In this work, a total of 115 oat samples, collected over the years 2022-2023 in Ireland, were analysed in order to investigate the occurrence of 32 mycotoxins, including the regulated mycotoxins, the masked mycotoxins T-2 toxin-3-glucoside (T2G) and deoxynivalenol-3-glucoside (D3G), and the emerging mycotoxins such as the enniatins, beauvericin and moniliformin. The samples included organic, conventional, spring and winter oats from different varieties and locations, and were analysed using a simple dilute-and-shoot sample preparation protocol, followed by ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) determination. The analytes were extracted using a mixture of acetonitrile:water:acetic acid (79:20:1, v/v/v), followed by a 1 min vibrational shaking and 90 min horizontal shaking. After centrifugation, an aliquot of the samples was diluted with acetonitrile:water:acetic acid (20:79:1, v/v/v), filtered and injected onto the UHPLC-MS/MS system. The residues most commonly found were: T-2 toxin (range 10.2-300  $\mu\text{g kg}^{-1}$ ); HT-2 toxin (range 11.2-527  $\mu\text{g kg}^{-1}$ ); enniatin A1 (range 3.2-117  $\mu\text{g kg}^{-1}$ ); enniatin B (range 3.4-481  $\mu\text{g kg}^{-1}$ ); enniatin B1 (range 4.0-385  $\mu\text{g kg}^{-1}$ ); and moniliformin (range 6.7-88  $\mu\text{g kg}^{-1}$ ). Results showed that the enniatins were present in 60% of the samples, with a similar trend over the two years. Among the masked mycotoxins, T2G was found to be present in two samples only at levels ranging from 53 to 57  $\mu\text{g kg}^{-1}$ , while D3G was found at concentrations ranging from 6.7 to 42.9  $\mu\text{g kg}^{-1}$  in seven samples. This study is part of a more comprehensive survey that will be continued over the coming years, and highlights the need for monitoring for a wide range of mycotoxins, with a particular focus on the emerging compounds.

**Keywords:** mycotoxins, masked mycotoxins, emerging mycotoxins, UHPLC-MS/MS

**Acknowledgement:** We would like to thank the Mycotox-I project (Field to fork assessment and mitigation of mycotoxin exposure risk on the Island of Ireland) - DAFM DAERA funding 2022 - 2025.

S40

### A NEW PHYSICAL AND BIOLOGICAL STRATEGY TO REDUCE THE CONTENT OF ZEARELENONE IN INFECTED WHEAT KERNELS: THE EFFECT OF COLD NEEDLE PERFORATION, MICROORGANISMS, AND PURIFIED ENZYME

**Amandine Andre**<sup>\*(1)</sup>, **Katrin Hecht**<sup>(2)</sup>, **Sandra Mischler**<sup>(1)</sup>, **Rebecca Buller**<sup>(2)</sup>, **Susette Freimüller Leischtfeld**<sup>(1)</sup>, **Irene Chetschik**<sup>(1)</sup>, **Susanne Miescher Schwenninger**<sup>(1)</sup>, **Nadina Müller**<sup>(1)</sup>

<sup>1)</sup>ZHAW Zurich University of Applied Sciences, Department of Life Sciences and Facility Management, Institute of Food and Beverage Innovation, Switzerland

<sup>2)</sup>ZHAW Zurich University of Applied Sciences, Department of Life Sciences and Facility Management, Institute of Chemistry and Biotechnology, Switzerland

\*Corresponding author - E-mail: andn@zhaw.ch

With the aim of reintroducing wheat grains naturally contaminated with mycotoxins into the food value chain, a decontamination strategy was developed in this study. For this purpose, in a first step, the whole wheat kernels were pre-treated using cold needle perforation. The pore size was evaluated by scanning electron microscopy and the accessibility of enzymes and microorganisms determined using fluorescent markers in the size range of enzymes (5 nm) and microorganisms (10 µm), and fluorescent microscopy. The perforated wheat grains, as well as non-perforated grains as controls, were then incubated with selected microorganisms (*Bacillus megaterium* Myk145 and *B. licheniformis* MA572) or with the enzyme ZHD518. The two bacilli strains were not able to significantly reduce the amount of zearalenone (ZEA), neither in the perforated nor in the non-perforated wheat kernels in comparison with the controls. In contrast, the enzyme ZHD518 significantly reduced the initial concentration of ZEA in the perforated and non-perforated wheat kernels in comparison with controls. Moreover, *in vitro* incubation of ZHD518 with ZEA showed the presence of two non-estrogenic degradation products of ZEA: hydrolysed zearalenone (HZEA) and decarboxylated hydrolysed ZEA (DHZEA). In addition, the physical pre-treatment led to a reduction in detectable mycotoxin contents in a subset of samples. Overall, this study emphasizes the promising potential of combining physical pre-treatment approaches with biological decontamination solutions in order to address the associated problem of mycotoxin contamination and food waste reduction.

**Keywords:** mycotoxins, zearalenone, wheat kernels, cold needle perforation, biological decontamination

**Acknowledgement:** The authors would like to thank all the collaborators from the research groups of food chemistry, food biotechnology, food technology and biocatalysis of the ZHAW for their support during the course of the research project. The authors would also like to thank John Bennett for proofreading this paper.

## S41

### EFFECTIVENESS OF COMMERCIAL ADSORBENTS FOR THE ELIMINATION OF MYCOTOXINS

**Zbynek Dzuman**\*<sup>(1)</sup>, Milena Stranska<sup>(1)</sup>, Nela Prusova<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\*Corresponding author - E-mail: zbynek.dzuman@vscht.cz

The presence of toxic mycotoxins commonly occurring in feed (such as zearalenone and trichothecenes) and endotoxins, lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria, pose a significant threat animal health and productivity. These compounds can lead to a wide range of adverse effects in animals, including immunosuppression, reproductive issues, reduced growth rates, and in severe cases, death. However, these risks can be effectively reduced by a combination of preventive measures such as pre- and post-harvest crop management and the strategic use of mycotoxin adsorbents. These materials particularly made of clay minerals, activated charcoal, yeast cell wall components, and synthetic polymers are added to feed as supplements which form a complex of adsorbent and contaminant in the gastrointestinal tract, thus preventing their absorption into the bloodstream.

Within the presented study, a wide spectrum of mycotoxin adsorbents (n = 60) based on clay minerals and activated charcoal provided by a major local producer were tested *in vitro* to determine the effectiveness of adsorption of endotoxin and mycotoxins zearalenone, T-2 toxin and deoxynivalenol. The adsorption testing was based on a methodology using incubated shaking at 37°C in a phosphate buffer at pH = 3 for three hours followed by exchange of buffer with pH = 6,5 and further shaking which corresponds to conditions in different parts of animals' gastrointestinal tract. Subsequently, mycotoxins were determined by internally validated method utilizing liquid chromatography coupled with high resolution tandem mass spectrometry (U-HPLC-HRMS/MS); endotoxin was detected using recombinant factor C assay with fluorometric detection. Many of tested sorbents showed promising results regarding adsorption of endotoxin, zearalenone and T-2 toxin (mean adsorption of 100 %, 66 % and 55%, respectively) while results for deoxynivalenol were rather poor (adsorption of 11 %). In any case, the addition of such sorbents is a promising way to protect farm animals thus reduce economic losses.

**Keywords:** mycotoxins, endotoxin, adsorbents, liquid chromatography - high resolution mass spectrometry, fluorometric detection

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S42

## DEGLYCOSYLATION OF "MASKED" HT2/T2 TOXINS AND QUANTIFICATION IN CEREALS

**Tereza Dolezalova<sup>(1)</sup>, Nela Prusova<sup>(1)</sup>, Adam Behner<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Milena Stranska<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: zacharim@vscht.cz*

Mycotoxins, toxic secondary metabolites produced by microscopic filamentous fungi, are often modified by plant enzymes after infection. In the case of *Fusarium* mycotoxins, the most common mechanism of modification is conjugation with saccharides. While the determination of free mycotoxins is well established, the levels of conjugated forms remain unknown due to challenges such as unknown structures, limited availability of analytical standards and poor extractability of some forms. Therefore, the aim of our study was to develop and validate a method for the indirect quantification of the conjugated forms of HT-2 toxin (HT2) and T-2 toxin (T2) using alpha-amylase, glucoamylase, endo-1,3(4)-beta-glucanase and beta-glucosidase. The procedure consists of three steps, in which the enzymes are added directly to the homogeneous sample and the optimum conditions (temperature and pH) are always set for each enzyme/mixture of enzymes. Separation of the mycotoxins before and after hydrolysis was performed on Acquity UPLC<sup>®</sup> HSS T3 reversed-phase column (100 mm x 2.1 mm, 1.8 µm; Waters), with subsequent detection assured by Q-orbitrap mass spectrometer in full-spectral acquisition mode with systematic fragmentation of precursor ions in electrospray positive ionisation mode. Quantification was performed using isotopically labelled internal standards [<sup>13</sup>C<sub>22</sub>]-HT2 and [<sup>13</sup>C<sub>22</sub>]-T2 to compensate for matrix effects. As part of the method validation, the degradation of HT2 and T2 and the conversion of T2 to HT2 during hydrolysis were also investigated. The method was then applied to 10 barley samples. The proportion of HT2 and T2 originally 'hidden' in modified forms was approximately 50% of their total content.

**Keywords:** HT-2 glycosides, T-2 glycosides, enzymatic hydrolysis, high-resolution mass spectrometry, mycotoxins

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### S43

#### MATRIX EFFECTS WHEN QUANTIFYING MYCOTOXINS IN VARIOUSLY COMPLEX CRAFT BEER MATRICES - WHY DOES IT MATTER?

**Tereza Dolezalova<sup>(1)</sup>, Nela Prusova<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Milena Stranska<sup>\*(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: zacharim@vscht.cz*

Raw materials used in beer production can be contaminated with mycotoxins, which may transfer to the final product. Beer, especially the craft beers with rather high original wort extract (OWE) and various non-barley ingredients, is a complex matrix, and despite partial purification during sample preparation, there is a significant influence of matrix effects. The aim of the study was to analyse 57 mycotoxins in 135 samples of a broad range of craft beers with an OWE of 12 vol% or higher, made from different types of malt (barley, wheat, oats, and rye), and to assess the matrix effect in them. Separation of the target analytes was performed by ultra-high performance liquid chromatography on an Acquity UPLC® HSS T3 reversed-phase column (100 mm x 2.1 mm, 1.8 µm; Waters) and detection by high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) using Q-orbitrap mass analyser (Thermo Scientific). Data were acquired in *parallel reaction monitoring* (ESI-) and *fullMS-data dependentMS<sup>2</sup>* (ESI+). The method was validated for different beer types categorised according to the malt used, the colour of the beer (light and dark), and the OWE level, i.e., low (~12%), medium (~16%), and high (~23%). For this purpose, a total of 7 matrix calibration batches were prepared to represent each group. The results showed that especially the beer colour had significant influence on the matrix effects. Under the conditions of our method, more polar mycotoxins, mainly deoxynivalenol (DON), were more affected by matrix-induced signal suppression / enhancement, depending on the beer matrix. For DON, this could in some cases lead to a tenfold overestimation or underestimation of the calculated levels if an inappropriate matrix calibration series was used. On the other hand, for HT-2 and T-2 toxins (HT2, T2), eluting in later retention times, the signal suppression/enhancement was not as significant, on average 10% for HT2 and even less for T2. Among the mycotoxins detected in the samples, the most prevalent was DON (85% of all samples) with concentrations ranging from 0.5 to 50 µg/L and deoxynivalenol-3-glucoside (D3G) (41% of the samples) at levels from 1 to 91 µg/L. HT-2 and T-2 toxins were found in 12% of the samples at levels of 1-3 µg/L and in 7% of the samples at levels of 0.5-2 µg/L, respectively.

**Keywords:** beers, mycotoxins, matrix effect, UHPLC-HRMS/MS

**Acknowledgement:** *The work used [data/tools/services/facilities] provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064) and was supported from the grant of Specific university research - grant No. A1\_FPBT\_2024\_006 and No. A2\_FPBT\_2024\_052.*

S44

### A NOVEL STRATEGY FOR THE ANNOTATION OF UNKNOWN PYRROLIZIDINE ALKALOIDS

Leonie Straub<sup>\* (1)</sup>, Federico Padilla Gonzalez<sup>(1)</sup>, Han Zuilhof<sup>(1)</sup>, Laura Righetti<sup>(1)</sup>, Patrick Mulder<sup>(2)</sup>

<sup>1)</sup> Wageningen University & Research, The Netherlands

<sup>2)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: leonie.straub@wur.nl

Worldwide food safety is an ongoing concern, exacerbated by globalisation and climate change. One of the key issues are natural toxins such as pyrrolizidine alkaloids (PAs). They have been implicated in numerous incidents over the past 120 years, affecting both livestock and humans. More than 6000 plant species have been estimated to contain PAs, mostly members of the Boraginaceae, Asteraceae and Fabaceae families [1]. PAs exhibit carcinogenic and genotoxic properties following chronic exposure and hepatotoxic activity following acute poisoning. Particularly 1,2-unsaturated PAs are associated with higher toxicity, as they form DNA and protein adducts after metabolism in the liver [2]. Chemically, PAs are the mono- or diesters of four main necine bases with numerous necic acids. As a consequence of their chemical structure, there is a huge variety of potential compounds. The determination of unknown compounds is further complicated by the regular occurrence of isomers. Due to their wide distribution in nature, toxicity and structural, it is essential to have an overview which new emerging PAs occur and how the accumulation in different plant species might be triggered.

An important technique for the analysis of unknown PAs is untargeted high-resolution mass spectrometry (HRMS). Compared to targeted MS/MS scans, it allows the screening of all compounds present in the sample. However, it produces a huge amount of data, which complicates the processing. To address this challenge, novel approaches such as computational tools are required as prioritization strategies. Among these approaches, feature based molecular networking (FBMN) is a promising tool to group PAs based on the similarity of their MS<sup>2</sup> spectra, creating a molecular network. This allows the investigation of relationships between unknown and known compounds and therefore facilitates conclusions about their putative structure.

About 80 PA standards are commercially available, which account for only about 10% of the estimated number of existing PAs. However, these standards represent the different structural families, making them suitable for FBMN. As this approach relies heavily on the availability and quality of MS<sup>2</sup> spectra, a database (DB) will be created, since in Global Natural Products Social Molecular Networking (GNPS) only 20 HRMS PAs spectra are available<sup>3</sup>. This new DB will be validated by applying it to a set of known Jacobaea samples and comparing the results to previous experiments. Eventually, the DB can be used to build a repository containing information on the PA profiles correlated to different plant parts of the Jacobaea species. This will be a great improvement for food safety, as it allows the identification of the contaminating weed species in PA-containing food samples.

[1] Casado, et al. *Trends in Food Science & Technology* 120, 123-139 (2022).

[2] Fu, et al. *Drug Metabolism Reviews* 36, 1-55 (2004). <sup>3</sup> Wang, et al. *Nature Biotechnology* 34, no. 8 (2016).

**Keywords:** pyrrolizidine alkaloids, high-resolution mass spectrometry, molecular networking, spectral library

### S45

#### CO-OCCURRENCE OF T-2 AND HT-2 TOXINS AND THEIR GLUCOSIDES IN WHEAT AND OAT GRAINS

Adam Pierzgalski<sup>(1)</sup>, Marcin Bryła<sup>\*(1)</sup>, Benedikt Cramer<sup>(2)</sup>, Hans-Ulrich Humpf<sup>(2)</sup>, Magdalena Twaruzek<sup>(3)</sup>

<sup>1)</sup> Institute of Agricultural and Food Biotechnology, Poland

<sup>2)</sup> Institute of Food Chemistry, University of Münster, Germany

<sup>3)</sup> Faculty of Biological Sciences, Kazimierz Wielki University, Poland

\*Corresponding author - E-mail: marcin.bryla@ibprs.pl

The aim of this study was the simultaneous determination of T-2 and HT-2 toxins and the  $\alpha$  and  $\beta$  anomers of their glycosides to assess their content in wheat and oat grains harvested in Poland (2020-2022). Of 298 wheat samples, only 14 (5%) contained the sum of T-2 and HT-2 toxins (average 34.2  $\mu\text{g}/\text{kg}$ ; 10.6-67.7  $\mu\text{g}/\text{kg}$ ). In oats ( $n = 129$ ), these compounds were detected much more frequently (70% of samples) at an average level of 107.5  $\mu\text{g}/\text{kg}$  (6.9-949.1  $\mu\text{g}/\text{kg}$ ). The sum of T-2 and HT-2 glucosides was detectable in 3% of wheat samples (mean 16.3  $\mu\text{g}/\text{kg}$ ; 7.1-39.4  $\mu\text{g}/\text{kg}$ ) and 65% of oat samples (mean 35.1  $\mu\text{g}/\text{kg}$ ; 4.0-624.1  $\mu\text{g}/\text{kg}$ ). Following the study, T-2-3- $\alpha$ -glucoside was identified as the only naturally occurring anomer, while both HT-2-3-glucoside anomers were detected with a higher content and frequency of HT-2-3- $\beta$ -glucoside than the  $\alpha$ -anomer of this compound.

The study confirmed previous reports of higher levels of HT-2 in cereals compared to T-2, as well as the relatively high content of these toxins in oat grains. Furthermore, it was indicated that T-2-3- $\alpha$ -Glc is the only naturally occurring anomer of this compound in wheat and oat grains, and that HT-2-3- $\beta$ -Glc is present in these cereals in much higher amounts than HT-2-3- $\alpha$ -Glc. Correlation analysis between the sum of T-2 and HT-2 content and their glycosides in oat grains indicates that these values are highly correlated with each other. It remains to be determined in the future whether these anomers are formed as a result of the metabolism of T-2/HT-2 by the plants or whether they are secreted by the *Fusarium* fungi that infect them. In addition, the formation of HT-2-glucoside anomers, which may be formed by deacetylation of the corresponding T-2-glucoside anomers or by deacetylation of T-2 to HT-2 and subsequent glycosylation to C-3 or C-4, remains unclear. It is very important to know the toxic properties of T-2- and HT-2-glucoside anomers. Key aspects are cytotoxicity, cell membrane permeability and the ability to hydrolyse with the release of native toxins.

**Keywords:** occurrence, HT-2 and T-2 toxins, glucosides of HT-2 and T-2 toxins, *Fusarium* toxins, cereal grain

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S46

### OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN ROOIBOS TEA FROM THE EUROPEAN MARKET

**Alessandro Volkov<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>, Ondrej Brabenec<sup>(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: hajslovj@vscht.cz*

Pyrrrolizidine alkaloids (PAs) are secondary metabolites produced by plants and can be found in various food commodities, including herbal teas. These alkaloids are of high concern as they are associated with chronic toxicity in humans causing hepatotoxicity, carcinogenic and mutagenic activity. The European Union has recently established maximum limits for PA in rooibos tea, with a maximum concentration of 400 µg/kg calculated as the sum of 35 PA listed in European regulation 915/2023. The present study focused on monitoring of PAs in rooibos tea on the European market. In fact, 203 samples from 18 European countries have been collected and analyzed. For the analysis, highly sensitive (with limit of quantification of 1 µg/kg) ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) was employed and a total of 32 PAs were monitored. Target PAs were detected in 201 out of 203 samples analyzed (99% positivity) however, all the contaminations were below the limit imposed by the European regulation. The main alkaloid responsible for contamination is senecionine N-oxide, which is present in 97% of positive samples, while other alkaloids with relevant concentrations often found are: senecionine (89%), retrorsine N-oxide (66%) and senecivernine N-oxide (40%).

**Keywords:** pyrrolizidine alkaloids, rooibos, herbal tea, mass spectrometry, liquid chromatography

S47

### MONITORING OF PYRROLIZIDINE AND TROPANE ALKALOIDS IN CZECH HONEY

**Ondrej Rubas**<sup>\*(1)</sup>, **Zbynek Dzuman**<sup>(1)</sup>, **Ondrej Brabenec**<sup>(1)</sup>, **Dalibor Titera**<sup>(2)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

<sup>2)</sup> *Czech Bee Research Institute, Czech Republic*

\*Corresponding author - E-mail: rubaso@vscht.cz

Honey has been popular functional food since ancient times due to its health benefits based on the presence of bioactive substances with antioxidant, antimicrobial and anti-inflammatory properties. Despite its many health benefits, honey may also contain contaminants that are potentially hazardous to human health. In most cases, the cause of contamination is due to anthropogenic activity, as in the case of pesticide residues, polycyclic aromatic hydrocarbons and heavy metals; bacterial spores or toxic plant substances such as isoquinoline, quinoline, tropane and pyrrolizidine alkaloids may also be present in honey.

Pyrrolizidine alkaloids are produced as secondary metabolites by some dicotyledonous plants mainly of the families *Boraginaceae*, *Asteraceae*, and in the case of tropane alkaloids *Solanaceae* and *Convolvulaceae*. Pyrrolizidine and tropane alkaloids are transferred to honey from nectar or pollen carried by honeybees (*Apis mellifera*).

In this study, target analysis of 32 pyrrolizidine alkaloids and 16 tropane alkaloids was performed on a set of 173 samples of authentic honey from Czech breeders using U-HPLC-MS/MS. In addition, target screening of 134 additional pyrrolizidine alkaloids was performed using U-HPLC-HRMS/MS. Pyrrolizidine alkaloids were detected in 12 % of samples, nevertheless, their concentrations were low in the range from 1 to 26 µg/kg. Echimidine was detected as the most abundant contaminant. Target screening showed possible presence of 4 additional toxins of this group. In 3 % samples also tropane alkaloids were detected. Their concentrations ranged from 2 to 10 µg/kg. Interestingly, this study is the first to report detection of norscopolamine in 2 samples of honey in addition to atropine and scopolamine.

**Keywords:** pyrrolizidine alkaloids, tropane alkaloids, mass spectrometry, honey

**Acknowledgement:** *The work used [data/tools/services/facilities] provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064).*

S48

## STRUCTURAL MODIFICATIONS OF CERTAIN PYRROLIZIDINE ALKALOIDS DURING SAMPLE EXTRACTION AND ITS IMPACT ON ANALYTICAL RESULTS

Florian Kaltner<sup>\*(1)</sup>, Lisa Monika Klein<sup>(1)</sup>

<sup>1)</sup> Chair of Food Safety and Analytics, Faculty of Veterinary Medicine, LMU Munich, Germany

\*Corresponding author - E-mail: florian.kaltner@lmu.de

Pyrrrolizidine alkaloids (PAs) are a group of plant secondary metabolites with toxic properties to humans and livestock. PAs can occur as contaminants in food or feed and are typically extracted from such samples using liquid extraction. The crude extracts are then often purified using solid-phase extraction (SPE) cartridges before being analysed by LC-MS/MS. During the development of analytical methods based on strong cation exchange SPE, certain structurally related PAs showed unexpectedly low or significantly increased recoveries, suggesting that transformation reactions have been occurred [1,2].

To investigate this hypothesis, sample preparations were conducted using PA-free milk as a food matrix, water, or organic solvents into which PA reference standards were spiked before or after critical steps of the following general protocol: First, crude extracts were obtained via liquid-liquid extraction. These were further purified using preconditioned polymeric strong cation exchange SPE cartridges. The alkaline methanol eluates were dried under vacuum, reconstituted, filtered and analysed via LC-MS/MS. To also test non-protic eluent solvents, acetonitrile with or without ammonia was directly spiked, evaporated and analysed.

The results revealed a significant decrease in acetylated PA N-oxides to their corresponding deacetylated compounds as well as the formation of epoxydic PAs from PA compounds containing chlorine and hydroxyl groups in the  $\alpha$  position. Evaporation of the alkaline SPE eluates, combined with the use of the protic solvent methanol in cases of deacetylation, was responsible for these phenomena. An alkaline ester hydrolysis mechanism was hypothesised for the deacetylation, while an internal S<sub>N</sub>2 reaction, similar to the chlorohydrin reaction [3], was suggested for the formation of epoxy PA compounds. Consequently, using different sample preparation methods may inadvertently influence the determined PA patterns in food samples.

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**Keywords:** pyrrolizidine alkaloids, analytical method, modification

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S49

### TOXIC ALKALOID OR INTERFERENCE? PITFALLS IN LC-MS ANALYSIS OF TROPANE AND PYRROLIZIDINE ALKALOIDS

**Ondrej Brabenec**\*<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\*Corresponding author - E-mail: Brabeneo@vscht.cz

Tropane (TAs) and pyrrolizidine alkaloids (PAs) are toxic secondary metabolites that can be transferred into human food chain. Currently, approximately 200 TAs and 660 PAs are known, for 2 TAs and 35 PAs (the dominating representatives of these toxins) maximum limits have been set in selected food commodities (Commission Regulation (EU) 2023/915). LC-MS/MS methods are commonly used for the analysis of PAs, and obtaining high-quality unbiased data, specifically in the latter case, is a challenging task since number of isomeric species exists, and for many of them analytical standards are not commercially available. Chromatographic and/or spectral resolution is the key condition for accurate analysis. This study illustrates various troubleshooting scenarios and demonstrates the need to employ complementary principles, both in terms of separation (reversed phase and HILIC) and detection (QqQ and orbitrap). Both targeted UHPLC-MS/MS method and UHPLC-HRMS/MS targeted screening were validated for several plant matrices and honey.

**Keywords:** tropane alkaloids, pyrrolizidine alkaloids, isomers, liquid chromatography, mass spectrometry

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S50

### VALIDATION OF SINGLE EXTRACTION METHOD FOR THE SIMULTANEOUS ANALYSIS OF MYCOTOXINS USING IMMUNOAFFINITY COLUMNS

Dave Leeman<sup>(1)</sup>, Andrew Allen<sup>(1)</sup>, Helen Cameron<sup>(1)</sup>, Carol Donnelly<sup>(1)</sup>, Claire Milligan<sup>(1)</sup>, Adam Tramseur<sup>(2)</sup>, Joanna Stratton<sup>(2)</sup>, Susan MacDonald<sup>(2)</sup>, Elizabeth Mannaing<sup>\*(1)</sup>

<sup>1)</sup> R-Biopharm Rhone Ltd, United Kingdom

<sup>2)</sup> Fera Science Ltd, United Kingdom

\*Corresponding author - E-mail: Elizabeth@r-biopharmrhone.com

This validation evaluated the 11+Myco MS PREP® immunoaffinity column with LC-MS/MS for simultaneous determination and confirmation of aflatoxins B1, B2, G1, G2, and M1; deoxynivalenol, fumonisins B1, B2, and B3; ochratoxin A; T-2; HT-2; and zearalenone from a single sample. Matrices tested includes corn, wheat, cereal-based baby food, paprika, chili powder and animal feed. An independent laboratory verified method performance on corn and animal feed.

11+Myco MS PREP® uses a single extraction method that requires no pH adjustment that can be applied to a range of matrices giving excellent clean-up. Solvent based standards can be used for calibration removing the need for expensive isotopic standards to correct recoveries. Its ability to analyse 11+ mycotoxins simultaneously is highly advantageous as it saves significant time, reagents and consumables without compromising on results.

Once extracted the sample analysis was done using LC-MS/MS with Electrospray Ionization mode Scheduled Multiple Reaction Monitoring in positive polarity. Data were analysed for recovery, repeatability precision, LOD<sub>est</sub>, LOQ, and method selectivity.

For acceptable bias, there was no case where a ≥20% matrix effect on a calibration curve was observed. Therefore, the solvent-based mixed standard calibration curve was sufficient. Selectivity results demonstrated the ability of the 11+Myco MS-PREP® method to react to all variants of the analytes and exclude similar compounds or other mycotoxins that could be encountered in the claimed matrixes. There were no significant positive or negative interferences observed from the challenges.

**Keywords:** validation, single extraction, 11+, LC-MS/MS



S51

## IMPROVING INTERNAL LABORATORY PROCESSES AND SUSTAINABILITY USING RAPID ONLINE AUTOMATION FOR AFLATOXIN ANALYSIS

Sebastian Wissmueller<sup>(1)</sup>, Alexander Burkon<sup>(1)</sup>, Markus Kopp<sup>(1)</sup>, Elizabeth Manning<sup>(2)</sup>, Naomi McLachlan<sup>(2)</sup>, Carol Donnelly<sup>\*(2)</sup>

<sup>1)</sup> Chemical Institute BURkon, Germany

<sup>2)</sup> R-Biopharm Rhone Ltd, United Kingdom

\*Corresponding author - E-mail: Carol@r-biopharmrhone.com

Automation is becoming an increasingly important tool for laboratories to manage growing sample numbers and to maintain the highest quality results. There are also added pressures on the lab to make best use of valuable technician time, to manage overall costs and improve workflow.

At Institute Burkon, there has been an increase in sample analysis for mycotoxins creating a bigger demand on staff resources, consumable and solvent costs. Like many laboratories we are dedicated to improving and using state-of-the-art methods wherever possible for the analysis of food products. Following a review of various automation options the CHRONOS Symbiosis RIDA®CREST was selected due to consistency of results with a wide variety of matrices. The re-usability of the IMMUNOPREP® ONLINE immunoaffinity cartridge also requires less solvent, reduced storage and transport costs helping to maintain our policy on sustainability.

As part of the system and method validation, several FAPAS samples were analysed achieving good z-scores. In addition, a variety of spiked matrices were tested, and the methods measured for specificity, precision, trueness, linearity, and limit of quantitation.

Maize, prunes, ginger, cardamom and cashews were extracted using a single extraction method with 80% methanol. This extraction method was previously used in conjunction with immunoaffinity columns at the bench and was easily transferred to IMMUNOPREP® ONLINE AFLATOXIN cartridges with CHRONOS Symbiosis RIDA®CREST increasing the speed of conversion to an automated system. In terms of method performance, EU 2023/2782 has significantly simplified requirements from those in EC 401/2006. Apart from some specific cases of aflatoxins, ochratoxin A and ergot alkaloids, limits of quantification (LOQs) must be less than half of the maximum limits and preferably less than 20 % of the maximum limits.

Institute Burkon work to an LOQ which is 10 % of the maximum regulatory limits in foodstuff (except for infant formula) and successfully achieved this target using the automated IMMUNOPREP® ONLINE AFLATOXIN method.

At spiking levels of 0.2 ppb, recoveries ranged from 87 % - 118 % with a RSD of between 0.99 % - 7.75 %. At spiking levels of 5 ppb, recoveries ranged from 85 % - 105 % with a RSD of between 0.91 % - 3.32 %. Therefore, complying with the new regulations.

The CHRONOS Symbiosis RIDA®CREST was found to be easy to use, providing faster results, reducing manual handling whilst improving quality and consistency of result.

**Keywords:** automation, aflatoxin, rapid analysis, re-usability, sustainability

### S52

#### ASSESSMENT OF DIFFERENT EXTRACTION METHODS FOR THE VALIDATION OF AN ANALYTICAL MULTI-METHOD FOR THE DETERMINATION OF ALTERNARIA MYCOTOXINS IN WHEAT BY LC-MS/MS

Vojislava Bursic <sup>(1)</sup>, Aleksandar Vatazevic <sup>(2)</sup>, Gorica Vukovic\*<sup>(2)</sup>, Bojan Konstantinovci <sup>(1)</sup>, Tijana Stojanovic <sup>(1)</sup>, Natasa Samardzic <sup>(1)</sup>, Milena Popov <sup>(1)</sup>

<sup>1)</sup> University of Novi Sad, Faculty of Agriculture, Serbia

<sup>2)</sup> Filed Test Ltd., Serbia

\*Corresponding author - E-mail: gorica.vukovic@fieldtest.rs

A multi-analyte method for the identification and quantification of *Alternaria* mycotoxins was developed in wheat samples by high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Three extraction methods were evaluated for the extraction of alternariol (AOH), tentoxin (TEN), and alternariol monomethyl ether (AME) mycotoxins including three different methods QuEChERS (dSPE), QuEChERS (SPE) and solid-phase extraction (SPE) methods. All the methods were validated in the terms of the limits of detection (LOD), limits of quantification (LOQ), linearity, repeatability, reproducibility and recovery in accordance with the Regulation (EU) 2023/2782. The blank wheat samples were spiked at two levels 0.02 and 0.1 mg kg<sup>-1</sup> in six replicates. The QuEChERS (dSPE) sample preparation method demonstrated optimal performance, yielding average recoveries and precisions (expressed as RSDr, %) of 107.6 ± 6.84% for AOH, 108.0 ± 6.78% for TEN, and 110.1 ± 6.50% for AME. The achieved linearity for procedural calibration across all analytes was R<sup>2</sup> > 0.9997. The CrossTOX cartridge produced results comparable to the QuEChERS (dSPE) method, with recoveries of 96.1 ± 16.10% for AOH, 95.9 ± 9.04% for TEN, and 102.1 ± 11.84% for AME. Using the Bond Elut Mycotoxin cartridge, the recovery and precision for TEN were 108.4 ± 11.97%, and for AME, 96.9 ± 9.53%. However, the detection and identification of alternariol was not possible with the Bond Elut Mycotoxin cartridge, indicating a potential area for further research.

**Keywords:** plan toxins, alternaria, LC-MS/MS, QuEChERS

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S53

### OCCURRENCE OF PYRROLIZIDINE ALKALOIDS IN BLACK PEPPER

**Aleksandar Vatazevic**<sup>(1)</sup>, **Gorica Vukovic**\*<sup>(1)</sup>, **Sanja Novkovic**<sup>(1)</sup>, **Vojislava Bursic**<sup>(2)</sup>, **Nikola Puvača**<sup>(3)</sup>, **Bojana Spirovic-Trifunovic**<sup>(4)</sup>

<sup>1)</sup> *Field Test Ltd., Serbia*

<sup>2)</sup> *University of Novi Sad, Faculty of Agriculture, Serbia*

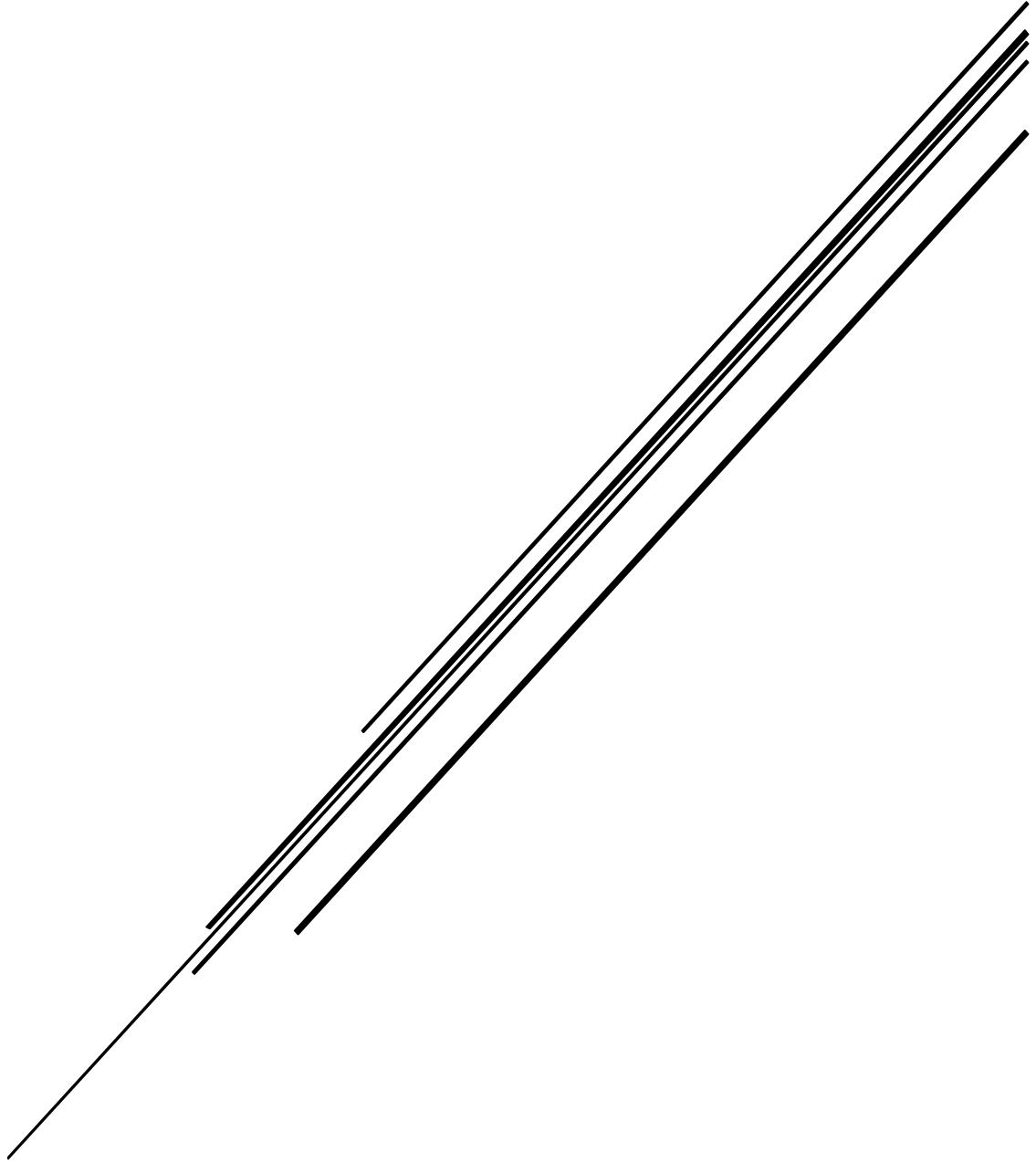
<sup>3)</sup> *Faculty of Economics and Engineering Management, University Business Academy, Serbia*

<sup>4)</sup> *University of Belgrade, Faculty of Agriculture, Serbia*

\*Corresponding author - E-mail: [gorica.vukovic@fieldtest.rs](mailto:gorica.vukovic@fieldtest.rs)

Pyrrrolizidine alkaloids (PAs) are toxins exclusively biosynthesized by plants. Over 6,000 plant species are known to contain PAs, and they are produced by plants as a defense mechanism against herbivores, apropos they are typical plant secondary metabolites. PAs are believed to be present in 2% of all flowering plants, especially occurring in the families Asteraceae (tribe Senecioneae and Eupatorieae), Boraginaceae, Apocynaceae (tribe Echiteae) and Fabaceae (genus *Crotalaria*). PAs can contaminate food and feed. Especially honey, pollen, tea, herbal teas, herbal food supplements, spices, and aromatic herbs are well-known sources of PAs exposure to humans, wildlife and livestock. In recent years, an increasing number of reports revealed relatively high contaminations with PAs in food and herbal infusions and teas not prepared from so-called 'pyrrolizidine alkaloids containing plants', which is mainly due to cross contamination during harvesting. The main toxic effects of PAs are on the liver and lungs, but EFSA has concluded that some PAs may act as carcinogens in humans. In this study, a previously validated LC-MS/MS analytical method for the determination of pyrrolizidine in black pepper was used in the analysis of 10 samples of this spice. The experimental procedure involves a simple and fast QuEChERS extraction followed by LC-MS/MS analysis. To avoid matrix effect, a matrix-matched calibration was used. The method was validated according to the requirements Commission Regulation (EU) 2023/2783 for terms of linearity ( $R^2 > 0.995$ ), precision (%RSDr from 4,78(lm) to 12,9(Er-Ox)), and Recovery (from 83,4%(Em) to 116,6%(Sn-Ox)). The LOQs ( $25 \mu\text{gkg}^{-1}$ ) were found to be below the maximum levels specified by the Commission Regulation (EU) 2023/915, proving this method to be highly suitable for ensuring effective control of black pepper. Ten black paper samples were analyzed of which pyrrolizidine alkaloids were detected in two of them. None of detected concentrations were above the maximum level.

# NANOPARTICLES



## T1

### PHYSICOCHEMICAL CHARACTERISATION OF IRON OXIDES AND HYDROXIDES APPLIED AS FOOD ADDITIVE E 172

**Lisa Siciliani**<sup>(1)</sup>, **Francesco Fumagalli**<sup>(2)</sup>, **Eveline Verleysen**<sup>(3)</sup>, **Jessica Ponti**<sup>(2)</sup>, **Francesco Cubadda**<sup>(4)</sup>, **Andrea Haase**<sup>(5)</sup>, **Sara Bals**<sup>(6)</sup>, **Jan Mast**<sup>(3)</sup>

<sup>1)</sup> *Sciensano / University of Antwerp, Belgium*

<sup>2)</sup> *Joint Research Centre (JRC), Italy*

<sup>3)</sup> *Sciensano, Belgium*

<sup>4)</sup> *Istituto Superiore di Sanità - National Institute of Health, Italy*

<sup>5)</sup> *Bundesinstitut für Risikobewertung - German Federal Institute for Risk Assessment, Germany*

<sup>6)</sup> *University of Antwerp, Belgium*

\*Corresponding author - E-mail: [siciliani.lisa@gmail.com](mailto:siciliani.lisa@gmail.com)

Iron oxides and hydroxides are a group of inorganic substances allowed for use as food additive E 172 in the European Union. They were re-evaluated by EFSA as food additive in 2015, following the classification of the EU specifications, namely iron oxide yellow: hydrated iron (III) oxide ( $\text{FeO}(\text{OH})\cdot\text{H}_2\text{O}$ ), iron oxide red: anhydrous ferric oxide ( $\text{Fe}_2\text{O}_3$ ), and iron oxide black: ferrosol ferric oxide ( $\text{FeO}\cdot\text{Fe}_2\text{O}_3$ ). After the publication of the 2015 re-evaluation, ensemble methods and counting methods, including electron microscopy, reported particle sizes in the nano- and micrometre size range, and showed high amounts of iron oxide nanoparticles (NP) in commercially available E 172. In this context, a detailed physicochemical characterisation of six different E 172 materials (2 yellow, 3 red and 1 black) available on the European market was performed.

For qualitative and quantitative (scanning) transmission electron microscopy ((S)TEM) analysis, the materials were dispersed following an adapted Nanogenotox protocol ensuring electrosteric stabilisation. This resulted in stable dispersions, with maximal de-agglomeration, for all materials except the black iron oxide. Based on their particle morphology, at least 4 different forms of E 172 were identified. The manually measured constituent particle size distributions showed that all materials contained a fraction of nanoparticles. In addition, STEM-tomography showed pores at the surface of the rod-like hematite materials. The diffraction patterns of the yellow, red and black E 172 materials measured by both electron diffraction and X-ray diffraction matched with goethite, hematite and magnetite, respectively.

Surface chemistry of E 172 materials was analysed via X ray photoelectron spectroscopy (XPS) and Time-of-flight Secondary Ions Mass Spectrometry (ToF-SIMS). Analysis of high-resolution Fe 2p (3/2) allowed to determine the iron chemical species present on the samples surface. Experimental peak shapes were modelled, considering multiplets splitting, and an estimation of valence states mix composition was obtained following an approach by Biesenger et al. Multi-variate analysis of ToF-SIMS spectra allowed to group materials based on correlations between their surface chemistry and revealed the presence of organic and inorganic contaminants. Volume specific surface area and pore sizes were determined by BET analysis, and compared with the STEM data.

Several types of iron oxides and hydroxides with differences in particle size, shape, crystal structure, elemental composition and surface properties were shown to be used as food additive E 172.

**Keywords:** iron oxides and hydroxides, food additive, physicochemical characterisation, nanoparticles, E 172

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## T2

### ANALYSIS OF (NANO)PARTICLES IN FOOD CONTACT MATERIALS USING ELECTRON MICROSCOPY

Lisa Siciliani<sup>(1)</sup>, Eveline Verleysen<sup>(2)</sup>, Sara Bals<sup>(3)</sup>, Heidi Demaegdt<sup>(2)</sup>, Salvatore Ciano<sup>(2)</sup>, Els Van Hoek<sup>(2)</sup>, Jan Mast<sup>(2)</sup>

<sup>1)</sup> Sciensano / University of Antwerp, Belgium

<sup>2)</sup> Sciensano, Belgium

<sup>3)</sup> University of Antwerp, Belgium

\*Corresponding author - E-mail: siciliani.lisa@gmail.com

The aim to reduce the use of fossil-based plastics is driving innovation in food packaging and prompting the food contact materials (FCM) industry to develop substitute materials. Such substitutes include bio-based and/or biodegradable polymers, fibrous materials and wood analogues. While these substitute materials need to be environmentally friendly, they also have to be safe for consumers. Although the use of FCM is regulated under the Framework Regulation (EU) 1935/2004, this Regulation does not take into account all the different types of materials used as FCM. Furthermore, addition of metal oxide nanoparticles into polymers has been reported to improve the characteristics of the material, which is especially important for biobased FCM. The use of nanomaterials in the polymer nanocomposites for FCM is accompanied by safety concerns as to whether metals, nanomaterials or small-sized fractions of them may be released from FCM into food. In this context, an approach to monitor the presence and migration of inorganic (nano)particles from FCM is presented. The properties of the (nano)particles in FCM were characterised using electron microscopy, where ultra-thin sections of FCM were prepared by embedding them in an epoxy resin, followed by sectioning using ultramicrotomy. The sections were analysed by scanning transmission electron microscopy and energy dispersive X-ray spectroscopy (STEM-EDX). (Nano)particles were identified based on their elemental composition, their size, shape and agglomeration state were measured, and their location in the FCM matrix (e.g. inside fibres, at the surface) was assessed. The approach was tested on 11 FCM samples including palm, bagasse, bioplastic, silicone, bamboo and textile. The results from STEM-EDX allowed to identify inorganic (nano)particles in 10 of the selected FCM. It was possible to measure their size and shape properties and to demonstrate their precise localisation in the FCM. This information is useful to assess the functionality of this application of (nano)particles in FCM and is also necessary for risk assessment of this type of application.

**Keywords:** food contact material, nanoparticles, electron microscopy, plastic alternatives

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## T3

### STUDY OF DETOXIFICATION IN AQUATIC ENVIRONMENTS FOR THE ELIMINATION OF DECARBAMOYLSAXITOXIN (DCSTX), USING MAGNETIC NANOPARTICLES

Carmen Lopez<sup>(1)</sup>, Alejandro Cao<sup>(1)</sup>, Ana Botana<sup>\*(1)</sup>, Luis Botana<sup>(1)</sup>

<sup>1)</sup> University of Santiago de Compostela, Spain

\*Corresponding author - E-mail: anamaria.botana@usc.es

Algal blooms in freshwater bodies can release paralytic shellfish toxins (PSP), including decarbamoylsaxitoxin (dcSTX). The presence of this toxin in drinking water poses a significant health risk, highlighting the need for effective detoxification methods. Current methods in water treatment plants are not fully effective against dcSTX, necessitating the exploration of new techniques.

The primary objective was to assess the capacity of different nanostructured magnetic particles to adsorb dcSTX from freshwater, aiming to apply these techniques to human drinking water samples. Various nanostructured particles, including activated carbon and graphene, were used. The efficacy of these particles was tested in both Milli-Q water and natural water. Methods such as hydrogen peroxide oxidation and HPLC-FLD analysis were used to measure dcSTX concentration before and after treatment.

The results showed that one specific particle had a high adsorption efficiency in both water samples, with an adsorption percentage of 97.87% in Milli-Q water and 92.64% in natural water. The pH and presence of organic matter in the water influenced the adsorption efficiency of the particles. The effectiveness of nanostructured particles depends on several factors, such as pore size and ionic charge of the particles and the toxin. Mesoporous particles showed low adsorption, likely due to fewer contact points with dcSTX. Additionally, the presence of organic matter in natural water can saturate the binding sites of the particles, reducing their adsorption capacity.

This study provides a solid foundation for the application of adsorption techniques using magnetic nanoparticles in the detoxification of freshwater, highlighting the importance of considering factors like pH and organic matter to optimize process efficiency.

**Keywords:** detoxification, magnetic nanoparticles, decarbamoylsaxitoxin

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# NANOPARTICLES

## T4

### PREPARATION AND CHARACTERIZATION OF VITAMIN E/CALCIUM/SOY PROTEIN ISOLATE NANOPARTICLES FOR SOYBEAN MILK BEVERAGE FORTIFICATION

**Neveen Agamy**<sup>(1)</sup>, **Amira Darwish**\*<sup>(2)</sup>

<sup>1)</sup> High Institute of Public Health, Alexandria University, Egypt

<sup>2)</sup> (1) Faculty of Industrial and Energy Technology, Borg Al Arab Technological University BATU, Alexandria, Borg El Arab, Egypt , (2) Food Technology Department, Arid Lands Cultivation Research Institute, Ci, Egypt

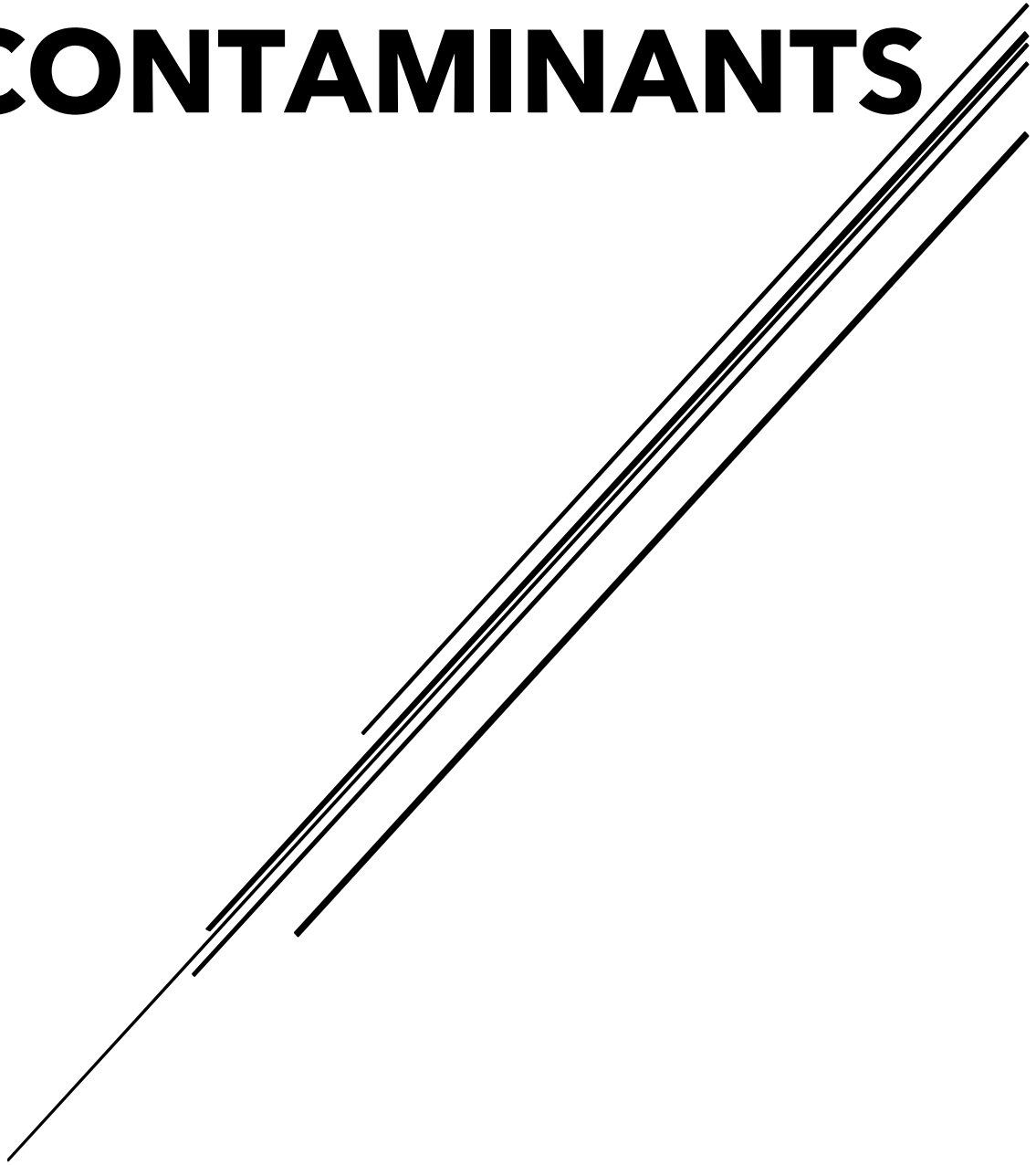
\*Corresponding author - E-mail: amiragdarwish@yahoo.com

Soybean milk is a rich plant-based source of protein, and phenolic compounds. This study compared the nutritional value of soybean milk, flour, soy protein isolate (SPI) and evaluated the impact of prepared vitamin E/calcium salt/soy protein isolate nanoparticles (ECSPI-NPs) on fortification of developed soybean milk formulations. Results indicated that soybean flour protein content was 40.50 g/100 g, that fulfills 81% of the daily requirement (DV%), the unsaturated fatty acids (USFs), oleic and linoleic content was 21.98 and 56.7%, respectively, of total fatty acids content. In soybean milk, essential amino acids, threonine, leucine, lysine achieved 92.70, 90.81, 77.42% of amino acid scores (AAS) requirement values respectively. Ferulic acid was the main phenolic compound in soybean flour, milk and SPI (508.74, 13.28, 491.78 µg/g). Due to the moisture content of soybean milk (88.50%) against (7.10%) in soybean flour, the latest showed higher nutrients concentrations. The prepared calcium (20 mM/10 g SPI) and vitamin E (100 mg/g SPI) nanoparticles (ECSPI-NPs) exhibited that they were effectively synthesized under transmission electron microscope (TEM), stability in the zeta sizer analysis and safety up to IC50 value (202 µg/mL) on vero cell line. ECSPI-NPs fortification (NECM) enhanced significantly phenolic content (149.49 mg/mL), taste (6.10), texture (6.70) and consumer overall acceptance (6.54). Obtained results encourage the application of the prepared ECSPI-NPs for further functional foods applications.

**Keywords:** soybean milk and isolate (SPI), phenolic content, vitamin E/Ca nanoparticles, amino acids, fatty acids



# **PROCESSING CONTAMINANTS**



# PROCESSING CONTAMINANTS

## U1

### APPROACHES TO MINIMIZE ACRYLAMIDE IN OXIDIZED CALIFORNIA STYLE OLIVES

**Carmen Breitling-Utzmann<sup>(1)</sup>, Nadja Bauer<sup>(1)</sup>, Alexandra Treyer<sup>(1)</sup>**

<sup>1)</sup> Chemical and Veterinary Investigations Office (CVUA) Stuttgart, Germany

\*Corresponding author - E-mail: carmen.breitling-utzmann@cvuas.bwl.de

Acrylamide (AA) is an undesirable process contaminant classified by IARC as a probable human carcinogen (Group 2A). According to an EFSA opinion, the dietary intake of AA should therefore be reduced [1]. Hence, AA benchmark levels (BML) in food and mitigation measures were established in Commission Regulation (EU) 2017/2158 [2]. In addition to foods such as potato chips and French fries, which are commonly associated with AA, olives have also been identified by EFSA as a potential source of AA [1]. As there are insufficient data and thus no BML for olives so far, olives have been included in the Commission Recommendation (EU) 2019/1888 [3]. AA is formed during food processing primarily via the Maillard reaction. During this process, the free amino acid asparagine reacts with reducing sugars, particularly at temperatures above 120°C. In olives, an alternative formation pathway via precursors from fat degradation is probably responsible for AA formation [4]. The results of a German nationwide monitoring project in 2021 showed that significant amounts of AA were found in blackened "California style" olives. In contrast, green and naturally ripened black olives contained rather low AA amounts [5]. The wide spread in AA levels in different olive varieties is attributed to differences in production methods. Raw olives have a very bitter taste, which is removed by treatment with brine or lye baths. During the production of "California style" olives, the oxidation of phenolic compounds is stimulated by an additional supply of air, which gives the olives their characteristic black colour. It is assumed that the subsequent sterilization in combination with the oxidation process is responsible for the formation of the high AA amounts [4]. Numerous olive samples from the retail sector were analysed at CVUA Stuttgart in 2019 to 2023, and their AA contents confirmed the results of the monitoring project. In addition, indications were found that there might be a relationship between the AA content in oxidized olives and the packaging and preservation that was used. In some samples of oxidized olives sold in plastic containers and/or to which preservatives had been added, significantly lower AA levels were detected. It can be assumed that these samples, in contrast to olives in jars, were not subjected to sterilization at high temperatures. Lower temperatures during heat preservation, also in combination with the use of preservatives, could thus represent a possible measure to mitigate the AA content in oxidized olive products.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM), EFSA Journal. 2015, 13(6), 321.

[2] Commission Regulation (EU) 2017/2158 of 20 November 2017.

[3] Commission Recommendation (EU) 2019/1888.

[4] S. Charoenprasert, A. Mitchell, J. Agric. Food Chem. 2014, 62, 8716.

[5] Berichte zur Lebensmittelsicherheit, BVL Report, 2021, 149.

**Keywords:** acrylamide, process contaminant, olives, California style olives, mitigation measures

**Acknowledgement:** The authors express thanks to the laboratory staff at the CVUA Stuttgart, especially Marion Bord, Simone Goetz, Ragna Gregorius, Cornelia Kobe, and Fiona Schert.

# PROCESSING CONTAMINANTS

## U2

### POLYCHLORINATED ALKANES (PCAS): INVESTIGATING LEVELS IN FOOD FROM THE DUTCH MARKET

**Wout Bergkamp**<sup>(1)</sup>, **Kerstin Krätschmer**\*<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: kerstin.kraetschmer@wur.nl

Chlorinated paraffins (CPs) are high production volume chemicals of growing concern because of their ubiquitous presence in food samples, their persistence, and their bioaccumulation potential. Their simple and cheap production established CP products as popular secondary plasticizers in PVC products, despite partial restrictions by the United Nations Stockholm Convention. Studies on the occurrence of their main constituent, polychlorinated alkanes (PCA's), in (European) food are sparse, but indicate their presence in vegetable oils and in other commodities [1,2]. Thus, our goal was to investigate PCA levels in the main food groups of interest from the Dutch market, including fats, meat, fish, eggs, and milk.

**Materials and Methods:** Materials and sample preparation have been described elsewhere [3]. In brief, the extracted fat from the samples was spiked with 0.1 mL of <sup>13</sup>C<sub>10</sub>-1,5,5,6,6,10-hexachlorodecane and dissolved in 10 mL n-hexane. To remove lipids, concentrated sulphuric acid was added before repeated liquid/liquid extraction with n-hexane was performed. Following clean-up on a deactivated silica column, the eluate was collected and evaporated to 1 mL after a solvent change to iso-octane. Analysis was done using a LC-ESI-HRMS instrument (R=140.000 FWHM, m/z 120-1500, full scan) with linear regression based on the average chlorination degree of the sample, using a customized R script for data processing.

**Results:** In half of the vegetable oil samples, elevated PCA levels above 100 ng/g product were observed. In contrast, results of most other food groups were close to or below chain length specific LOQs, with the exception of spreadable fats ( $\Sigma$ PCAs-C<sub>14-17</sub> max. 78 ng/g) and eggs ( $\Sigma$ PCAs-C<sub>14-17</sub> max. 130 ng/g,  $\Sigma$ PCAs-C<sub>18-20</sub> max. 170 ng/g).

**Discussion and conclusion:** While not all food types were included in the other published studies, PCA levels observed in the samples are in agreement with literature data, though on the lower end of the European concentration ranges. The highest PCA levels determined in food from the Dutch market were comparatively low, though the high findings in eggs and oils and elevated levels in especially spreadable fats call for further investigation. While not as pronounced, there was a higher amount of  $\Sigma$ PCAs-C<sub>14-17</sub> found in spreads with palm oil than in those without. Still, PCA levels in spreads were orders of magnitude lower than previously reported levels in palm oils. Unfortunately, the number of egg samples was insufficient to indicate differences between regions or farming types. Such information might help identify PCA sources or other areas of interest within the food production chain and support mitigation measures in the future.

[1] Krätschmer et al. (2020). Environ. Pollut., 272, 116019.

[2] McGrath et al. (2021). Environ. Pollut., 291, 118236.

[3] Yang et al. (2023). Food Control, 153, 109889.

**Keywords:** chlorinated paraffins, polychlorinated alkanes

**Acknowledgement:** The Dutch Ministry of Agriculture, Nature and Food Quality is gratefully acknowledged for providing financial support to the authors (WOT-02-001-015).

## PROCESSING CONTAMINANTS

### U3

#### EXPERIMENTAL INVESTIGATION AND MODELLING OF PROCESS PARAMETERS EFFECT ON GINGERBREAD QUALITY AND SAFETY

Lene Duedahl-Olesen<sup>\*(1)</sup>, Elena Hakme<sup>(1)</sup>, Eda Topaloglu<sup>(1)</sup>, Denis Sehovic<sup>(1)</sup>, Aberham Hailu Feyissa<sup>(1)</sup>

<sup>1)</sup> National Food Institute, DTU, Denmark

\*Corresponding author - E-mail: lduo@food.dtu.dk

Gingerbread is a traditional festive cake based on flour, honey, eggs and ammonium hydrogen carbonate as a baking agent. Like other bakery products gingerbread is subject to Maillard reactions during baking, resulting in color formation, and, undesirably, acrylamide formation [1]. Danish gingerbread producers are interested in understanding the mechanisms behind acrylamide formation during baking and storage, as part of their mitigation strategies.

Our studies on industrial prepared dough focused on baking parameters, storage parameters, pan and free-standing baking methods to evaluate quality and safety. An experimental design involving three factors was employed to collect data for modelling kinetic of acrylamide formation during baking, considering process conditions such as time and temperature.

For gingerbread quality evaluation, acrylamide concentrations, texture, color and moisture content were correlated with process conditions. The analysis of acrylamide, based on LC-MS/MS, showed an increase with baking time and temperature, following kinetic modelling. Notably, baking at lower temperatures resulted in a soft texture (assessed using Texture Profile Analysis (TPA)) and minimal color formation (lower L\* value).

The findings of this study enable us to provide recommendations for industrial production regarding optimal baking temperature, time, and storage conditions for gingerbread, to ensure minimal acrylamide levels and a safe product.

[1] Amrein, TM., Schönbächler, B., Escher, F., and Amadò R.(2005). Factors influencing acrylamide formation in gingerbread in Chemistry and Safety of Acrylamide in Food (Friedman and Mottram, Eds) pp 431-445, Springer Science.

**Keywords:** acrylamide modelling, colour, texture analysis, DOE, temperature and time

## PROCESSING CONTAMINANTS

### U4

#### INFLUENCE OF THE BAKING PROCESS ON CANNABINOID CONTENTS AND PROFILES IN HEMP-CONTAINING BREAD AND SURVEY OF MARKET SAMPLES

**Niklas Lindekamp<sup>(1)</sup>, Nadja Triesch<sup>(1)</sup>, Tim Rautenberg<sup>(1)</sup>, Alexander Voß<sup>(2)</sup>, Sascha Rohn<sup>(3)</sup>, Stefan Weigel<sup>\*(1)</sup>**

<sup>1)</sup> German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Germany

<sup>2)</sup> Institute for Food and Environmental Research (ILU) e.V., Germany

<sup>3)</sup> Department of Food Chemistry and Analysis, Institute of Food Technology and Food Chemistry, TU Berlin, Germany

\*Corresponding author - E-mail: stefan.weigel@bfr.bund.de

Since 2023, Commission Regulation (EU) 2023/915 has regulated  $\Delta^9$ -THC equivalents ( $\Delta^9$ -THC +  $0.877 \times$ THCA) with maximum levels in hemp seeds and processed hemp seed products.  $\Delta^9$ -THC (tetrahydrocannabinol) is the most relevant psychoactive cannabinoid in the hemp plant. Cannabinoid acids can be converted to neutral cannabinoids by thermal decarboxylation. This is particularly relevant in the case of THCA (tetrahydrocannabinolic acid), where the psychoactive  $\Delta^9$ -THC can be formed. Hemp breads are becoming increasingly popular.

The aim of this study was to quantify cannabinoid contents and profiles of hemp-containing breads. It is the first systematic description of the influence of the baking process on cannabinoid contents and profiles. Hemp seed breads were manufactured at small-business scale and analysed using a validated HPLC-MS/MS method. Rye-wheat breads containing 10% unhulled hemp seeds (based on total flour weight) were baked at 180-260 °C for 40-60 min. The breads were separated into crust, crumb, whole slices, and the initial raw dough sample was retained (84 samples in total). In addition, 26 bakery products containing hemp were purchased and analysed.

Baking under different conditions revealed a dependence of acidic cannabinoid decarboxylation on temperature and time. Compared to the acid/neutral ratio (A/N) in the raw dough (A/N 4.0), the ratio decreased to A/N 2.0 in the 180 °C/40 min bread and to A/N 0.9 in the 260 °C/60 min bread. At the same time, the  $\Delta^9$ -THC content increased by up to 48% (260 °C/60 min bread) compared to the raw dough, further emphasising the importance of decarboxylation. Decarboxylation and  $\Delta^9$ -THC formation were more pronounced in the crust. Comparing raw dough and the 260°C/60 min bread, in the crust A/N decreased from 4.0 to 0.4 and  $\Delta^9$ -THC increased by 105%. In the corresponding crumb, A/N decreased to 1.2 and  $\Delta^9$ -THC increased by 43%. The difference is explained by the different thermal profiles in the two compartments, highlighting the effects of baking parameters on cannabinoid contents and profiles in bakery products. The total cannabinoid content in the market samples ( $n = 26$ ) ranged from 68 to 4,603  $\mu\text{g}/\text{kg}$  and the  $\Delta^9$ -THC equivalents were up to 66  $\mu\text{g}/\text{kg}$ . The sample with the highest  $\Delta^9$ -THC equivalents was a gingerbread covered with dehulled hemp seeds. The highest total cannabinoid content was found in a wholemeal rye bread, which is the most common baked product containing hemp found during sample collection.

**Keywords:**  $\Delta^9$ -THC, thermal processing, HPLC-MS/MS, tetrahydrocannabinol, quantification

**Acknowledgement:** The authors thank Sebastian Kemter for his support in the laboratory.

# PROCESSING CONTAMINANTS

## U5

### TRACE ELEMENTS AND BISPHENOLS IN PROCESSED FOOD (MEAT AND FISH PRODUCTS)

Vincenzo Lo Turco<sup>(1)</sup>, Vincenzo Nava\*<sup>(2)</sup>, Federica Litrenta<sup>(1)</sup>, Rossana Rando<sup>(2)</sup>, Angela Giorgia Potorti<sup>(2)</sup>, Giuseppa Di Bella<sup>(2)</sup>

<sup>1)</sup> Department of Biomedical and Dental Sciences and of Morphological and Functional Images (BIOMORF), University of Messina, Italy

<sup>2)</sup> Department of Biomedical, Dental, Morphological and Functional Images Sciences (BIOMORF), University of Messina, Viale Annunziata, 98122 Messina, Italy., Italy

\*Corresponding author - E-mail: vnava@unime.it

Processed food is now an integral part of the food system [1]. Processing makes it possible to extend the shelf life of a product while maintaining its quality. However, it can also be a source of various types of contamination (chemical, process, environmental) [1,2]. There are many different types of processed food, but among the most consumed processed foods are certainly meat and fish, which are important in the diet as a source of protein and other nutrients. However, these matrices can be subject to inorganic contamination (toxic and potentially toxic elements), but also to bisphenol and derivatives, which can be used as coatings in cans and packaging for certain foods. It is therefore important to monitor these processed products to protect the health of consumers [2,3]. This study focused on the monitoring of trace elements and bisphenols in 120 different processed foods (meat and fish) of EU and non-EU origin purchased in supermarkets and ethnic food shops in Messina (Italy) and assessed the potential toxicological risk to humans and the non-carcinogenic risk. The results showed that the most representative trace elements in processed fish samples were zinc and iron, followed by aluminium, copper and manganese. This trend was different for processed meat samples: Fe > Zn > Al > Cu > Mn. The Commission Regulation (EU) 2023/915 sets a maximum limit for some elements. All processed fish samples showed concentrations within the legal limits for cadmium, only the sample "cuttlefish ink" had a lead content close to the limit (0.30 mg/Kg). As regards samples of processed meat, some species exceeded the maximum limit for Pb and Cd. The most representative bisphenols found in all samples were analogues A, F, and B. The analogues S, E, Z, P, AP, and AF were not found in any sample. Concerning the ether derivatives of bisphenols (BADGEs), BADGE, and its hydrolysis and chlorination products BADGE·2H<sub>2</sub>O, BADGE·HCl·H<sub>2</sub>O and BADGE·2HCl were detected in both fish and meat samples. The assessment of the potential toxicological risk showed that the average consumption of 100g results in a safe level of mineral intake. However, the same cannot be said for BPA, for which two samples (mackerel and crab) exceeded the reference value (0.2 ng BPA/kg body weight). Finally, for the non-carcinogenic risk assessment, the HQ did not exceed the threshold value of 1 for any contaminant potentially ingested by adults from processed foods, except for BPA, for which most samples had HQ >1. Considering the great success of these processed products and the results obtained, continuous monitoring is necessary to assess the safety status of these foods and to protect the health of consumers.

[1] Nava V., Di Bella G., Fazio F., Potorti A.G., Lo Turco V., Licata P, (2023). Hg Content in EU and Non-EU Processed Meat and Fish Foods. *Applied Sciences*, 13, 793.

[2] Kowalska G., Pankiewicz U., Kowalski R, (2020). Determination of the Level of Selected Elements in Canned Meat and Fish and Risk Assessment for Consumer Health. *J. Anal. Methods Chem*, 2148794.

[3] Collado-Lopez S., Betanzos-Robledo L., Tellez-Rojo M.M., Lamadrid-Figueroa H., Reyes M., Rios C., Cantoral A, (2022). Heavy Metals in Unprocessed or Minimally Processed Foods Consumed by Humans Worldwide: A Scoping Review. *Int. J. Environ. Res. Public Health*, 19, 8651.

**Keywords:** trace elements, bisphenols, processed food, potential toxicological risk, hazard quotient

### U6

## MONOCHLOROPROPANEDIOL AND GLYCIDYL FATTY ESTERS IN A DIVERSE RANGE OF BABY FOODS

**Dimitra Diamantidou**\*<sup>(1)</sup>, **Arvid Fromberg**<sup>(1)</sup>

<sup>1)</sup> National Food Institute, Denmark

\*Corresponding author - E-mail: dimdi@food.dtu.dk

The presence of 2- and 3-monochloropropanediol and glycidyl fatty acid esters (2- and 3-MCPDEs and GEs) in refined fats and oils has been investigated in recent years due to their potential health risks. Baby foods, often fortified with refined fat and oils to meet nutritional requirements, may contain these contaminants. The low body weight of infants and young children combined with a relative high intake of often a limited selection of food items, makes them particularly vulnerable to such contaminants, necessitating monitoring of these compounds in baby food products. Currently, no maximum limits for the free and bound MCPDs and GEs are established for infant and young children food, highlighting the need for additional occurrence data.

This study presents the validation of a previously developed GC-MS/MS method [1] in four different baby matrices: biscuit, wet porridge, vegetable and meat jars. The objective was to establish a low limit of quantification (LOQ) across all studied food categories. The method validated in terms of linearity, sensitivity, recovery and precision, and the results were evaluated according to Commission Regulation (EC) 333/2007 [2]. The results demonstrated satisfactory linearity ( $R^2 > 0.99$ ) and achieved an LOQ at 3 µg/kg determined as the lowest successfully validated level. Precision was evaluated through matrix spiking with native standards at three concentrations levels, yielding coefficients of variation below 20% at the lower concentrations. Recoveries ranged from 76 to 110%, indicating the method's accuracy.

The validated method offers a robust tool for the detection and quantification of 2- and 3-MCPDEs and GEs in different baby foods, contributing to enhanced food safety and regulatory compliance. This advancement is critical for protecting the health of infants and young children, ensuring that baby food products remain within safe contaminant limits.

[1] K. H. Nguyen and A. Fromberg, "Monochloropropanediol and glycidyl esters in infant formula and baby food products on the Danish market: Occurrence and preliminary risk assessment," *Food Control*, vol. 110, p. 106980, 2020, doi: <https://doi.org/10.1016/j.foodcont.2019.106980>.

[2] Commission Regulation (EC) No. 333/2007, laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. Consolidated version of 30/04/2024 *Official Journal of the European Union*, 2024.

**Keywords:** MCPD esters, glycidyl esters, baby food, method validation, processing contaminants

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### U7

#### EVALUATION OF MINERAL OIL HYDROCARBONS (MOSH AND MOAH) CONTAMINATION IN SYNTHETIC CASINGS USING GCXGC-MS

Ana M. Rodrigues<sup>(1)</sup>, Fabio L. Gonçalves<sup>(2)</sup>, Carolina Ventura<sup>(2)</sup>, António Ferreira<sup>(2)</sup>, Naiara Fernandez<sup>(2)</sup>, Catia Carmo<sup>(2)</sup>, Maria Rosario Bronze<sup>\* (2)</sup>

<sup>1)</sup> BET, Instituto de Biologia Experimental e Tecnologica (iBET), Portugal

<sup>2)</sup> Instituto de Biologia Experimental e Tecnologica (iBET), Portugal

\*Corresponding author - E-mail: mbronze@ibet.pt

Mineral oil hydrocarbons (MOH) are complex mixtures of compounds obtained mainly from petroleum distillation and refining. They are categorized into two main groups: saturated MOH (MOSH) and aromatic MOH (MOAH). MOH can enter the food chain through multiple sources: natural origin, environmental contamination, contamination in the production chain (e.g., use of lubricants), and food contact materials. Synthetic casings used in the food industry are usually made of collagen, cellulose, or plastic. Collagen casings are edible if derived from animal sources, but all others should be removed before consumption. Furthermore, all casings are in direct contact with the food product; therefore, it is important to detect the presence of MOSH and/or MOAH contamination. The latest risk assessment report by the European Food Safety Agency (EFSA) [1] provisionally concluded that MOAH compounds with three or more aromatic rings may act as a genotoxic carcinogen and are potentially harmful to human health. Thus, a deep characterization of MOSH and MOAH compounds is crucial to assess food safety.

The current method for MOSH and MOAH analysis is based on online liquid chromatography coupled to gas chromatography with flame ionization detection (LC-GC-FID). Due to the complexity of MOH mixtures, this method is not suitable for the identification of individual compounds.

In this work a comprehensive two-dimensional GC coupled to mass spectrometry (GC×GC-MS) approach was used for the further characterization of MOH in artificial casings. The MOH extraction from synthetic casings using *n*-hexane was performed followed by chromatographic fractionation of MOSH and MOAH using silver nitrate silica gel as stationary phase prior to their analysis. Results on the differentiation between the sub-classes within the MOSH and the MOAH fraction will be presented.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM) et al. 2023. Update of the risk assessment of mineral oil hydrocarbons in food. EFSA Journal, 21, e08215. DOI: 10.2903/j.efsa.2023.8215.

**Keywords:** mineral oil hydrocarbons, MOSH, MOAH, Food contaminants, GCxGC

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# PROCESSING CONTAMINANTS

## U8

### SURVEY ON MINERAL OIL HYDROCARBONS (MOH) CONTAMINANTS IN VIRGIN AND EXTRA VIRGIN OLIVE OILS

Ana M. Rodrigues<sup>(1)</sup>, Daniel Reyes Diaz<sup>(2)</sup>, Amulya Baniya<sup>(1)</sup>, Sofia Lourenço<sup>(3)</sup>, Paula Allen Lopes<sup>(3)</sup>, Luis Aparicio<sup>(3)</sup>, Naiara Fernandez<sup>(1)</sup>, **Maria Rosario Bronze**\*<sup>(1)</sup>

<sup>1)</sup> Instituto de Biologia Experimental e Tecnológica (iBET), Portugal

<sup>2)</sup> Sovena España, S.A.U., Spain

<sup>3)</sup> Sovena Group, Portugal

\*Corresponding author - E-mail: mbronze@ibet.pt

Mineral oil hydrocarbons (MOH) comprise a wide range of chemical compounds obtained mainly from petroleum distillation and refining. They can be divided into mineral oil saturated hydrocarbons (MOSH) or mineral oil aromatic hydrocarbons (MOAH). Contamination with MOH can occur at any stage of food processing, from harvesting to packaging (e.g., machinery lubricants, agricultural chemicals, food contact materials). These compounds have been found in various foods, including edible oils. The latest risk assessment report by the European Food Safety Agency (EFSA) provisionally concluded that MOSH do not pose a health concern. However, MOAH compounds with three or more aromatic rings may act as a genotoxic carcinogen and are potentially harmful to human health. The EU commission guidelines recommend a maximum of 13 mg MOSH/kg and 2 mg MOAH/kg in products with high-fat content (> 50%) [1], such as olive oil. Furthermore, the EU Commission is currently working on a draft regulation to establish matrix-specific maximum limits for MOAH, across various food categories.

The aim of this study was to survey the levels of MOSH and MOAH in virgin and extra virgin olive oils [AMR1] available on the market in Portugal. Results were compared with recommended benchmark levels. Commercial olive oils were acquired and analysed using online-coupled liquid chromatography-gas chromatography with flame ionization detection (HPLC-GC-FID), which is the reference method for MOSH and MOAH quantification [2]. The results showed that about 50% of the olive oils exceeded the recommended MOSH limit of 13 ppm, while about 40% surpassed the recommended MOAH limit of 2 ppm.

The assessment of MOH contamination in olive oil, especially in virgin and extra virgin varieties, is essential for ensuring food safety compliance. Current regulatory frameworks and industry efforts are vital in ensuring that contamination with MOH is minimized to comply with food safety regulations, protect consumer health, and maintain the integrity of high-quality olive oil products. For the olive oil companies it is crucial that rapid methods of analysis are evaluated, implemented, and validated to be used as screening methodologies and ensure compliance with legal limits for olive oil acceptance.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM) et al. 2023. Update of the risk assessment of mineral oil hydrocarbons in food. EFSA Journal, 21, e08215. DOI: 10.2903/j.efsa.2023.8215.

[2] ISO20122:2024(en) Vegetable oils – Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online-coupled high performance liquid chromatography-gas chromatography-flame ionization detection (HPLC-GC-FID) analysis – Method for low limit of quantification.

**Keywords:** mineral oil hydrocarbons, MOSH, MOAH, Olive oil, Food contaminants

**Acknowledgement:** Funding: MOH (WP2| 2.3.), from VIIAFOOD Agenda, financed by the Portuguese Recovery and Resilience Plan (PRR), iNOVA4Health – UIDB/04462/2020 and UIDP/04462/2020 (funded by FCT/MCTES) and the Associate Laboratory LS4FUTURE (funded by FCT).

## PROCESSING CONTAMINANTS

### U9

## CHALLENGES IN THE PRODUCTION OF PROFICIENCY TESTING AND REFERENCE MATERIALS FOR FURAN, ALKYL FURANS AND BENZENE IN FOOD

Isabella Zübner<sup>\*(1)</sup>, Martin Kaminski<sup>(1)</sup>

<sup>1)</sup> Federal Office of Consumer Protection and Food Safety, Germany

\*Corresponding author - E-mail: [isabella.zuebner@bvl.bund.de](mailto:isabella.zuebner@bvl.bund.de)

Furan and alkylfurans are formed from reducing sugars, amino acids, ascorbic acid, unsaturated fatty acids, and carotenoids during processes such as sterilization, baking, and roasting. These compounds are of concern in foods such as roasted coffee, canned goods, baked goods, and infant formula. Benzene can be formed from the thermal decomposition of benzoic acid, as seen in the production of carrot-based baby foods.

The toxicological effects of these compounds are of concern. Studies show that furan can cause liver damage and cancer, leading the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EFSA to raise health concerns about exposure levels in food. Benzene, classified as a Group 1 carcinogen by the IARC, is a known human carcinogen.

Due to the health concerns, the EU Monitoring Recommendation 2022/495 established requirements for the analysis of furan, its alkyl derivatives in food. While analytical methods for furan itself as well as benzene are well established in Europe, the inclusion of alkyl furans is a recent development. However, there is still a lack of proficiency tests (PTs) for furan and alkylfurans in food, and there are no PTs for benzene.

The German Federal Office for Consumer Protection and Food Safety (BVL) is currently developing a PT program focusing on benzene and furans in food. A successful preliminary study on baby food matrix was completed in 2022. As no uncontaminated materials were identified for this matrix, cooking tests were performed to produce blank materials. A gas-tight spiking device was developed to allow spiking of furans and benzene under stirring and cooling conditions to minimize loss of these highly volatile analytes.

Different packaging materials and methods were tested and a specific analytical method with low variability and high sensitivity (LOQ < 5 µg/kg for furan and its alkyl derivatives and < 0.3 µg/kg for benzene) was developed to characterize the reference material candidates. A packaging and shipping concept for frozen transport was also established.

Despite the use of an inert, gas-tight spiking device, analyte losses were observed during spiking, even for less volatile compounds, suggesting that adsorption rather than evaporation was the primary cause of these losses. In the 2022 trial using a plastic piping bag, a trend of decreasing analyte concentration over the sample filling process was observed, also suggesting adsorption losses. During the first weeks of freezer storage, furan and benzene concentrations in the samples decrease until they stabilize, even when coated glass bottles are used. Assigned analyte concentrations could not be derived from the material preparation process, although maximum concentrations were determined for all analytes studied in the trial, providing valuable information to participants.

A proficiency test on furans and benzene in baby food is currently being conducted in 2024 to implement the knowledge and improvements gained from the 2022 study.

**Keywords:** furan, alkylfurans, proficiency testing, reference material, benzene

## PROCESSING CONTAMINANTS

### U10

#### COMMITMENT TO EXCELLENCE: ENSURING FOOD SAFETY AND INNOVATION IN MOSH/MOAH ANALYSIS THROUGH A DUAL APPROACH

Carlos Martin-Alberca<sup>(1)</sup>, Zuzana Zelinkova<sup>\*(2)</sup>, Raquel De Llanos<sup>(1)</sup>, Benjamin Horemans<sup>(3)</sup>, Inna Stambula<sup>(2)</sup>, Marian Steverink<sup>(1)</sup>

<sup>1)</sup> Cargill Food Solutions EMEA, Product and Process Development - Edible Oils Europe, The Netherlands

<sup>2)</sup> Cargill Food and Bio-industrial, FSQR Analytical Chemistry Group EMEA, The Netherlands

<sup>3)</sup> Cargill, Core R&D, Belgium

\*Corresponding author - E-mail: [Zuzana\\_Zelinkova@cargill.com](mailto:Zuzana_Zelinkova@cargill.com)

Contamination of food with mineral oil hydrocarbons (MOH) pose a potential health hazard to consumers and in past decades has gained more attention from food safety authorities, food producers and researchers. The analysis of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) presents significant analytical challenges due to heterogenous analyte composition, complex sample preparation, challenging results interpretation, and selectivity issues caused by a broad range of co-eluting interfering substances.

In this work a comprehensive overview of a dual approach for MOSH/MOAH analysis is presented, combining a standard method for routine analysis and innovative research and development (R&D) efforts.

Cargill global internal capabilities for MOSH/MOAH analysis by LC-GC-FID with automated and manual sample preparation were successfully implemented within Cargill following one standard, thoroughly validated, and critically assessed in terms of method performance and quality control. Routine MOH monitoring ensures food safety and high-quality products delivered to customers. High sample throughput of diverse products in Cargill QC labs revealed difficult matrices with complex interfering substances and will be further discussed.

In parallel, R&D initiatives aimed to push the boundaries of MOSH/MOAH analysis. The implementation of advanced techniques, such as online-LC-GCxGC-TOF-MS/FID, has enabled us to achieve significant breakthroughs in understanding the nature and origin of these contaminants and complex co-eluting substances, as well as exploring new processing methods to remove them from oils and fats.

The synergies between the QC labs and R&D teams will be discussed, providing examples on how thorough root cause analysis (RCA) and rapid internal feedback mechanisms contribute to our overall analytical excellence and contaminant management strategy.

**Keywords:** MOSH/MOAH, mineral oil, contaminants, LC-GC-FID, online-LC-GCxGC-TOF-MS/FID

# PROCESSING CONTAMINANTS

## U11

### DETERMINATION OF MINERAL OIL AROMATIC HYDROCARBONS BY GCxGC-FID. USED ROUTINELY FOR 1.5 YEARS: AN OVERVIEW ABOUT EXPERIENCES, LIMITATIONS AND RESULTS

Michael Koch<sup>(1)</sup>, Kristin Wenzel<sup>(1)</sup>, Eileen Schulz<sup>\*(1)</sup>, Agnès Buczkowski<sup>(1)</sup>, Thomas Behnke<sup>(1)</sup>, Erik Becker<sup>(1)</sup>

<sup>1)</sup> Institut Kirchoff Berlin GmbH a Mérieux NutriSciences Company, Germany

\*Corresponding author - E-mail: agnes.buczkowski@mxns.com

Mineral oil hydrocarbons are mixtures of saturated and aromatic hydrocarbons: MOSH (mineral oil saturated hydrocarbons) are paraffin-like (open-chain, mostly branched) and naphthenic (cyclic) hydrocarbons, MOAH (mineral oil aromatic hydrocarbons) are aromatic hydrocarbons, mainly consisting of highly alkylated systems. The toxicological risks of mineral oil aromatic hydrocarbons (MOAHs) and in particular 3- to 7-ring MOAHs have been increasingly emphasized by EFSA [1]. Currently different drafts for regulatory measures on MOAH are under discussion. Limits are planned to become legal with start of 2026. The proposed limits were mostly adopted from already known SCOPAFF report from 2022 [2]. The values for the MOAH as a sum comprise following: 0.5 mg/kg for dry foods with a low fat/oil content ( $\leq 4\%$  fat/oil), 1 mg/kg for foods with a higher fat/oil content ( $> 4\%$  and  $\leq 50\%$  fat/oil) and 2 mg/kg for fats/oils as well as products with  $> 50\%$  fat/oil content.

Contaminants with mineral oil hydrocarbons are detectable in many foods [3], but can have different matrix interferences depending on the raw material [4]. These cannot be eliminated despite a variety of modern purification techniques such as epoxidation. This makes it difficult to quantify mineral oil hydrocarbons using the standard LC-GC-FID method. Which poses a problem for the manufacturers with regard to the planned legal limits described above. With the two-dimensional gas chromatography with flame ionization detection (GCxGC-FID), the remaining interferences can be separated from the MOAH, which makes quantification possible.

Routine application of such a complex analytical technique over 1.5 years was overviewed and the obtained data summarized and analyzed. The separation of interfering compounds within the second chromatographic dimension is not always successful. Specific factors such as the sample matrix and the degree of refinement of fats are crucial and represent a limitation of this otherwise very efficient chromatographic technique. These limitations were addressed and discussed closer regarding the possibility of the quantification on the affected samples.

[1] EFSA Update of the risk assessment of mineral oil hydrocarbons in food, 12th. July 2023.

[2] Standing Committee on Plants, Animals, Food and Feed Section Novel Food and Toxicological Safety of the Food Chain 21 April 2022.

[3] Biedermann M., Fiselier K., Grob K., Journal Agric. Food Chemistry, 2009, 57 (19), 8711-8721.

[4] Biedermann, M.; Grob, K., Journal of Chromatography A, vol. 1255, 2012, 56-75.

**Keywords:** mineral oil contaminants, MOSH/MOAH, GCxGC-FID, interfering compounds

# PROCESSING CONTAMINANTS

## U12

### NITROSAMINES 2030: FROM PHARMA & PACKAGING TO FOOD & PACKAGING. RISKS TO THINK ABOUT.

**Emiliano De Dominicis**<sup>(1)</sup>, Marialuisa Piva<sup>(1)</sup>, Mario Dante<sup>(1)</sup>, Elisa Gritti<sup>(1)</sup>, Gianluigi Boschello<sup>(1)</sup>, Valentina Menegon<sup>(1)</sup>, Manuel Rubino<sup>(1)</sup>, Laura Zampieri<sup>(1)</sup>, Silvia Toffoletto<sup>(1)</sup>, Emanuele Conte<sup>(1)</sup>, Stefano Cazzaro<sup>(1)</sup>, Marco Redaelli<sup>(1)</sup>, Enrico Nieddu<sup>(1)</sup>, Cinzia Pellicorio<sup>(1)</sup>, Francesca Faraon<sup>(1)</sup>, Valentina Abbondandolo<sup>(1)</sup>, Sergio Fasan<sup>(1)</sup>, Sylvain Dahiot<sup>(1)</sup>

<sup>1)</sup> Mérieux NutriSciences Italy, Italy

\*Corresponding author - E-mail: [emiliano.de.dominicis@mxns.com](mailto:emiliano.de.dominicis@mxns.com)

The N-Nitrosamines (N-NAs) are process-related contaminants that primarily originate from precursor molecules containing secondary amine groups. They can form in the presence of nitrites (or other nitrosating agents) under specific production process conditions, such as high temperature, high pressure, or particular pH, ionic strength, etc.

Over the past five years, attention to N-Nitrosamines (N-NAs) has been increasingly focused on by the pharmaceutical industry (both regulatory and industrial) concerning two critical categories of N-Nitrosamines:

- "small nitrosamines" (as for example NDMA = N-Nitroso Dimethylamine, etc.);
- "complex NDSRIs" (Nitrosamine Drug Substance-Related Impurities as for example N-Nitroso Phenylephrine, etc.).

Mérieux NutriSciences Pharma & Cosmetics Division has over 70 GMP-validated analytical methods using LC-MS/MS and LC-MS/HRMS, which are instrumental in assessing, managing, and mitigating the risk of nitrosamine presence in the pharmaceutical industry.

Today, we observe a growing awareness that the N-NAs extends beyond the pharmaceutical domain to include food, packaging and cosmetic sectors.

In particular, EFSA in 2023 has investigated the presence of more than 30 N-NAs in food (10 out of the 23 carcinogenic N-NAs were detected in food) [1]. At the European Commission level, a discussion is underway regarding a possible Recommendation for monitoring and establishing maximum levels of nitrosamines in food.

This is why Mérieux NutriSciences Food Division developed and validated a targeted quantitative methods for the determination of these N-NAs in baby food products.

Summarizing, if N-Nitrosamines are present in pharmaceutical products as process-related contaminants, why wouldn't they also be found in food, novel food, packaging, water, and also environment? [1,2]

Nitrosamines are indeed present; the attention has simply not yet been focused on this aspect. However, we believe that this focus will soon shift, and therefore, we are preparing accordingly.

Here is an example: the levels of NMOR found in pharmaceutical and food products due to packaging (N-nitrosomorpholine = a process-related contaminant from unintentionally added secondary amines) are significantly lower than those potentially present in complex finished apple food products, such as apples treated with "morpholine fatty acid salt" as an additive (N-nitrosomorpholine = a process-related contaminant from intentionally added secondary amines)2. Let's talk about it together.

[1] EFSA Scientific Opinion, Risk assessment of N-nitrosamines in food, EFSA Journal 2023;21(3):7884 doi: 10.2903/j.efsa.2023.7884.

[2] C. Han et Al., Determination of morpholine residue in fruit and fruit juices by gas Chromatography–Tandem mass spectrometry, LWT, Volume 161, 2022, 113369, ISSN 0023-6438, <https://doi.org/10.1016/j.lwt.2022.113369>.

**Keywords:** nitrosamines, NNAs, NDSRIs, mass spectrometry, food

## PROCESSING CONTAMINANTS

### U13

#### FIRST CERTIFIED REFERENCE MATERIAL FOR LOW LEVELS OF MCPD FATTY ACID ESTERS IN INFANT FORMULA

**Berit Sejerøe-Olsen**<sup>(1)</sup>, **Penka Shegunova**<sup>(1)</sup>, **Andrea Held**<sup>(1)</sup>, **Arvid Fromberg**<sup>(2)</sup>

<sup>1)</sup> *European Commission-Joint Research Centre, Belgium*

<sup>2)</sup> *European Reference Laboratory for Processing Contaminants, Denmark*

*\*Corresponding author - E-mail: Berit.SEJEROE-OLSEN@ec.europa.eu*

Infants are a particularly vulnerable part of the population and the EU sets special measures to protect their health. For certain contaminants in food, the low maximum levels allowed to minimise their exposure can pose a challenge for laboratories during analysis.

Infant formula is a food matrix prepared to reach a fat composition similar to breast milk. Vegetable oils such as palm oil, coconut oil or pomace olive oil serve this purpose. The oil ingredients can carry levels of contaminants generated during their thermal refinement process, as they may form at the high temperature utilised in oil deodorisation in the presence of a chlorine donor.

Monochloropropanediol (MCPD), its fatty acid esters and glycidyl fatty acid esters are food processing contaminants (PC) potentially contained in vegetable oils used for infant food formulation. Infants have a risk of contact with the processing contaminants if present in the infant formula feeding. This potential exposure is of a high concern as MCPD fatty acid esters and glycidyl fatty acid esters are hydrolysed in the human gastrointestinal tract into their corresponding free form MCPD and glycidol, which are classified as possibly carcinogenic to humans.

The Commission Regulation (EU) 2023/915 set maximum levels for 3-MCPD, 3-MCPD fatty acid esters and glycidyl fatty acid esters in foodstuffs and food ingredients. The maximum levels of 3-MCPD and 3-MCPD fatty acid esters are regulated as the sum of the compounds expressed as 3-MCPD. For infant formula (powder) the maximum permitted level is 125 µg/kg, 10-20 fold lower to those established in other foods.

The Joint Research Centre of the European Commission (EC-JRC) has developed the first Certified Reference Material for low levels of 2- and 3-MCPD fatty acid esters in infant formula. That involves a highly processed food matrix with contaminant levels close to the maximum permitted at the EU. The CRM allows control laboratories to validate their measurement methods according to Regulation (EU) 333/2007, which lays down the methods of sampling and the minimum performance requirements for the analysis of 3-MCPD, 3-MCPD fatty acid esters and glycidyl fatty acid esters in foodstuffs. The main findings during material characterisation for ERM-BD087, produced under ISO 17034:2016, are presented along with the certified values.

**Keywords:** processing contaminants, certified reference material, infant formula, MCPD fatty acid esters

## PROCESSING CONTAMINANTS

### U14

#### POLISH APPROACH OF THE TRANS FATS (I-TFA) ELIMINATION FROM FOOD

Hanna Mojska<sup>(1)</sup>, Edyta Jasinska-Melon\*<sup>(1)</sup>

<sup>1)</sup> National Institute of Public Health NIH - National Research Institute, Poland

\*Corresponding author - E-mail: [ejasinska@pzh.gov.pl](mailto:ejasinska@pzh.gov.pl)

Trans fatty acids (TFA) are defined as "fatty acids with at least one non-conjugated carbon-carbon double bond in the trans configuration". TFA are naturally present in food products derived from ruminant animals (r-TFA). TFA are also produced industrially (i-TFA). The primary dietary source of i-TFA are partially hydrogenated vegetable oils (PHVOs) and food products manufactured with their use.

TFA increase the concentration of LDL-cholesterol and reduce HDL-cholesterol in blood serum. High dietary TFA intake increases the risk of all-cause death, death from coronary heart disease, and coronary heart disease by 34%, 28%, and 21%, respectively. It is also a risk factor for the development of, among others, overweight or neurodegenerative diseases. Elimination of i-TFA was identified by World Health Organization (WHO) as a priority target in public health. WHO published the REPLACE Programme to eliminate i-TFA from the world's food supply and the Validation Programme to assess the status of countries in their efforts to eliminate i-TFA by granting them a Certificate. In November 2023, Poland received this certificate in the first edition of the WHO Validation Programme, as one of five countries in the world.

The Polish approach to eliminating i-TFA from food included 5 effective main lines of action:

I. Monitoring studies and official food control have been conducted in Poland since 2004. Food samples are selected randomly throughout Poland by sanitary inspection employees. Samples were tested in one laboratory which use GC-MS or GC-FID method, accredited by Polish Centre of Accreditation method. So far, 1327 food samples were analyzed, including 633 infant formula and follow on formula.

II. Legal regulations regarding TFA content in food includes Regulation (EU) 2019/649 of 24 April 2019, Regulation (EU) No 609/2013, Regulation (EU) No 1169/2011 and Regulation (EC) No 1924/2006

III. Database of TFA content in Polish food (e-Base) was created in 2017 under the National Health Programme and currently contains data on the TFA content in 1246 products belonging to 11 food categories. Data on TFA are presented as median and minimum and maximum value, in g/100g, g/whole product and g/portion. The e-Base is updated twice a year with the analytical results of TFA content in food.

IV. Educational activities includes dissemination of knowledge about the TFA through: National Center for Nutrition Education, conferences, sending messages about the e-Base to over 5,000 representatives of target groups, Nutrition Standards for the Polish Population or training for State Sanitary Inspectorate employees or teachers.

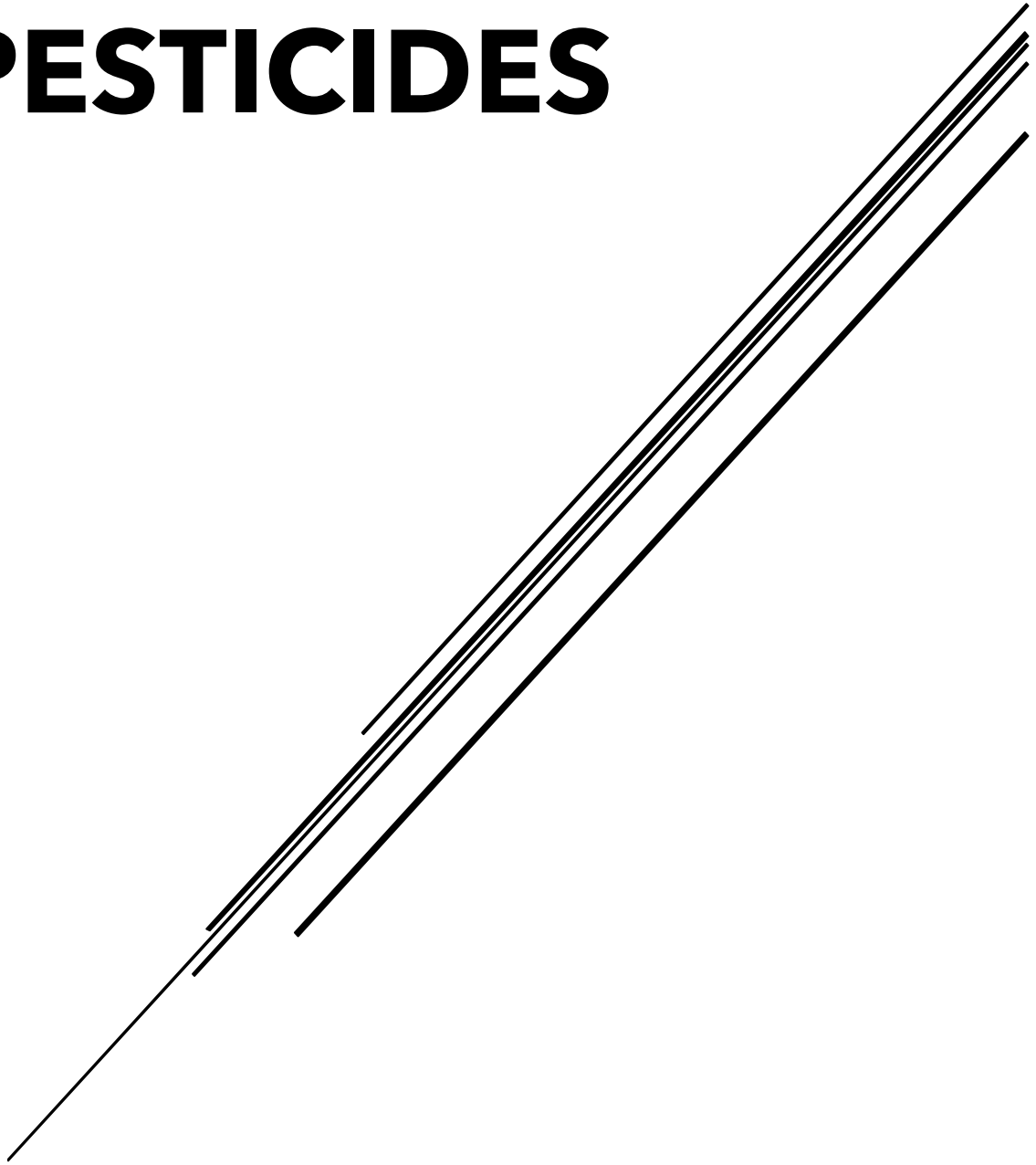
V. Scientific and research activities primarily include the publication of long-standing research results on the TFA content in food and biological fluids (blood, serum, human milk).

Thank to above mentioned activities, reduction in the TFA content in Polish food has been observed for many year. Also dietary TFA intake has decreased.

**Keywords:** i-TFA elimination, polish approach

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# **RESIDUES - PESTICIDES**





### V1

## MONITORING BIOCIDAL CONTAMINATION IN FOOD AND FEED USING A COMPREHENSIVE LC-HRMS METHOD

**Paul Zomer<sup>(1)</sup>, Ingrid Duarte Marques<sup>(1)</sup>, Joëlle Wieman<sup>(1)</sup>, Hans Mol<sup>(1)</sup>**

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: paul.zomer@wur.nl

Biocides are products used to control microorganisms (viruses, bacteria, fungi) and pests (rodents, insects) that can be harmful to human health, the environment, or human activities. While biocides are not intended for direct use on crops, they can still enter the food chain. Examples include not completely rinsing food producing machinery after cleaning with biocidal products, improper application of rodenticides in farm animal housing, or contamination of water bodies with compounds used to prevent fouling on the hulls of ships.

Due to dual use of biocidal active substances in plant protection products and veterinary drugs, maximum limits are set for some compounds that are also used as biocides. However, many approved biocidal compounds have no maximum limits set in food and/or feed. Because of the absence of regulatory limits, many of these compounds are hardly or not at all monitored. To gain insight into the occurrence of biocidal compounds in (processed) food and feed products, analytical methods for their monitoring are needed.

Here, we present a multi-method suitable for detecting more than 110 biocidal compounds in relevant matrices. The method is based on the widely used QuEChERS extraction, as this approach can extract compounds with a wide range of physicochemical properties. For instrumental analysis, LC-HRMS was chosen. The MS method combines full scan analysis of the precursor ions with variable Data Independent (vDIA) acquisition, meaning that all MS1 ions are fragmented. This method can be used for both quantitative analysis of known target compounds and (retrospective) screening for additional compounds that may become of interest at a later stage.

The method is validated for food and feed matrices that have a higher change of becoming contaminated with biocides due to the way they are processed: dairy (milk and milk powder, ice cream), two types of feed (slurry feeds and potato by-products), and fish filets.

Validation was performed according to the SANTE/11312/2021 v2 document. All matrices were validated at three levels, and the LOQ was set as the lowest level that met the criteria for recovery and repeatability. For the majority of the compound/matrix combinations, a LOQ of 0.01 mg/kg was achieved. The feed samples (especially the slurry feed) had some higher LOQs compared to the other matrices, mostly due to lower signals in the MS caused by matrix suppression during the electrospray ionization.

The method was successfully applied to quantify biocides in dairy products, feed, and fish. In 2022, sixty-two samples were analyzed, with DDAC-C10 and/or imazalil detected in five samples. In 2023/2024, 20 fish samples were analyzed, revealing lauryldimethylamine oxide and myristamine oxide in 17 samples. The method's application in real samples demonstrates its suitability for routine analysis. Additionally, it has the potential to be expanded to include other biocides and combined with existing pesticide residue methods.

**Keywords:** biocides, LC-HRMS, multi method, food, feed

### V2

## PESTICIDE REMOVAL FROM WATER USING DIFFERENT TYPES OF ADSORBENT MATERIALS

**Alejandro Cao**<sup>(1)</sup>, **Natalia Vilarino**\*<sup>(2)</sup>, **Lisandra De Castro Alves**<sup>(3)</sup>, **Jose Rivas**<sup>(3)</sup>, **Yolanda Pineiro**<sup>(3)</sup>, **Luis Rodriguez-Santos**<sup>(2)</sup>, **M. Carmen Louzao**<sup>(2)</sup>, **Sandra Raposo-García**<sup>(2)</sup>, **Luis M. Botana**<sup>(2)</sup>

<sup>1)</sup> Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain, Spain

<sup>2)</sup> Departamento de Farmacología, Farmacia y Tecnología Farmacéutica, Facultad de Veterinaria, Universidad de Santiago de Compostela, 27002 Lugo, Spain., Spain

<sup>3)</sup> Departamento de Física Aplicada, Facultad de Física, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain, Spain

\*Corresponding author - E-mail: natalia.vilarino@usc.es

Pesticides are chemical agents used in agriculture to control pests, weeds, and diseases, thereby enhancing crop yield and quality. Among these, imidacloprid, atrazine, and metazachlor are notable due to their widespread application in Europe and their persistence in water, which suppose an impact on both the environment and public health. Although water must be directed to a treatment station before consumption, water treatment processes do not eliminate all pesticides present in the water. To mitigate this problem, a complementary method for toxin removal is investigated which consists of using magnetic nanostructured composites containing an adsorption material. Different adsorption materials have been evaluated: several activated carbons, mesoporous carbon, graphene, chitosan and pectin.

The experiments were carried out by keeping each type of particle in contact with a known concentration of each of the pesticides evaluated, taking samples at 10, 30, 60 and 120 min after particle addition. The amount of compound remaining in solution at these time points was quantified using ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). Panreac-activated carbon nanostructured particles were the best adsorbing the three pesticides, with an effectiveness greater than 85% for the 3 pollutants. The high number of micropores in activated carbon makes it suitable for adsorption of small molecules, such as pesticides, with superior efficiency vs mesoporous carbon, graphene, chitosan and pectin. Subsequently, *in vitro* toxicity of Panreac-activated carbon pulverized composites was evaluated with four cell lines: renal CAKI-1, neuroblastoma SH-SY5Y, liver HepG2 and intestinal CACO-2 cells. No *in vitro* toxicity was observed in the cell lines evaluated.

In conclusion, the use of magnetic-activated carbon composites presents an effective method for removing pesticides from water and enhancing drinking water safety. These composites did not show *in vitro* toxicity, which suggests that they are a safe material to humans and the environment.

**Keywords:** magnetic nanoparticles, pesticide removal, drinking water safety

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### V3

#### EXPLORING PESTICIDE RESIDUE ANALYSIS IN FOOD USING A NITROGEN-ONLY SYSTEM FOR GC-MS/MS

Peter Hancock\*<sup>(1)</sup>, David Gould<sup>(1)</sup>, Douglas Stevens<sup>(1)</sup>, Stuart Adams<sup>(1)</sup>

<sup>1)</sup> Waters, United Kingdom

\*Corresponding author - E-mail: peter\_hancock@waters.com

Gas chromatography coupled to tandem quadrupole mass spectrometry (GC-MS/MS) is a powerful analytical technique used for the detection and quantification of pesticide residues in food samples. The technique has traditionally relied on helium as a carrier gas but, in recent years, there have been difficulties with availability and increased costs in sourcing high purity helium. This situation has increased the level of interest in using alternative carrier gases for gas chromatography. Nitrogen is readily available, relatively inexpensive, and safe compared to other options

We will demonstrate the ease of transferring helium carrier gas GC-MS/MS methods, utilizing an atmospheric pressure ionization source (APGC), to nitrogen carrier gas. APGC achieves improved selectivity and sensitivity when compared to electron ionization (EI) with equivalent performance demonstrated when using either carrier gas. For ease of use, the system was also run using nitrogen as a collision gas meaning all reagent gases can come from one source.

A method for 300 pesticide residues was evaluated on the Xevo™ TQ-XS System with APGC source using both carrier gases on fruit and vegetable food extracts prepared using a modified acetonitrile QuEChERS protocol. Parameters such as column (Restek Rxi-SVOCms) diameter and scaled gas flows were investigated and, once equivalence between the carrier gases was proven, the analysis was repeated comparing argon and nitrogen as collision gases for fruit and vegetable and infant food extracts. All nitrogen analysis showed equivalent calibration coefficients for the compounds as well as equivalent resolution of critical pairs. Scaling the method also meant that retention times could be matched, leading to a reduction in the overall time for method development.

Proving equivalent chromatographic separation and performance when switching to nitrogen carrier and collision gases with APGC can introduce a viable alternative for GC-MS/MS pesticide residue analyses for labs that are under pressure caused by the increased cost and scarcity of helium.

**Keywords:** GC-MS/MS, nitrogen carrier, pesticide residues, APGC

### V4

#### NON-TARGETED ANALYSIS OF FOOD SAMPLES BY A DUAL IONIZATION GC-HRMS USING SIMULTANEOUS EI AND CI FOR UNAMBIGUOUS IDENTIFICATION OF UNKNOWNNS

**Eliska Ceznerova**\*<sup>(1)</sup>, **Sonja Klee**<sup>(1)</sup>, **Steffen Bräkling**<sup>(1)</sup>, **Marlene Moskowitz**<sup>(2)</sup>, **Javier Lopez**<sup>(2)</sup>, **Arnd Ingendoh**<sup>(2)</sup>, **Carsten Baessmann**<sup>(2)</sup>

<sup>1)</sup> TOFWERK AG, Switzerland

<sup>2)</sup> Bruker Daltonics GmbH & Co KG, United States of America

\*Corresponding author - E-mail: [eliska.ceznerova@tofwerk.com](mailto:eliska.ceznerova@tofwerk.com)

The detection and quantification of pesticide residues and contaminants in agricultural products are critical for ensuring food safety and compliance with global regulations. The growing demand for these requirements, driven by consumer awareness and stringent regulations, increases the demands on analytical screening. Chromatographic techniques coupled to mass spectrometry are the most prevalent ones due to their reliability and wide spread application. Especially high-resolution mass spectrometry (HRMS) techniques are becoming one of the most reliable analytical platforms for pesticides analysis, mainly due to their comprehensive detection, and identification of both, known and unknown residues with high sensitivity and specificity. In this study, we present the non-targeted analysis of complex food matrices with different chemical variety by using a novel gas chromatography (GC)-HRMS system for gaining information about pesticide residues.

Different agricultural products were collected and prepared based on common QuEChERS extractions protocols. Blank and standard mixture analysis for an established non-targeted analysis were carried out. The GC-HRMS analysis were measured as triplicates using a GC 8500 system (Bruker, Bremen, Germany) equipped with PAL autosampler (CTC Analytics AG, Zwingen, Switzerland), and an ecTOF (TOFWERK AG, Thun, Switzerland) as mass spectral detector. The ecTOF operates an electron ionization (EI) and a chemical ionization (CI) source in parallel for simultaneous generation of structural and molecular information in a single GC run. A specifically for GC-MS developed medium pressure HRP (helical resonator plasma) CI source allowed the automated selection of different CI reagents ions ( $N_2H^+$ ,  $H_3O^+$ ,  $NH_4^+$ ), which enabled the adjustment of reactant selectivity and the degree of fragmentation for increased molecular information generation. For post-processing of the collected data, AnalyzerPro XD (Spectralworks, Cheshire, UK) was used for target, suspect and non-target analysis based on compound detection & deconvolution as well as statistical analysis.

Several examples are presented which illustrate the advantages of incorporating CI alongside traditional EI in a single GC-HRMS pesticide analysis to enhance measurement reliability, particularly when dealing with complex samples. The simultaneous generation of molecular and structural information by the mass spectrometer within one single GC-MS experiment shows an improved performance for compound identification. Whereas the dual ionization GC-HRMS approach easily identifies commonly investigated pesticides of interest using standard procedures, it simultaneously enables to identify and distinguish other volatile and semi-volatile compounds which may play a crucial role in the food safety. Both standard pesticide screening as well as improved compound identification of unknown compounds within the different, complex agricultural products are presented.

**Keywords:** dual ionization GC-HRMS, pesticide residues, ecTOF, non-targeted analysis, food safety

### V5

#### HIGHLY EFFICIENT LC-MS/MS ANALYSIS OF ORGANOPHOSPHOROUS PESTICIDES UTILIZING ARC-18 COLUMN SELECTIVITY WITH INERT COLUMN TECHNOLOGY

Tina Brandscher<sup>(1)</sup>, Hansjörg Majer<sup>(1)</sup>, Paul Connolly<sup>(2)</sup>, Diego Lopez<sup>\*(2)</sup>, Jan Pschierer<sup>(1)</sup>

<sup>1)</sup> Restek GmbH, Germany

<sup>2)</sup> Restek Corporation, United States of America

\*Corresponding author - E-mail: diego.lopez@restek.com

Organophosphorus pesticides (OPPs) play a pivotal role in modern agriculture, aiding in pest control and enhancing crop yield. OPPs are inexpensive and efficient and account for a significant portion of pesticides used globally. Despite their benefits, these pesticides are toxic, and their extensive use raises concerns regarding environmental contamination and human health risks. Therefore, careful analysis and monitoring is necessary.

However, the analysis faces sensitivity and recovery issues due to their reactivity with metal surfaces in conventional columns, resulting in tailing peaks and low sensitivity. In this work, Restek look to establish the benefits of coated column technologies by comparing methods developed on Inert and Standard Stainless-Steel hardware. The coating combined with our selective stationary phases resulted in exceptional analyte recoveries, lower detection limits and better peak shape. The signal heights and areas compared to conventional columns were doubled. In addition, time-consuming conditioning and complicated passivation belong to the past.

**Keywords:** inert column hardware, organophosphorus pesticides, liquid chromatography, pesticides

### V6

#### **ASSESSING THE PERFORMANCE OF NEW SORBENT COMBINATION IN $\mu$ SPE CARTRIDGES FOR PESTICIDES ANALYSES IN CEREAL BASED MATRIXES**

**Ederina Ninga<sup>\*(1)</sup>, Mette E. Poulsen<sup>(1)</sup>**

<sup>1)</sup> EURL-CF, National Food Institute, Technical University of Denmark, Denmark

\*Corresponding author - E-mail: edni@food.dtu.dk

Pesticide residue methods are constantly being improved and new methods that increase productivity, such as miniaturized and robotic analytical techniques have emerged during the last years. From 2022, newly designed micro-solid phase extraction cartridges are now available, reflecting the increasing shift towards laboratory automation, especially in the clean-up step for the analysis of pesticide residues in food and feed.

In the present study, the introduction of different sorbents on the newly designed PAL  $\mu$ SPE CTC cartridges was investigated for the removal of matrix interferents and the recovery of pesticides in cereal samples.  $\mu$ SPE cartridges containing different sorbent combinations and different amounts were used including PSA, Zsep, C18, and GCB in combination with  $MgSO_4$ .

A preliminary evaluation was conducted to evaluate the effect of different amounts of PSA (5, 10, 15 and 20 mg combined with 20 mg  $MgSO_4$ ) on matrix removal efficiency, pesticides recoveries and RSD. Extracts from two different cereal matrices, wheat and oat, were cleaned-up through different cartridges and results from spiked samples were compared.  $\mu$ SPE containing 10 mg PSA and 20 mg  $MgSO_4$  was chosen for further optimization.

Additionally, we optimise the amount of extract loaded at the cartridges and the elution speed. The evaluation focused on parameters which gave the best results in the removal of co-extractives while maintaining the recovery of pesticides.

Finally, findings from validation studies using clean-up of the same extract on  $\mu$ SPE PSA cartridges containing 10 mg PSA and 20 mg  $MgSO_4$  and comparison with cartridges containing other sorbent combination (20 mg Zsep/20 mg  $MgSO_4$  and 12 mg PSA/12 C18/ 1 mg GCB/ 20 mg  $MgSO_4$ ) analyzed in both GC-Orbitrap and LC-MS/MS analysis will be presented.

### V7

#### **ENHANCED WORKFLOW FOR COMPREHENSIVE PESTICIDE RESIDUE ANALYSIS IN FOOD WITH CROSS-CONFIRMATION USING GC-MS/MS AND EITHER LC-MS/MS OR LC-HRMS**

**Delphine Thomas**\*<sup>(1)</sup>, **Bénédicte Gauriat**<sup>(1)</sup>, **Julie Moriceau**<sup>(1)</sup>, **Maryline Carvalho**<sup>(1)</sup>, **Valerie Thibert**<sup>(1)</sup>, **Aristide Ganci**<sup>(1)</sup>, **Jean-François Garnier**<sup>(1)</sup>

<sup>1)</sup> *Thermo Fisher Scientific, France*

\**Corresponding author - E-mail: delphine.thomas@thermofisher.com*

An innovative approach by cross-confirmation with a new pre-configured pesticide workflow has been developed for multi-class pesticides analysis using Thermo Scientific GC-MS/MS and either LC-MS/MS or LC-HRMS instruments and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS).

More than a thousand pesticides are used in agriculture. To ensure consumer health and regulatory control, it is crucial to define and monitor pesticide residue targets. Laboratories are responsible for developing methods to detect and quantify those pesticides and their transformation products in various sample matrices, in compliance with Maximum Residue Levels set by the European Union Reference Laboratory (EURL). The unique feature for cross-confirmation of identity, by combining GC-MS/MS and either LC-MS/MS or LC-HRMS data, provides increased confidence in results and reduces the need for re-analysis while minimizing the possibility of false positive and false negative results.

The Chromeleon CDS is a versatile software optimized for a pesticide analysis workflow, offering efficient data processing, customized for laboratory process, and a user-friendly interface.

These workflows include the analysis of up to 700 pesticides, 450 in a single GC-MS/MS analysis and 600 in LC-MS/MS or LC-HRMS analysis with a cross-confirmation rate of up to 50% with hardware, software, acquisition and customizable data processing methods, allowing for fast implementation. The approach enables the detection, identification, and quantitation by combining results in a software interface, increasing confidence in data and improving productivity by reducing the need for repeat sample injections, especially in the presence of interference from difficult samples with high matrix co-extractives.

The analysis of Proficiency Test (PT) samples was carried out to validate the workflow. The BIPEA PT samples consisted of French green beans and wheat, containing various unspecified pesticides within a specific concentration range. The results obtained from the PT test, analyzed using both GC-MS/MS and either LC-MS/MS or LC-HRMS, demonstrated satisfactory outcomes with all pesticide measurements falling within the acceptable range (Z-scores), thus highlighting the accuracy, precision, and reliability of the integrated pesticides workflow.

**Keywords:** pesticide multiresidue analysis, proficiency test, LC and GC cross-confirmation, innovative workflow, chromatography data system

## RESIDUES - PESTICIDES

### V8

#### JOINT MONITORING PROGRAM OF PESTICIDE RESIDUES IN HONEY (MRM - COMPOUNDS)

**Anja Muzyka<sup>(1)</sup>, Anna Buettner<sup>\*(1)</sup>**

<sup>1)</sup> *European Union Reference Laboratory für Pesticides in Food of Animal Origin and Commodities with High Fat Content, Germany*

*\*Corresponding author - E-mail: anna.buettner@cvuafr.bwl.de*

Honey differs from the classic animal origin matrices as it has a higher sugar content and a different pesticide spectrum. Around 250.000 t of honey are produced every year in the EU. Moreover, is honey one of the most popular spreads. The aim of this monitoring was to get an overview about the pesticide situation in honey. Therefore, EURL SRM and EURL AO performed a monitoring study of the most important commercially available honeys on the european market.

In total, 169 honey samples were analysed for MRM (multiple reaction monitoring) compounds by EURL AO using QuEChERS method and an LC-Q-TOF system for LC amenable pesticides and SweEt method and GC-Orbitrap for GC amenable pesticides.

In 60% of the samples the amitraz metabolite N-(2,4-Dimethylphenyl)formamide and in 21% of the samples coumaphos could be detected above the LOQ of 0.001 mg/kg. Only in a view samples other common analytes like acetamiprid, boscalid and thiacloprid were found. 44% of the collected honey samples were from EU countries and 48% from non-EU countries. Between the different origins, no major differences could be observed. 7% of the samples were blends from EU and non-EU countries and the origin of one sample was not specified.

Regarding MRM compounds, there were no findings above the valid maximum residue levels (MRLs).

**Keywords:** honey, monitoring, pesticide residues, pesticide analysis



### V9

## INVESTIGATION OF THE POTENTIAL EXPOSURE OF DANISH BROWN HARES TO PESTICIDES

Elena Hakme<sup>\*<sup>(1)</sup></sup>, Victor Cutillas Juarez<sup>(2)</sup>, Mette Erecius Poulsen<sup>(1)</sup>

<sup>1)</sup> National Food Institute, Technical University of Denmark, Denmark

<sup>2)</sup> Pesticide Residue Research Group, University of Almeria, Spain

\*Corresponding author - E-mail: elehak@food.dtu.dk

The brown hare has become an endangered species in many European countries. Hunting bag records of the brown hare have declined throughout most of Europe since the 1960s. The continuous decline in hares rises concerns and prompts questions about the root causes. The initial and primary factors contributing to the decline in the hare population are probably associated with alterations in the agricultural landscape such as larger field sizes and the reduction of field borders and small wild biotopes between fields. Nevertheless, the ongoing decrease in the hare population could also be linked to the use of pesticides.

The objective of this study was to assess the concentrations and patterns of pesticides found in Danish brown hares. The study aims to identify these pesticides in various hare organs, including urine, liver, lung, and pelt, and explore potential pathways of exposure. A total of over 50 samples from each organ were analyzed, except for the liver, where 100 samples were analyzed. This broader liver sample was included to assess consumption exposure, as the hare's liver is also consumed as food in EU. Validated methods were employed, including multi-residue analysis, to analyze the samples. Additionally, for urine samples, single-residue methods were used to detect polar pesticides such as glyphosate and its metabolites.

A total of 20 pesticides and metabolites were detected in pelt and 13 pesticides and metabolites were detected in urine, 12 pesticides were detected in liver, and seven were detected in lung. Geometric means of detected pesticides varied between 5.02 and 67.4 µg/kg in pelt, 4.94 and 195 µg/ml in urine, 5.58 and 85.8 µg/kg in liver, and 8.35 and 26.6 µg/kg in lung. The highest number of pesticides in pelt suggests that the exposure was mainly due to contact. The presence of pesticides in urine, and at relatively higher concentration than in pelt suggests that the exposure to some pesticides is mainly due to diet. The most frequently detected pesticides were the fungicide tebuconazole in pelt, the herbicide glyphosate in urine, and the acaricide fenazaquin in liver and lung. The results were further interpreted, and correlations were examined between qualitative information of the samples e.g., the season when the samples were collected and the physiological status of hares, in relation to the presence of pesticides.

The findings of this research provide valuable information on the exposure of brown hares to pesticides, contributing to a better understanding of routes of exposure and better evaluation of the situation.

**Keywords:** pesticides, environment, exposure

**Acknowledgement:** This work has been funded by the Danish Environmental Protection Agency.

### V10

#### IMPACT OF CLIMATE CHANGE AND SUSTAINABLE APPROACHES ON PESTICIDE RESIDUES

**Thomas Behnke<sup>(1)</sup>, Andreas Hentschel<sup>(1)</sup>, Paolo Matteini<sup>\*(2)</sup>, Giuseppe Sammarco<sup>(3)</sup>, Jay Alappat<sup>(4)</sup>, Juan Carrasco<sup>(5)</sup>, Sophie Robert<sup>(6)</sup>, Emiliano De Dominicis<sup>(7)</sup>, Cinzia Pellicorio<sup>(8)</sup>, Wessel Kriek<sup>(9)</sup>, Francesca Bellini<sup>(10)</sup>, Massimo Frera<sup>(10)</sup>**

<sup>1)</sup> *Mérieux NutriSciences Berlin, Germany*

<sup>2)</sup> *Mérieux NutriSciences Global, Italy*

<sup>3)</sup> *Mérieux NutriSciences Global, Italy*

<sup>4)</sup> *Mérieux NutriSciences North America, United States of America*

<sup>5)</sup> *Mérieux NutriSciences Global, Chile*

<sup>6)</sup> *Mérieux NutriSciences, Global, France*

<sup>7)</sup> *Mérieux NutriSciences Resana, Italy*

<sup>8)</sup> *Mérieux NutriSciences Italy, Italy*

<sup>9)</sup> *Mérieux NutriSciences Somerset West, South Africa*

<sup>10)</sup> *Mérieux NutriSciences Verona, Italy*

*\*Corresponding author - E-mail: paolo.matteini@mxns.com*

Climate change is affecting most production sectors at all geographical latitudes, often in unpredictable ways. In this context the type, distribution and concentration of pesticides are also very likely to be affected. All this happens in a general framework of growing sensitivity and concern among people regarding the quality of food and environmental protection, which has triggered progressive restrictions in some pesticides regional regulations.

From our observation point at Mérieux NutriSciences (a multinational company engaged with analytical testing and services) we have noticed an increase in the number of alerts, with possible intensification of crisis situations, and potential variations in the use of pesticides in particular climatic situations. Furthermore, alternative solutions such as bio-pesticides may become more important.

In this scenario, we believe that for analytical laboratories dealing with pesticide monitoring it is essential to maintain a proactive approach: extensive and updated lists, ability to test "every" matrix, cutting-edge technology for high performance methods and fast TAT, and eventually the capacity to provide specialist consultancy services, including support for field treatment.

**Keywords:** climate change, pesticides, residues, bio-pesticides

### V11

#### NOVEL TRIPLE QUAD APPROACHES FOR RELIABLE AND SENSITIVE QUANTIFICATION OF A LARGE NUMBER OF PESTICIDES IN A SINGLE RUN

Diego Martin Ortiz<sup>(1)</sup>, Javier Loepz Flores<sup>\*(1)</sup>

<sup>1)</sup> Bruker, Spain

<sup>\*</sup>Corresponding author - E-mail: javier.lopez@bruker.com

Pesticides represent the most frequently analyzed hazardous compounds in food and feed globally. The Rapid Alert System for Food and Feed (RASFF) network reports nearly 1,000 instances of pesticide presence in fruits and vegetables every year, both at border control and within the market. A key challenge is the vast number of diverse pesticide types (multi-class analytes) to be analyzed in a single run. Consequently, screening techniques are gaining popularity, particularly for organic and pesticide-free products. These techniques push rapid detection while still allowing for quantification of positive samples. Proposed here is a methodology for the quantitative analysis of a large number of pesticides in food using a novel triple quadrupole mass spectrometer.

A UHPLC (Elute 2, Bruker) was used with mobile phase A (water with 0.01% formic acid and 2 mM ammonium formate) and B (methanol, same buffer) at a 15-100% B gradient in 0.5-8min. Data acquisition time was 9.2 min with subsequent 2.5 min equilibration. 2  $\mu$ L sample were injected on a Bruker Intensity Solo 2.0 100 x 2.0 mm column at 45 °C temperature. An EVOQ-DART TQ+ with pulser-HESI was used in MRM Compound Based Scanning. At a scan time for each compound of 5 - 11 ms, the method that can accommodate more than 1000 pesticides in fast polarity switching with at least 2 transitions in a single run. A probe temperature program was developed to reduce the impact for thermolabile compounds eluting at the end of the chromatogram, like Abamectin, Ivermecting or Sprimesifen. A calibration curve containing more than 500 pesticides in the mix was prepared in a pepper matrix (QuEChERS extraction) with five calibration levels between 0.2 and 50 ppb. Thirty real samples prepared with the same extraction method were injected afterward to quantify the potential pesticides.

The calibration curve showed an  $R^2 > 0.99$  for 95% of the compounds with typical ion ratios at  $< 10\%$  deviation. For 10 consecutive injections, an RSD  $< 10\%$  for most pesticides was achieved in the real matrix spiked with 2 ppb in total. Different methods have been concluded for 520 pesticides in a single run (positive and negative ionization). Due to of novel electronics and the rapid polarity switching, the intensity and ion ratio remained consistent regardless of the scan speed. All these parameters indicate a robust and reliable method for screening and quantitation up to 1000 pesticides in a single run.

**Keywords:** pesticide, high throughput, triple quadrupole, QuEChERS

### V12

#### NON-TARGET SCREENING OF FOOD FOR HALOGENATED SUBSTANCES BY LC-HRMS APPLIED IN THE EFSA SCREENER PROJECT

**Paul Zomer**<sup>(1)</sup>, **Hans Mol**<sup>(1)</sup>, **Wout Bergkamp**<sup>(1)</sup>, **Anna Undas**<sup>(1)</sup>, **Arjen Lommen**<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: paul.zomer@wur.nl

The EFSA SCREENER project aimed to screen various food commodities for the presence of 212 prioritised REACH chemicals, to additionally screen them for halogenated chemicals, to perform a quantitative confirmatory analysis for selected chemicals found during screening, and to assess their human health risks. This work describes the LC-HRMS-based non-target screening (NTS) of halogenated chemicals.

Over 900 raw food items from plant (fruits/vegetables, wheat) or animal origins (meat, eggs, milk, fish) were collected across four countries. At the commodity level, samples were pooled (max. 5) resulting in 194 samples for analysis. For the non-target measurement a generic extraction, QuEChERS-based method was used. The extracts were analysed by RPLC-Q-HRMS, combining full scan and data independent fragmentation (vDIA).

Data processing and annotation were carried out using an in-house software tool, MetAlign. Initial feature detection was based on MS1 data. Elemental composition analysis was performed on the isotope patterns. From the resulting elemental compositions, only molecular formulas with Cl, Br, and F were kept. These were searched against in-house food toxicant databases (including metabolites). The remaining molecular formulas were analysed using the PubChem database and literature to establish their relevance to the analysed food matrices. This resulted in 96 annotations: 87 compound names (some at multiple retention times), 4 presumed marine compounds, and 5 unresolved molecular formulas. The majority of the tentative detects were pesticides (39) and their metabolites (25), with additional findings including PFAS, antibiotics and a human antifungal drug. In total 1122 annotations were made across all samples, with 90% found in fruits/vegetables.

Whereas for the SCREENER project the pesticides and their metabolites were not considered emerging chemicals in the context of the project, these tentative detects were taken as case to demonstrate the feasibility of the NTS approach. For this, a confirmatory analysis by LC-MS/MS against reference standards was performed for 14 pesticides and 5 metabolites in 29 samples. Of the 220 tentative detects, 131 were confirmed (matching retention time and correct ion ratio of two transitions). Estimated concentrations, based on quantification against solvent standards, were mostly below 10 or even below 1 µg/kg. The 89 tentative detects that could not be confirmed were mostly very low-intensity peaks for which no qualifier ion could be obtained in LC-MS/MS, rendering the confirmatory analysis inconclusive. However, there were also several cases where the tentative detects were clearly false positives.

In conclusion, halogenated compounds can be successfully detected through LC-HRMS based non-target screening. However, manual curation of software-based annotations is required. Except for low-intensity signals, most tentative detects were confirmed through re-analysis against reference standards.

**Keywords:** pesticides, LC-HRMS, screening

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### V13

#### HIGH-THROUGHPUT AUTOMATED OFFLINE $\mu$ -SPE FOR CLEANUP OF COMPLEX QUECHERS EXTRACTS IN GC-BASED MULTI-RESIDUE ANALYSIS

Ivan Aloisi<sup>\*1)</sup>, Marc Tienstra<sup>(1)</sup>, Michel Willemsen<sup>(1)</sup>, Hans Mol<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

<sup>\*</sup>Corresponding author - E-mail: [ivan.aloisi@wur.nl](mailto:ivan.aloisi@wur.nl)

Analysis of pesticide residues in food can be considered as a tedious task. What makes it challenging is often the combination of low concentration of the analytes of interest ( $\mu\text{g kg}^{-1}$ ) and high concentration of matrix components (interferences). Especially in GC-based methods, co-extractants can deteriorate instrumental method performance. Improvement of the robustness of the GC-MS/MS performance can be achieved in several ways including a cleanup using sorbents which have the ability to remove the matrix components from the raw extract. The use of sorbent materials can be performed in dispersive mode (d-SPE) or using SPE cartridges. d-SPE is quick, while SPE cartridges can be more effective but involve a more laborious manual procedure. Recently, a miniaturized and automated version of SPE,  $\mu$ -SPE, was introduced. The latter can be coupled online to any chromatographic system but also operated offline as a benchtop configuration. The aim of this work is to investigate and optimize the use of offline automated  $\mu$ -SPE cleanup for the analysis of GC-amenable pesticides in complex food/feed matrices, such as wheat, linseeds and insects. Different cartridge compositions, loading volumes and elution rates were tested and compared in order to find the best conditions for the cleanup of highly complex QuEChERS raw extracts. Furthermore, the manual d-SPE and the automated  $\mu$ -SPE procedure were compared in terms of analytical performance, throughput and operational costs.

**Keywords:**  $\mu$ SPE, cleanup, pesticide residues

### V14

#### DEVELOPMENT AND VALIDATION OF A RESIDUE ANALYSIS METHOD FOR GLYPHOSATE, ITS METABOLITE AMPA, AND GLUFOSINATE IN HONEY BY DERIVATIZATION AND LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY

**Marianna Martinello**<sup>(1)</sup>, Sara Zanella<sup>(2)</sup>, Franco Mutinelli<sup>(1)</sup>

<sup>1)</sup> *NRL for honey bee health, Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

<sup>2)</sup> *Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Italy*

\*Corresponding author - E-mail: [mmartinello@izsvenezie.it](mailto:mmartinello@izsvenezie.it)

For several years, the European Commission has been paying attention and asking for support from European laboratories in the search for glyphosate in honey, a food for which there are still gaps in the data relating to its presence and concentration. Glyphosate and glufosinate are among the most widely used herbicides in the world but due to their chemical-physical characteristics they cannot be included in classic multi-residue methods. In this study we developed and validate an accurate and rugged LC with tandem MS (MS/MS)-based method for the analysis of glyphosate, its main metabolite aminomethylphosphonic acid (AMPA), and glufosinate after derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) in honey.

The developed method includes acidification and neutralization of the sample, 1 hour derivatization step, extraction and cleanup through HLB SPE cartridges, followed by reverse phase UHPLC-MS/MS determination of the analytes. The method validation was performed in compliance with the SANTE/11312/2021 document. Several quali-quantitative performance criteria, such as linearity, limit of quantification, specificity, recovery, repeatability, robustness and ion ratio were carefully evaluated. The recovery samples, spiked at three different levels, were assessed in honey of different botanical origin (multi-flower, acacia, chestnut and eucalyptus honey).

The method showed satisfactory results according to SANTE/11312/2021 requirements. The linear working range was validated from 20 to 500 µg/kg and the deviation of back-calculated concentration from true concentration of the calibration curve was  $\leq \pm 20\%$ . The average recoveries were within 70-120% and precision (RSDr) <20%. The LOQs achieved during the validation were 0.025 mg/kg for all the three analytes, and are all lower than the MRLs established in honey (0.050 mg/kg for both glyphosate and glufosinate) according to Regulation (EC) No 396/2005 as last amended. The matrix effect is compensated by using matrix-matched calibration curves and deuterated internal standard for each analyte added before processing the samples, like surrogate standards.

The present method allows the quantification of glyphosate, AMPA and glufosinate in honey with an instrument commonly available in food analysis laboratories, LC-MS/MS, at concentrations lower than MRLs level. It could be useful to increase the analytical coverage for this determination, as request by European Commission.

**Keywords:** glyphosate, AMPA, glufosinate, LC-MS/MS, honey

### V15

#### CHLORATE, PERCHLORATE AND BROMATE IN FOOD USING A MIXED MODE REVERSED-PHASE ANIONIC EXCHANGE COLUMN

**Peter Hancock\***<sup>(1)</sup>, **Ofure Idialu**<sup>(1)</sup>, **Simon Hird**<sup>(1)</sup>, **Eimear Trivedi**<sup>(1)</sup>, **Euan Ross**<sup>(1)</sup>

<sup>1)</sup> Waters, United Kingdom

\*Corresponding author - E-mail: peter\_hancock@waters.com

Chlorate, perchlorate and bromate are oxyanions used in food production, rocket fuel, pyrotechnics and fertilizers that have been detected in food. It is important to routinely monitor these compounds as chlorate and perchlorate pose a high risk to human health especially amongst infants and children by inhibiting iodine uptake while bromate has been classed as a possible human carcinogen. The analysis of oxyanions can be challenging because of their low molecular weight and ionic nature.

Chlorate and perchlorate were previously determined in infant milk using the Anionic Polar Pesticide (APP) Column. However, the developed method involved the use of a high concentration ammonium formate buffer which can sometimes result in signal suppression. The objective of this study was to establish an efficient method for the analysis of chlorate, perchlorate and bromate using a novel mixed mode reversed-phase anionic exchange column, Atlantis™ Premier BEH™ C18 AX Column. Separation and detection were via the ACQUITY™ Premier UPLC™ System coupled to Xevo™ TQ Absolute Tandem Quadrupole MS System. Data acquisition and processing utilized waters\_connect™ for Quantitation Software.

Method performance was successfully evaluated using matrix-matched standards prepared in cucumber and infant formula using the EURL Quick Polar Pesticides (QuPPE) PO and AO methods, respectively. Excellent selectivity and retention were achieved for the three analytes using the novel mixed mode reversed-phase anionic exchange column. Responses for the three compounds were linear over a range of 5 - 200 µg/kg with coefficients of determination  $r^2 > 0.990$ . Retention times, ion ratios and residuals were all within the SANTE acceptance criteria. The sensitivity of the method was further evaluated by assessment of the response of matrix-matched standards prepared in infant formula at 0.1 - 20 µg/kg. Excellent sensitivity (LOQ ≤ 0.1 µg/kg) was achieved allowing method flexibility to be realized when sample dilution or reduced sample injection volumes are required.

**Keywords:** oxyanion, polar pesticide, infant formula

### V16

#### **A COMPREHENSIVE HRMS METHODOLOGY FOR THE WIDE-SCOPE TARGET SCREENING OF >750 PESTICIDES IN OLIVE OIL USING LC-(ESI)-/GC-(APCI)-QTOF MS COMPLEMENTARY PLATFORMS**

**Sofia K. Drakopoulou<sup>(1)</sup>, Stefanos E. Kokolakis<sup>(1)</sup>, Apostolos L. Karagiannidis<sup>(1)</sup>, Marilena E. Dasenaki<sup>(2)</sup>, Niki C. Maragou<sup>(1)</sup>, Carsten Baessmann<sup>(3)</sup>, Nikolaos S. Thomaidis<sup>\*(1)</sup>**

<sup>1)</sup> National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771, Greece

<sup>2)</sup> National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Food Chemistry, Panepistimiopolis Zografou, 15771, Greece

<sup>3)</sup> Bruker Daltonics GmbH & Co KG, Germany

\*Corresponding author - E-mail: ntho@chem.uoa.gr

Pesticides play a crucial role in modern agriculture, widely used worldwide to increase production and improve the quality of agricultural products. However, the extensive use of these chemicals and the non-compliance to good agricultural practises may endanger human health safety. In the case of olive oil in particular, producers often resort to pesticides' administration in an attempt to increase the agricultural production and eliminate the risk of crop loss. Considering that olive oil's commercial price and availability varies according to its production per year, pesticides are often extensively used, thus posing serious risks to consumer health. Indeed, part of them often pass into crude olive oil at alarming concentrations, with reference to Maximum Residues Limits (MRL) (*Regulation (EC) No 396/2005*).

In the present work, a comprehensive methodology using liquid chromatography with electrospray ionization, operating in positive and negative mode, and gas chromatography with atmospheric-pressure chemical ionization in positive mode, both coupled to quadrupole-time-of-flight mass spectrometry (LC-(ESI)- / GC-(APCI)-QTOF MS) was developed, enabling the detection of 771 pesticides. The sample preparation involved a QuEChERS-based protocol, which was common for both techniques, differentiated only on the reconstitution step, thus making the method highly applicable in routine analysis. Taking advantage of high-resolution mass spectrometry (HRMS) potential, a strong post-acquisition evaluation of the data was implemented considering all criteria available through LC/GC-HRMS analysis (i.e. mass accuracy, retention time, isotopic pattern, MS/MS fragmentation), aiming at high-confidence identification. A smart evaluation of method's performance was carried out, with 65 representative analytes comprising the validation set. The method was validated in terms of linearity, accuracy, matrix effect and precision, while the limits of detection and quantification of the method were estimated. Finally, twenty Greek olive oil samples were analysed with the proposed methodologies and were found to be in compliance with European legislation, detecting pesticides' residues below the established MRLs.

To the best of our knowledge, this is the widest method developed so far in terms of number of pesticides, applied for the first time in olive oil. It is also worth mentioning that thanks to the HRMS workflow introduced, retrospective analysis of the samples is also enabled. Namely, pesticides not yet evaluated and authorised in the European Union (EU), or unknown pesticides' degradation products and metabolites could be detected in the already analysed samples, thus constituting an important asset towards pesticide control.

**Keywords:** pesticides, olive oil, HRMS, LC-ESI-QToF-MS, GC-APCI-QToF-MS

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### V17

#### **QUECHERS MICRO-SPE CLEAN-UP METHOD FOR THE DETERMINATION OF PESTICIDES IN FEED SAMPLES IN GC-ORBITRAP-BASED ANALYSIS-COLLABORATIVE STUDY**

**Ederina Ninga**<sup>(1)</sup>, **Ivan Aloisi**<sup>(2)</sup>, **Michel Willemsen**<sup>(2)</sup>, **Marc Tienstra**<sup>(2)</sup>, **Julio Cesar Espana Amortegui**<sup>(1)</sup>, **Hans Mol**<sup>(2)</sup>, **Mette E Poulsen**<sup>(1)</sup>

<sup>1)</sup> EURL-CF, National Food Institute, Technical University of Denmark, Denmark

<sup>2)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, Netherlands Antilles

\*Corresponding author - E-mail: edni@food.dtu.dk

To enhance precision and streamline sample preparation in routine analysis, many laboratories are adopting automation tools for sample clean-up. In 2022, the European Reference Laboratory for Pesticides in Cereals and Feedstuff (EURL-CF), in collaboration with CTC Analytics, developed and implemented a new fully automated clean-up workflow utilizing a Stand-Alone Multipurpose Autosampler. This automated procedure includes clean-up followed by dilution of the purified extract and addition of quality standards.

A collaborative study between EURL-CF and Wageningen Food Safety Research (WFSR) aimed to assess the performance of the QuEChERS- $\mu$ SPE clean-up method using the stand-alone system across various feed samples. Comparisons were made between cartridges containing and not containing graphitized carbon black (GBC) in their sorbent composition. The usage of GCB visually showed a notable improvement in pigment removal, particularly in fish feed, likely due to the elimination of carotenoids such as astaxanthin. Different loading volumes were evaluated at different elution rates to find the most reliable in terms of matrix interferences removal. Validation data using PAL  $\mu$ -SPE cartridges as clean-up for the majority of compounds involved in the study met the requirements outlined in the SANTE/11312/2021 (V2) guidelines. The results of this collaborative study, conducted in four different feed matrices, demonstrate the successful implementation of automatic QuEChERS- $\mu$ SPE clean-up in routine analyses for feed samples.

**Keywords:** QuEChERS- $\mu$ SPE clean-up, automatic sample preparation, pesticides residues, feed, GC-Orbitrap

### V18

#### EVOLUTION OF A ROBUST AND SENSITIVE METHOD FOR THE DIRECT ANALYSIS OF POLAR PESTICIDES IN FOOD

**Daniel McMillan**\*<sup>(1)</sup>, Jianru Stahl-Zeng<sup>(1)</sup>, Yoann Fillatre<sup>(1)</sup>, Wim Broer<sup>(2)</sup>

<sup>1)</sup> SCIEX, United Kingdom

<sup>2)</sup> Nofalab, The Netherlands

\*Corresponding author - E-mail: [daniel.mcmillan@sciex.com](mailto:daniel.mcmillan@sciex.com)

The prevalence of multi-residue LC-MS/MS analyses for the quantification of pesticides in food and environmental samples has been steadily increasing for many years, and they are now considered to be a minimum requirement of most laboratories working in these fields. Modern tandem quadrupoles are capable of detecting such regulated compounds at very low levels with minimal sample preparation, such as QuEChERS, thereby enabling labs to process large numbers of samples for many analytes with a fast turnaround. However, some very polar analytes which are not amenable to the extraction procedure, chromatographic method or are poor ionisers require additional single-residue methods which involve time-consuming preparation and separation and often involve derivatisation to improve detection.

Recent increase in public concern regarding the presence of glyphosate has significantly increased the requirement to analyse it and its metabolites in food, feed and the environment, so has accelerated the need for a more efficient and robust analytical method. The extraction and chromatography of these compounds is well described in the EURL-QUPPE method, but the separation is not robust in practice, so system and method maintenance are intensive. Several different HPLC or HILIC based methods have failed to address the issues of reproducibility and sensitivity, so FMOC derivatisation prior to analysis is often still employed for glyphosate, AMPA and glufosinate. Although possible to automate, this procedure is still time consuming or expensive, and is not applicable to the other polar pesticides of interest.

This poster shows how the method has evolved and been improved over the years with experience from its use in many different routine food testings laboratories

**Keywords:** glyphosate, LC-MS/MS polar pesticides, underivatised, quppe

### V19

#### SIMULTANEOUS DETERMINATION OF MYCOTOXINS AND PESTICIDES IN FISH FEED AND BEEKEEPING PRODUCTS BY QUECHERS-LC-MS

Maria Alvarez-Romero<sup>(1)</sup>, Jesus Marin-Saez<sup>(2)</sup>, Antonia Garrido-Frenich<sup>(3)</sup>, Ana M. Garcia-Campana<sup>(1)</sup>, Laura Gamiz-Gracia<sup>(1)</sup>, Maykel Hernandez-Mesa\*<sup>(4)</sup>

<sup>1)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, E-18071 Granada, Spain, Spain

<sup>2)</sup> (1) Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, E-18071 Granada, Spain. (2) Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, CIAIMBITAL, ceiA3, University of Almeria, E-04120, Almeria, Spain, Spain

<sup>3)</sup> Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, CIAIMBITAL, ceiA3, University of Almeria, E-04120, Almeria, Spain, Spain

<sup>4)</sup> Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain

\*Corresponding author - E-mail: maykelhm@ugr.es

Mycotoxins and pesticides are chemical substances found in food and animal feed that can pose health risks. Fish farming, which is crucial for reducing pressure on marine ecosystems, has regulated maximum levels for mycotoxins and pesticide residues in fish feed (Recommendation (EU) 576/2006). On the other hand, beekeeping products, such as pollen and honey, are valued for their nutritional benefits. Studies have detected mycotoxins and pesticides in these products, though there are no established maximum concentration levels for mycotoxins in honey or pollen. Maximum residue limits for pesticides in honey are set by Regulation (EC) 396/2005, but bee pollen remains unregulated.

This work proposes a multi-analyte method for the determination of 28 compounds, including 10 pesticides and 18 mycotoxins, in fish feed, bee pollen, and honey. The method involves a QuEChERS-based extraction, followed by liquid chromatography (LC) separation on a Hypersil GOLD aQ column. Detection was performed using Q-Exactive Orbitrap mass spectrometry (MS) for fish feed and by triple quadrupole (QqQ)-MS for beekeeping products. Method validation was carried out according to the SANTE/11312/2021 guideline, with determination coefficients above 0.990 for matrix-matched calibration curves of all compounds. Limits of quantification (LOQs) ranged from 0.1-100 µg/kg for fish feed and 0.1-289 µg/kg for beekeeping products, with extraction recoveries above 62% and satisfactory precision (RSDs < 20%) in all cases.

The method was applied to 36 fish feed samples, detecting pesticides such as pirimiphos-methyl in 75% of samples (maximum concentration 16.5 µg/kg), carbendazim (27%), tebuconazole (25%), and acetamiprid (15%). Mycotoxins detected include enniatin B and B1 (81%), zearalenone (19%), aflatoxin B1 (11%), alternariol (11%) and deoxynivalenol (8%). Ochratoxin α was found at the highest concentration (202 µg/kg), but no maximum limits from EU were exceeded. Additionally, non-targeted analysis revealed the presence of piperonyl butoxide, a compound used to enhance pesticide action, in 81% of samples. In beekeeping products, 28 honey and 5 pollen samples were analyzed. Acetamiprid and azoxystrobin were detected in 29% and 32% of honey samples, respectively, but their concentrations were within EU maximum limits. No pesticide residues or mycotoxins were found in bee pollen.

**Keywords:** pesticides, mycotoxins, tandem mass spectrometry, fish feed, beekeeping products

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### V20

#### SCREENING OF PESTICIDES AND MYCOTOXINS TRANSFER FROM FEED INTO EDIBLE INSECTS

Jingwen Han<sup>(1)</sup>, Dana Schusterova<sup>(1)</sup>, Tereza Dolezalova<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: hajslovj@vscht.cz*

Insects have been consumed as food for centuries, particularly in regions outside Europe. As of January 1st, 2018, Regulation (EU) 2015/2283 of the European Parliament and Council on Novel Foods has been in effect within the European Union. The regulation defines some insects and products thereof as novel foods. Until now, four species have been certified. Nevertheless, it is imperative to consider the potential food safety issues associated with the possibility of hazardous chemicals being transferred into insects through contaminated feed or from environment they are farmed. This study aimed to investigate the potential for the occurrence of pesticide residues and mycotoxins in the larvae of the yellow mealworm (*Tenebrio molitor*). Ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry was employed to develop and validate a method for the simultaneous analysis of 402 pesticide residues, pesticide metabolites, and 45 mycotoxins. First, the presence of pesticide residues was monitored in the larvae following their feeding by two batches of carrots with incurred residues of azoxystrobin, difenoconazole, fluazifop-P, pirimicarb, and tebuconazole. However, only fluazifop-P was detected in mealworm samples. Following the hydrolysis of conjugated or bound residues present in the larvae, an increase in the concentration of fluazifop-P was observed, indicating that it can be metabolized and accumulated in the larvae. The amount of total fluazifop-P residues in yellow mealworm larvae correlated with extent of carrot contamination (p

**Keywords:** edible insects, pesticide residues, mycotoxins, yellow mealworm, *Tenebrio molitor*

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### V21

#### COMPARISON OF SPE- AND QUECHERS-BASED EXTRACTION FOR THE ANALYSIS OF PESTICIDE METABOLITES IN HUMAN URINE

**Kamila Stehnova**<sup>(1)</sup>, **Darina Dvorakova**<sup>(1)</sup>, **Denisa Parizkova**<sup>(1)</sup>, **Jana Pulkrabova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: kamila.stehnova@vscht.cz*

Human biomonitoring (HBM) has become an important tool to assess and evaluate the extent of internal exposure of the population to chemical pollutants such as pesticide residues or their metabolites. Pyrethroids (PYRs) and organophosphates (OPs) are among the most commonly used insecticides over the last 30 years, although some of the highly toxic OPs have been replaced by neonicotinoid pesticides (NNIs) in recent decades. Their extensive use has led to human exposure to these chemicals and their metabolites are frequently detected in human biological matrices (e.g. urine, blood, breast milk). For this reason, the major metabolites of PYRs, OPs and NNIs are included in HBM projects such as the recently completed European Human Biomonitoring Initiative (HBM4EU) and the ongoing Partnership for the Assessment of Risks from Chemicals (PARC).

In a first step of our study, a U-HPLC-MS/MS-based method for the simultaneous determination of metabolites of PYRs, OPs and NNIs in human urine samples was developed. A total of ten biomarkers represented by metabolites of PYRs [cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis-DBCA), cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis-DCCA), trans-DCCA, 3-phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), and cis-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylic acid (CIF3CA)], OPs [3,5,6-trichloro-2-pyridinol (TCPy)] and NNIs [acetamiprid, acetamiprid-N-desmethyl (DMAP) and imidacloprid] were included in the method. The second aim of this work was to select the optimal sample preparation method. After an extensive literature search of studies conducted in the last 5 years, two extraction/clean-up approaches were selected for comparison: i) solid phase extraction (SPE) performed on Oasis HLB (3 cm<sup>3</sup>, 60 mg) cartridges and ii) the QuEChERS-based method. For both methods, isotopically labelled compounds were used to compensate for potential losses during sample preparation as well as ion suppression observed in urine samples. Both extraction protocols with subsequent U-HPLC-MS/MS analysis were critically evaluated with regard to validation parameters such as analyte recovery, repeatability and limit of quantification. The optimised and validated method with the best characteristics will be used for the analysis of urine samples from adults and children in the Czech Republic, obtained in cooperation with the National Institute of Public Health in Prague. The data obtained will provide important information on the exposure of the Czech population to selected pesticides and, thanks to participation in the PARC project, will enable comparison at international level.

**Keywords:** pesticide metabolites, human biomonitoring, SPE, QuEChERS, LC-MS/MS

**Acknowledgement:** *The result was achieved thanks to the support of the Junior Grant of the Rector of the University of Chemistry and Technology, Prague, for the year 2024 from the funds of the Institutional Plan.*

### V22

#### POST-HARVEST PROCESSING TECHNOLOGY OF POPPY TO ENSURE QUALITY AND SAFETY OF FINAL PRODUCT - PILOT STUDY

Dana Schusterova<sup>(1)</sup>, Katerina Sebelova<sup>(1)</sup>, Petr Mraz<sup>(1)</sup>, Leos Uttl<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: schusted@vscht.cz*

The Czech Republic is one of the world's leading producers and exporters of non-opium poppy. The importance of this commodity is also confirmed by the fact that the designation 'Český modrý mák' (Czech blue poppy) has been registered as a Protected Geographical Indication since 2021. The poppy plant contains opium alkaloids, but the mature seeds of non-opium poppy varieties are almost free of alkaloids. Elevated levels of alkaloids may be present on the surface of the seeds, either as a result of insufficient cleaning or contamination by poppy juice released when the seeds are disturbed due to mould or insect infestation. The use of insecticides and fungicides (such as acetamiprid, fluopyram, prothioconazole or tebuconazole) as chemical protection against diseases and pests is therefore considered desirable by growers, not only to increase yields but also to improve the quality and safety of the poppy. The application of herbicides is also common in poppy cultivation. Residues of the active substances applied may subsequently be present in the poppy seeds. After harvest, poppy seeds are cleaned of poppy straw and other impurities and in some cases treated with thermostabilisation (Commission Recommendation 2014/662/EU). Although these processes used in poppy processing have been previously studied at the University of Chemistry and Technology (e.g., in relation to the reduction of opium alkaloids), data are not available to comprehensively assess the impact of different processes on pesticide residue levels.

**Keywords:** pesticide residues, poppy seed, post-harvest processing technology

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### V23

## MONITORING OF PESTICIDES IN OILSEEDS AND THEIR TRANSFER INTO OIL

**Petr Mraz**\*<sup>(1)</sup>, **Leos Uttl**<sup>(1)</sup>, **Dana Schusterova**<sup>(1)</sup>, **Vladimir Kocourek**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

\*Corresponding author - E-mail: Petr.Mraz@vscht.cz

The popularity of oilseeds for direct consumption has been growing in recent years due to their content of biologically active compounds with beneficial effects on human health. Also, the demand for cold-pressed oils from these seeds is growing. However, oilseeds can contain residues of pesticides, used for their protection against harmful organisms, and can be transferred into oil.

The aim of this study was to get a comprehensive knowledge of the occurrence of pesticide residues in different types of oilseeds, which can be obtained from Czech food stores. The samples were extracted by QuEChERS method followed by a clean-up process (dSPE with C18 silica). The extracts were screened by both GC-MS/MS and LC-MS/MS for more than 450 target pesticides. From 97 oil seed samples, 65 % contained at least one pesticide residue, 48 % of the contaminated ones contained more than one residue. Poppy seeds, sesame seeds, and chia seeds were most often contaminated. No pesticide residue was found in flax seeds.

In the next phase, the oil pressed from 28 most contaminated samples was analysed. 29 pesticide residues were detected in these samples and 12 were transferred into pressed oil. All detected types of pesticide residues are capable of being transferred into oil. The process factors were calculated for all of them. The highest process factor was calculated for chlorpyrifos-methyl detected in the case of pumpkin seeds.

**Keywords:** pesticide residues, oilseeds, process factors, cold-pressed oils, LC-MS/MS, GC-MS/MS

**Acknowledgement:** *The work used [data/tools/services/facilities] provided by the METROFOOD-CZ Research Infrastructure (<https://metrofood.cz>), supported by the Ministry of Education, Youth and Sports of the Czech Republic (Project No. LM2023064) and by grant of Specific university research - grant No A2\_FPBT\_2020\_036.*

### V24

#### ARE FREEZE-DRIED STRAWBERRIES AND RASPBERRIES REALLY A HEALTHY SNACK?

Eva Czmorik Ludvickova<sup>(1)</sup>, Dana Schusterova<sup>(1)</sup>, Petr Mraz<sup>(1)</sup>, Zbynek Dzuman<sup>(1)</sup>, Jana Ryparova Kvirencova<sup>(1)</sup>, Vladimir Kocourek<sup>(1)</sup>, Jana Hajslova<sup>(1)</sup>, Vojtech Hrbek<sup>(1)</sup>

<sup>1)</sup> Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic

\*Corresponding author - E-mail: Eva.Czmorik.Ludvickova@vscht.cz

Small red fruits like strawberries and raspberries are popular for their attractive color and taste. While these fruits were traditionally consumed seasonally, advancements in preservation technologies, particularly freeze-drying, have made them available year-round. Freeze-dried fruit products are currently advertised as "healthy" snack for children and adults. Strawberries and raspberries, due to their water and sugar content, are prone to mold and that is why various agrochemicals, such as pesticides (fungicides), are used. During the freeze-drying process, substances with positive biological activity and possible harmful substances (e.g. pesticide residues) are concentrated and therefore these products can pose a certain health risk, especially for children with smaller body weight. For this reason, the chemical safety of these products needs to be evaluated.

This study was focused on the chemical safety evaluation of freeze-dried strawberries and raspberries available on the Czech market, particularly concerning pesticide residues and mycotoxins. A total of 44 freeze-dried fruit products were analyzed, including 32 samples of strawberries and 12 samples of raspberries, with some products being examined across different batches. For the determination of pesticide residues, the QuEChERS extraction procedure was chosen for multi-residue determination using LC-MS/MS and GC-MS/MS methods, and the QuPpe methodology for the determination of fosetyl residues using LC-MS/MS.

In total, 88 different pesticide residues (or their metabolites) were detected. The most frequently detected pesticide was phosphonic acid (fosetyl metabolite), found in 37 samples, followed by boscalid in 24 samples, with pyrimethanil in 19 samples and pyraclostrobin in 19 samples also commonly detected. The results indicate that strawberries are more contaminated with pesticide residues than raspberries. In addition to these findings, carbofuran, omethoate and haloxyfop residues were also detected, which pose a serious health risk. Among the mycotoxins, three were detected: alternariol, tenuazonic acid, and tentoxin, with tenuazonic acid being present in 31 samples.

The study highlights the importance of monitoring pesticide residues in freeze-dried fruits to ensure consumer safety, especially since small children often consume these products. Given the higher levels of contamination found in strawberries, it is crucial to consider the potential health risks, particularly for vulnerable groups such as young children who are more sensitive to chemical exposures.

**Keywords:** freeze-dried, strawberries, liquid chromatography, pesticides, mycotoxins

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### V25

#### FORMAT COMPARISON FOR DISPERSIVE SOLID PHASE EXTRACTION (DSPE) WORKFLOW IMPROVEMENTS FOR FOOD TESTING PRIOR TO GC/MS ANALYSIS

**Thomas Smith\***<sup>(1)</sup>, **Lucy Richards**<sup>(1)</sup>, **Lee Williams**<sup>(1)</sup>, **Geoff Davies**<sup>(1)</sup>, **Russell Parry**<sup>(1)</sup>, **Alan Edgington**<sup>(1)</sup>, **Adam Senior**<sup>(1)</sup>, **Helen Lodder**<sup>(1)</sup>, **Charlotte Hayes**<sup>(1)</sup>, **Zainab Khan**<sup>(1)</sup>, **Claire Desbrow**<sup>(1)</sup>, **Dan Menasco**<sup>(1)</sup>

<sup>1)</sup> *Biotage GB Limited, United Kingdom*

*\*Corresponding author - E-mail: thomas.smith@biotage.com*

**Introduction:** The diverse nature of solid samples in food testing makes method standardisation challenging. Pesticide analysis is centred around traditional QuEChERS salt extraction followed by dispersive solid phase extraction (dSPE) matrix scavenging. Both techniques are manual, labour intensive and typically difficult to automate. This poster will investigate the performance of various AOAC and EN prescribed dSPE clean up sorbent combinations covering a range of general, waxed, pigmented, and highly pigmented fruit and vegetable matrices. Traditional dSPE comprising loose sorbent will be compared to alternative column flow through formats, ISOLUTE® cSPE, in terms of analyte recoveries, RSDs, signal, matrix and pigment removal as well as processing and workflow advantages.

**Methodology:** The target analyte panel consisted of 85 commonly analysed "pesticides". Various matrices (apple, orange, broccoli, spinach and oats) were homogenized using the Biotage® Lysera bead mill homogenizer and extracted into acetonitrile (ACN) using standard AOAC or EN QuEChERS salt combinations. 1-2 mL of the resulting ACN fraction was cleaned up using standard dSPE or equivalent cSPE format. GC-MS analysis was performed using an Agilent 7890A GC coupled to a 5975C MSD equipped with a Quicksnap device. Chromatography was performed on a DB5 capillary column; 30 m x 0.25 mm ID x 0.25 µm using 1.2 mL/min helium at constant flow. Positive ions were acquired using electron ionization operated in SIM mode.

**Results:** A representative panel of 85 analytes were chosen, including pesticides, fungicides and insecticides. Bead mill homogenization was optimised for each matrix type at multiple weights in various tubes to maximize throughput using representative sample sizes. 7 mL tubes with up to 2.5g of matrix allowed for 12 simultaneous samples to be processed whereas 10-15g of matrix reduced capacity to 3 samples. Solid matrix extraction into ACN was performed using standard AOAC or EN QuEChERS salt blends for AOAC or EN methods, respectively.

GC/MS analysis demonstrated recoveries typically greater than 75% for both dSPE and cSPE. Traditional dSPE processing demonstrated slightly higher recovery in many cases. However, over recovery was often an issue when using dSPE, being greater than 120% depending on matrix and sorbent blend used. Improved matrix factors were generally observed for cSPE formats, resulting in increased overall signal compared to dSPE. Repeatability was generally good for both techniques with RSDs below 10%. In all cases, extract cleanliness demonstrated better visual clarity, turbidity and less pigment when using cSPE compared to dSPE. This translated to GC/MS baselines with less noise, interference peaks, levels of suppression and increased number of analytes being accurately quantitated. Frequency of GC/MS liner replacement and cleaning was reduced due to improved sample cleanliness. Finally, the cSPE column formats allow for increased sample throughput.

**Keywords:** pesticides, QuEChERS, dSPE, GC/MS, bead mill homogeniser

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### V26

#### ENANTIOSELECTIVE SEPARATION OF LAMBDA- AND GAMMA-CYHALOTHRIN BY LC-MS/MS FOR CONTROLS OF AGRICULTURAL PRACTICE

Pavλίna Landova<sup>(1)</sup>, Kristyna Ondrova<sup>(1)</sup>, Petra Kosubova\*<sup>(1)</sup>

<sup>1)</sup> Central Institute for Supervising and Testing in Agriculture, Czech Republic

\*Corresponding author - E-mail: [petra.kosubova@ukzuz.gov.cz](mailto:petra.kosubova@ukzuz.gov.cz)

Cyhalothrin is a synthetic pyrethroid insecticide composed of four stereoisomers. Only lambda-cyhalothrin (a 1:1 mixture of RS and SR isomers) and gamma-cyhalothrin (SR isomer only) are currently authorized for use in several EU countries, including the Czech Republic. When analyzing conventionally produced food, feed, and other agricultural commodities, detection is typically performed using GC-MS/MS or LC-MS/MS techniques with standard achiral chromatographic phases. Under these conditions, the RS and SR enantiomers are not separated, and the results are reported according to the residue definition (Reg. 396/2005) as lambda-cyhalothrin including the gamma-isomer (sum of RS and SR isomers). However, for integrated production, only the most insecticidally active enantiomer, gamma-cyhalothrin, is approved. In such cases, enantioselective chromatographic separation is essential to distinguish gamma-cyhalothrin from lambda-cyhalothrin. To meet this need, the laboratory adopted a method published by EURL-SRM, involving QuEChERS extraction, chromatographic separation on a ChiralArt Cellulose-SB column, followed by LC-MS/MS determination. The established method was applied for the analysis of plant material samples and spray liquids to confirm the presence of declared active substances. In addition to these results, the stability of the standard solutions will also be discussed.

**Keywords:** enantioselective separation, LC-MS/MS, lambda-cyhalothrin, gamma-cyhalothrin

### V27

## INNOVATIVE PESTICIDE SCREENING IN COMPLEX MATRICES: THE CASE FOR HIGH RESOLUTION ION MOBILITY MASS SPECTROMETRY

Oscar Cabrices<sup>(1)</sup>, Julie Wushensky<sup>(2)</sup>, Miriam Fico<sup>(2)</sup>, Frederick Strathmann<sup>\*(2)</sup>, Mario Stefanelli<sup>(3)</sup>

<sup>1)</sup> G-Flo Scientific, United States of America

<sup>2)</sup> MOBILion Systems, United States of America

<sup>3)</sup> Lab Instruments, Italy

\*Corresponding author - E-mail: frederick.strathmann@mobillionsystems.com

The burgeoning need to secure the food supply against unexpected contaminants has catalyzed the development of comprehensive screening methods using high resolution mass spectrometry (HRMS). These methods are particularly valuable in expanding the scope of pesticide detection in the laboratory setting in a cost-effective manner. Additionally, these methods facilitate the detection and identification of unexpected pesticides complementing the laboratories' existing quantitative methods that target frequently detected pesticides. Despite the comprehensive nature of available methods, issues such as chromatographic separation, specificity, matrix interference and chimeric spectra from isomers and isobars complicates their widespread adoption. These known limitations provide a path for the introduction of novel separation technologies where specificity and throughput can co-exist.

The combination of HRMS with high-resolution ion mobility (HRIM) provides substantial advantages in specificity and throughput over existing workflows. HRIM augments mass spectrometry's capabilities by separating molecules based on their shape and charge, enhancing the separation of complex matrices and removing the challenges of reliance on liquid chromatography (LC) for specificity not achievable by HRMS alone. HRIM digitally separates pesticide residues from matrix components while separating isobars and isomers that result in challenging chimeric spectra. By rapidly separating compounds before mass detection, HRIM considerably cuts down analysis time, solvent use and heightens throughput without losing specificity. Additionally, the structural information provided by HRIM dramatically improves both targeted and non-targeted workflow performance.

The goals of this project were multifaceted: 1) to use an established QuEChERS protocol for sample preparation of black pepper as an archetypal complex matrix; 2) to create a < 3 minute ballistic gradient LC method; 3) to demonstrate relative quantitation across a range of concentrations; and 3) to evaluate the utility of predicted CCS values for analyte annotation. This research aims to refine and extend existing workflows to handle an extensive list of over 600 pesticides, ensuring comprehensive coverage and compliance with food safety standards. Through this innovative approach, this project endeavors to significantly shift the pesticide testing paradigm for pesticide screening, setting a new benchmark for efficiency, reliability and speed in food safety testing.

**Keywords:** pesticides, HRMS, isobars, QuEChERS, 2D chromatography

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### V28

#### **“BEYOND TARGETED MONITORING: DUAL APPROACH FOR THE EARLY DETECTION OF TOXICOLOGICAL RISKS IN AGRICULTURAL PLOTS USING HIGH RESOLUTION MASS SPECTROMETRY”**

**Flavia Barragan**<sup>\*(1)</sup>, **Jesse L. Calvillo**<sup>(1)</sup>, **Jose M. Perez**<sup>(1)</sup>, **Oliver Stolper**<sup>(1)</sup>

<sup>1)</sup> *Analytica Alimentaria GmbH, Germany*

*\*Corresponding author - E-mail: flavia.barragan@aalimentaria.com*

Efforts towards “zero residue” in agriculture are primary topics in the modern evolution of the agri-food industry. This challenge, however, may push producers to pursue alternative and less-known tools for pest control, leading to emergent and unknown risks that may be toxicologically dangerous. Unknown, undeclared, or accidental residues (of active substances or contaminants) can enter the food chain if labs are not specifically testing for them within their targeted scopes.

In this work, we describe an innovative “qualitative + quantitative” approach to monitoring agricultural plots throughout the entire growing season. We have developed an in-house toxicological sampling technique and combined it with broad-spectrum, open-ended analysis using High Resolution Mass Spectrometry (Q-TOF) to tailor testing to the specific conditions and limitations of agricultural plots. This dual approach allows for the confirmation of degradation kinetics of plant protection products, monitoring of unexpected secondary impacts from overlapping applications, qualitative surveillance for emergent risks, and retrospective interrogation of historical data for specific risks.

This work presents preliminary results from one year of monitoring clementine, pepper, and tomato fields. The method has demonstrated its potential in early detection of irregular practices, such as the use of unapproved pesticides and biocides. Notably, clofentezin, an acaricide, was detected despite its non-renewal approval status under EU Regulation (EC) No 1107/2009, highlighting the importance of early detection in regulatory compliance and mitigating risks. These findings underscore the importance of early detection in enhancing the safety and sustainability of agricultural practices.

**Keywords:** field monitoring, high resolution mass spectrometry, integrated pest management, agricultural surveillance, non-target screening

### V29

#### DIFFERENT PESTICIDE TREATMENT STRATEGIES IN LOCALLY GROWN AND IMPORTED APPLES IN THE CZECH MARKET

**Petra Vackova<sup>(1)</sup>, Jana Hajslova<sup>\*(1)</sup>, Petr Mraz<sup>(1)</sup>, Leos Uttl<sup>(1)</sup>, Vladimir Kocourek<sup>(1)</sup>**

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: hajslovaj@vscht.cz*

To protect the crop from pests and diseases, the use of a pesticide on apples is essential. Pesticide application strategies can significantly affect the safety and quality of agricultural products. Different treatment approaches have an impact on the efficacy of protection, pesticide residue concentration, pesticide resistance, fruit quality and more. For this reason, the aim of the study was to assess the occurrence of pesticide residues in apples from the Czech Republic compared to other countries (mainly Poland) and to answer the question whether different treatment strategies can be identified based on pesticide profiles. To obtain up-to-date information about the presence of pesticide residues in apples on the Czech market, we compared pesticide residue pattern found in domestic apples and imported ones in a period 2019-2023. Both LC-MS/MS and GC-MS/MS methods were used to test for pesticide residues in apples of various varieties harvested in several regions of the Czech Republic. A set of 265 samples of apples of different varieties harvested in the Czech Republic (48.7% of the total number of samples) and imported from other countries (51.3% of the total number of samples) were analysed for 460 pesticide residues using LC-MS/MS and GC-MS/MS methods between 2019 and 2022. Pesticide residues were found in 260 of them (98.1% of all samples). A total of 71 different pesticides were found. The data show different pesticide treatment strategies in the EU countries. If fenpyroximate was detected in a number of imported apples, it was not found in a number of Czech apples. In contrast, pyriproxyfen was only detected in apples originating in the Czech Republic. The largest difference in pesticide use between Czech and imported apple producers was found for difenoconazole (50% of Czech samples and 19% of imported samples).

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### V30

#### DETERMINATION OF PESTICIDES USING GC-MS/MS WITH HYDROGEN AS AN ALTERNATIVE CARRIER GAS

Uwe Oppermann\*<sup>(1)</sup>, Waldemar Weber<sup>(1)</sup>, Dominika Gruszecka <sup>(2)</sup>, Victor Cutillas <sup>(3)</sup>, Chigusa Ichikawa <sup>(4)</sup>, Alan Owens <sup>(2)</sup>, Yoshiro Hiramatsu <sup>(2)</sup>

<sup>1)</sup> Shimadzu Europa GmbH, Germany

<sup>2)</sup> Shimadzu Scientific Instruments, United States of America

<sup>3)</sup> European Union Reference Laboratory for Pesticide Residues in Fruit & Vegetables, Agrifood Campus of International Excellence (ceiA3), University of Almeria, Spain

<sup>4)</sup> Shimadzu Corporation, Japan

\*Corresponding author - E-mail: [uo@shimadzu.eu](mailto:uo@shimadzu.eu)

When it comes to Gas Chromatography (GC) and Gas Chromatography-Mass Spec\_x0002\_trometry (GCMS), high purity gas is essential. Helium is the preferred choice due to its inertness, safety, high resolution and speed, and excellent sensitivity, making it the preferred carrier gas for most GC and GCMS applications. However, Helium re\_x0002\_sources have been depleting over the years, as it is extracted from natural gas and escapes our atmosphere, which has resulted in significant price increases over the recent years. In addition to helium-saving techniques, an alternative carrier gas such as hydrogen can be a viable option in the absence or shortage of helium in order to achieve reliable and reproducible chromatographic results. Of course, safety is an important subject and the Nexis GC-2030 helps to ensure that hydrogen carrier gas is used safely. Hydrogen can be a safe and highly effective carrier gas, with correct safety considerations and additional measures. All of Shimadzu's new GC and GCMS models include a built-in software-controlled hydrogen sensor, which constantly monitors the hydrogen concentration inside the GC oven and maintains a safe stand\_x0002\_by mode for early detection of potential leaks. When a leak is detected, the system will automatically shut off gas flow and lower the temperature, switching to a safe standby mode. Copper piping has a safety risk when using hydrogen as a carrier gas due to hydrogen embrittlement. In Shimadzu GCs, stainless steel material is used in the entire flow path so hydrogen can be used with no additional modifications to the main unit. For the determination of pesticides in fruits and vegetables hydrogen has been used as an alternative carrier gas in the Shimadzu GCMS TQ-8050 NX system for pesticide residue analysis [1]. A routine method has been used, achieving good chromatographic peak resolution attributable to the enhanced chromatographic performance of hydrogen as a carrier gas. Experiential work has been performed on different matrices. For the development of the multiresidue method the Shimadzu Smart Pesticides Database version 2.0 was used. Performance data for reproducibil\_x0002\_ity, matrix effects, and linearity will be presented and the suitability of hydrogen as a carrier gas in multiresidue pesticide analysis in fruits and vegetables by GC-MS/MS will be demonstrated.

[1] V. Cutillas, G. García-Gallego , M. Murcia-Morales , C. Ferrer and A. R. Fernández-Alba, Anal. Methods, 2024, 16, 1564-1569.

**Keywords:** pesticides, GCMS, hydrogen, carrier gas

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### V31

#### HIGHLY SENSITIVE ANALYSIS OF GLYPHOSATE, GLUFOSINATE, AND THEIR METABOLITES IN SOYBEANS USING ONLINE SOLID-PHASE DERIVATIZATION LC-MS/MS SYSTEM

Nozomi Maeshima<sup>\* (1)</sup>, Ryoichi Sasano<sup>(2)</sup>, Jun Watanabe<sup>(1)</sup>, Isao Saito<sup>(2)</sup>, Manami Kobayashi<sup>(1)</sup>, Hiroshi Akiyama<sup>(3)</sup>, Stephane Moreau<sup>(4)</sup>

<sup>1)</sup> Shimadzu Corporation, Japan

<sup>2)</sup> AiSTI SCIENCE, Japan

<sup>3)</sup> Hoshi University, Japan

<sup>4)</sup> Shimadzu Europa GmbH, Germany

\*Corresponding author - E-mail: nozomi\_m@shimadzu.co.jp

Glyphosate and glufosinate are worldwide used as herbicides in crop cultivation. Monitoring their residues in food and soil is crucial for food safety. Therefore, many analytical methods have been reported. Non-derivatization method using HILIC columns and the derivatization method using FMOC are generally employed for glyphosate, glufosinate, and their metabolites. However, these methods have time-consuming such as long reaction time, heating, and evaporation of water and solvent before the derivatization. We developed the analytical method system for glyphosate, glufosinate, N-acetyl glyphosate (Gly-A), N -acetyl glufosinate (Glu-A), and MPPA using LC-MS/MS with online solid-phase extraction and N -(tert-butyl dimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) derivatization automatically.

The sample was analyzed using a triple quadrupole mass spectrometer LCMS-8045 equipped with Nexera (Shimadzu corporation) and online-SPE interface (SPL-W100, AiSTI SCIENCE), which enables fully automated SPE conditioning, sample loading, elution, and direct LC injection. Using this Online-SPE interface, dehydration and derivatization by MTBSTA were performed automatically, and these processes are completed in approximately 10 minutes.

This on-line system was applied to analysis of glyphosate, glufosinate and their metabolites in soybeans. A calibration curve, recovery test, and reproducibility were good results. The derivatized glyphosate and glufosinate have high ionization efficiency and can be detected with high sensitivity.

**Keywords:** online SPE, herbicides, LC-MS/MS, MTBSTFA, derivatization

# **RESIDUES - VETERINARY DRUGS**





### W1

#### RESIDUE DEPLETION OF NITROFUZAZONE METABOLITES IN BROILER CHICKENS AFTER ORAL ADMINISTRATION

**Khaled El Hawari**<sup>(1)</sup>, Jean-François Taillandier<sup>(1)</sup>, Pierre Guichard<sup>(1)</sup>, Michel Laurentie<sup>(1)</sup>, Dominique Hurtaud-Pessel<sup>(1)</sup>, Eric Verdon<sup>(2)</sup>

<sup>1)</sup> Anses - Fougères Laboratory, France

<sup>2)</sup> Anses, French Agency for Food, Environmental and Occupational Health & Safety, France

\*Corresponding author - E-mail: khaled.elhawari@anses.fr

Nitrofurazone (NFZ) antibiotic is banned in food-producing animals and its protein-bound side-chain metabolite, the semicarbazide, is currently used as marker residue for nitrofurazone abuse. However, semicarbazide (SEM) analysis poses challenges as it can be generated during food processing where there was no veterinary treatment. Therefore, SEM cannot be considered as an unequivocal marker of NFZ abuse. This study aims at investigating the use of 5-nitro-2-furaldehyde (5-NF) as an additional marker for nitrofurazone in broiler chickens. The residue depletion of SEM and 5-NF in chickens was studied after oral administration of NFZ and 5-NF at 25 mg.kg<sup>-1</sup> BW for 10 consecutive days. Tissues (muscle and liver) and plasma were collected at different sampling time and analyzed by liquid chromatography-tandem mass spectrometry. In NFZ-treated chickens, 5-NF was detected within one hour in plasma at concentration peak level at 1.4±0.25 µg.kg<sup>-1</sup>, while in the 5-NF-treated ones, the maximum concentration was at 0.85±0.06 µg.kg<sup>-1</sup> after 2 hours. At the sampling time from 0 to 21 days, 5-NF was not detected in muscle and liver for both treated groups. However, alongside that, SEM persisted for up to 3 weeks in muscle (0.3±0.018 µg.kg<sup>-1</sup>), 2 weeks in liver (0.16±0.008 µg.kg<sup>-1</sup>) and 1 week in plasma (0.4±0.05 µg.kg<sup>-1</sup>). Tissue-bound SEM exhibits a faster elimination rate in liver tissues, with an elimination half-life of 4.6 days, compared to muscle tissues, where the elimination half-life is 6.5 days, indicating a slower rate of elimination in muscle. These findings suggest that SEM, compared to 5-NF, remains a better residue marker for detecting nitrofurazone abuse in chickens.

**Keywords:** 5-nitro-2-furaldehyde, semicarbazide, nitrofurazone, broiler chickens, residue marker

**Acknowledgement:** We express our gratitude to the Regional Council of Bretagne's - Department of Higher Education, Research and Development for the financial support. We wish to thank also the European Commission - Directorate General for Health and consumer (DG-SANTE) for its continuous scientific support throughout the project.

### W2

#### ITERATIVE DEVELOPMENT AND FINAL VALIDATION OF AN AUTOMATED METHOD FOR THE JOINT DETERMINATION OF 96 VETERINARY DRUGS FROM VARIOUS MATRICES BY LC-HRMS

**Nico Landsteiner**\*<sup>(1)</sup>, Clara John<sup>(1)</sup>, Jana Lücken<sup>(1)</sup>, Sonja Schittko<sup>(1)</sup>, Mareike Reichel<sup>(1)</sup>, Jan Sebastian Maenz<sup>(1)</sup>, Klaudija Cavlovic<sup>(1)</sup>

<sup>1)</sup> Eurofins WEJ Contaminants GmbH, Germany

\*Corresponding author - E-mail: NicoLandsteiner@eurofins.de

Veterinary drugs (VD) are used in animals to cure diseases. To protect animals, to avoid microbial resistance, and because residues of VD in food can have negative effects on human health, numerous legal limits have been set. Based on the needs of a commercial laboratory for speed, economy, and quality, as many analytes as possible should be detected in a simple, automatable method that still allows a stable, sensitive quantification in a large matrix variety.

An initial selection process of analytes took place based on technical production requirements and expected feasibility. After preliminary tests, some analyte groups were excluded, as no good quality compromise was to be expected in joint processing with the main group. Further analytes were included for which there was a growing analytical need. This resulted in a multi-residue method for the simultaneous determination of 96 analytes including the substance classes benzimidazoles, benzoylphenylureas, diaminopyrimidines, lincosamides, macrolides, salicylanilides, sedatives, and sulfonamides.

For sample preparation, a salting-out assisted liquid-liquid extraction (SALLE) without clean-up or evaporation step was developed in four modifications to fit the matrix categories eggs, fluids, fatty/fats, and all other. The extraction solvents, the choice and amount of salt and the measuring extract solvent were significant factors for recovery, repeatability, and robustness. Fatty matrices were degreased with heptane during the extraction step. A fully automated sample preparation system was developed to process the weighed samples into the measuring extract without any manual steps.

An efficient, fast chromatographic method was established that still allows baseline separation of the analytes with identical monoisotopic masses. Parameters of the HESI source and the high-resolution mass spectrometer (Thermo Scientific™ Orbitrap Exploris™ 120) were optimized to obtain the best compromise between intensity and interference-free signals. Time-dependent negative and positive measurement modes were implemented as parallel reaction monitoring (PRM) to measure and fragment all analytes with sufficient sensitivity and reproducibility.

The samples were quantified with the joint use of selected internal standards and standard addition. The method was validated according to the Commission Implementing Regulation (EU) 2021/808. All necessary performance criteria of the respective minimum method performance requirements (MMPRs) [1] and enforcement of (cascade) maximum residue limits (MRLs) [2] were considered.

[1] EURL (2022) EURL Guidance on MMPRs;

[https://eurl-residues.eu/wpcontent/uploads/2022/06/EURL\\_MMPR\\_guidance\\_endorsed.pdf](https://eurl-residues.eu/wpcontent/uploads/2022/06/EURL_MMPR_guidance_endorsed.pdf).

[2] EU Commission (2022) Current consolidated version of Commission Regulation (EU) 37/2010; EUR-Lex, Document 02010R0037-20240408; [http://data.europa.eu/eli/reg/2010/37\(1\)/2024-04-08](http://data.europa.eu/eli/reg/2010/37(1)/2024-04-08).

**Keywords:** veterinary drugs, LC-HRMS, multi-residue method, SALLE, automation

### W3

#### QUANTITATIVE ANALYSIS OF ANDROGENS AND ESTROGENS IN BOVINE AND PIG URINARY SAMPLES BY LC MS/MS: NEW ASPECTS IN VETERINARY DRUG RESIDUE ANALYSIS

Thomas Meikopoulos\*<sup>(1)</sup>, Mikael Pedersen<sup>(1)</sup>

<sup>1)</sup> National Food Institute, Technical University of Denmark, Copenhagen, Denmark, Denmark

\*Corresponding author - E-mail: thme@food.dtu.dk

Over the last six decades, steroid hormones have been used for anabolic purposes in animal production. However, studies in toxicology and epidemiology reveal adverse effects for consumers, posing a threat to public health. Numerous analytical techniques have been published to detect residues of unauthorized or prohibited pharmacologically active substances in various animal matrices. Therefore, it is crucial to improve and harmonize these methods according to the latest applied guideline and furthermore the MMPR values for many unauthorized substances were recently lowered. In this study we developed and validated a LC-MS/MS method for the quantification of 16 different steroids, including androgens and estrogens, in pig and bovine urine. Extraction optimization was aligned with the latest reports in the literature, highlighting the value of our protocol, as it involved a comprehensive evaluation and integration of the most current and relevant methodologies. Solid phase extraction (SPE), using HLB and NH<sub>2</sub> cartridges, was used to achieve the optimum extraction recoveries for all analytes of interest. An additional clean-up step with heptane was accomplished to minimize matrix effect. Pig urinary samples revealed significantly lower interferences than the bovine samples; hence, the extraction protocol was optimized separately for each animal. The method was validated in terms of linearity, trueness, precision, and sensitivity demonstrating satisfactory analytical figures of merit. Subsequently, LC-HRMS (Orbitrap LC-MS) profiling was applied to evaluate the sensitivity and performance of the method. Validation results indicated a successful implementation of the optimized workflow, with minimal variation.

**Keywords:** steroids, urine, LC-MS/MS, bovine and pig samples, quantitative analysis

### W4

#### **DETERMINATION OF CHLORPROMAZINE IN ANIMAL-DERIVED FOODS USING QUECHERS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)**

**Pak-wing Ricky Kong**<sup>(1)</sup>, Chi-shing Ng<sup>(1)</sup>

<sup>1)</sup> *Government Laboratory, Hong Kong, China, China*

*\*Corresponding author - E-mail: rickykong818@gmail.com*

A simple and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method using QuEChERS sample preparation for the determination of chlorpromazine in animal-derived foods is presented. The developed method was validated with a wide range of matrices covering animal muscle and offal, aquatic products, milk, honey, eggs and animal fat. Linearity, specificity, limit of quantification (LOQ), trueness and precision were examined in the method validation. Calibration curves at eight concentration ranged from ~ 0.05 ng/mL to ~10 ng/mL showed satisfactory linearity. Specificity was demonstrated by comparing chromatographs of blank samples of all considered matrices. The calculated LOQs of chlorpromazine in the validated matrices ranged from 0.3 µg/kg to 0.5 µg/kg. The trueness and precision of the method were evaluated by spike studies and relative standard deviation (RSD), which were carried out by spiking blank samples at 0.5, 1.0 and 10 µg/kg by different operators on different days. Recoveries were in the range of ~ 85% to ~120% and RSD was found to be <10%. The accuracy of the method was further verified by the analysis of a certified reference material (CRM). The validated method in this study is therefore suitable for accurately and precisely quantify chlorpromazine in animal-derived foods.

### W5

#### DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF 21 ANTIVIRAL DRUGS IN CHICKEN MUSCLE AND LIVER

Ana Castineira-Landeira<sup>(1)</sup>, Samantha Sasse<sup>(1)</sup>, Melissa Broeren<sup>(1)</sup>, Saskia Sterk<sup>(1)</sup>, Ane Arrizabalaga-Larranaga<sup>\*(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: ane.arrizabalagalarranaga@wur.nl

Outbreaks of animal viruses, such as African swine fever and avian influenza, have led to large economic losses worldwide due to the applied control measures, such as stamping out of animals, and a lack of available treatments for infected animals. In the past, approved human antiviral drugs, including amantadine, have been misused in Chinese poultry. In fact, one of the main concerns is that the use of antiviral drugs in food-producing animals lead to more drug-resistant strains in humans. Recently, the unauthorization of antiviral drugs in food-producing animals according to Commission Delegated Regulation (EU) 2022/1644 have increased the need for food control laboratories to develop analytical methods and perform official controls. In the present study, a simple and fast method was developed for the simultaneous determination of 21 antiviral drugs and their metabolites in chicken muscle and liver by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analytes included are anti-influenza drugs (amantadine, rimantadine, memantine, moroxydine, oseltamivir, oseltamivir acid, zanamivir, peramivir and laninamivir), broad-spectrum drugs (ribavirin, viremide, arbidol and arbidol sulfone), anti-herpes drugs (acyclovir, ganciclovir and penciclovir), antiretroviral drugs (efavirenz, nevirapine, saquinavir and lopinavir) and an immunomodulator (imiquimod). A generic sample preparation procedure was required to cover the multiple classes of antiviral drugs, in which the analytes were extracted from the homogenized chicken tissues using an acetonitrile:water (80:20, v/v) mixture followed by evaporation and reconstitution. Chromatographic separation was performed using a BEH amide column to retain the highly polar analytes. The analytes were detected using a triple quadrupole mass spectrometer operating in positive electrospray ionization mode, except for efavirenz in negative mode, and multiple reaction monitoring. The method was validated according to Commission Implementing Regulation (EU) 2021/808 as a quantitative confirmation method for chicken muscle and liver at lowest calibration levels of 0.15 µg/kg for amantadine, imiquimod, lopinavir, memantine and saquinavir, up to 25 µg/kg for ribavirin and zanamivir. To the best of our knowledge, it is the first time an analytical method was developed and validated with such a broad scope of antiviral drugs. The method was applied to 10 chicken muscle and 10 chicken liver samples from the Dutch National Residue Control Plan and resulted in no detected antiviral residues. In conclusion, the developed and validated LC-MS/MS method showed suitability for the monitoring of antiviral drugs abuse in chickens.

**Keywords:** antiviral drugs, food control, chicken muscle and liver, LC-MS/MS

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### W6

#### **ANABOLIC STEROIDS INDUCED CHANGES AT THE LEVEL OF PROTEIN EXPRESSION: EFFECTS OF PROLONGED ADMINISTRATION OF TESTOSTERONE AND NANDROLONE TO PIGS**

**Kristina Tosnerova**<sup>\*(1)</sup>, Jan Gebauer<sup>(1)</sup>, Nikola Hodkovicova<sup>(1)</sup>, Kristyna Skoupa<sup>(2)</sup>, Andrej Batik<sup>(2)</sup>, Iva Bartejsova<sup>(1)</sup>, Michaela Charvatova<sup>(1)</sup>, Lenka Leva<sup>(1)</sup>, Lea Jarosova<sup>(1)</sup>, Zbysek Sladek<sup>(2)</sup>, Martin Faldyna<sup>(1)</sup>, Kamil Stastny<sup>(1)</sup>

<sup>1)</sup> *Veterinary Research Institute, Czech Republic*

<sup>2)</sup> *Faculty of AgrSciences, Mendel University in Brno, Czech Republic*

*\*Corresponding author - E-mail: kristina.tosnerova@vri.cz*

Synthetic derivatives of steroid hormones, specifically anabolic-androgenic steroids (AAS), have gained prominence due to their observed benefits in enhancing meat quality. The study replicated the administration of banned AAS and investigated their impacts on pigs to contribute to the understanding of animal biochemistry and to explore the feasibility of detecting AAS administration by employing a non-targeted analysis. The effects were corroborated by evaluating changes in the expression of selected proteins, as well as examining haematological and biochemical profiles and histological alterations. Exposure to AAS influenced the expression of proteins related to drug-metabolizing enzymes, muscle and lipid metabolism, kidney function, reproductive processes, immune system functions, and carcinogenic changes. The effects of AAS appear intricate and contingent on factors such as the specific drug used, dosage, and duration of administration. The results underscore that protein expression analysis holds promise as a valuable tool for detecting illicit AAS use in the fattening process.

**Keywords:** drugs, histology, muscle, proteomics, testes

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### W7

#### DEVELOPMENT AND VALIDATION OF AN INDIRECT COMPETITIVE LATERAL FLOW IMMUNOASSAY FOR THE DETECTION OF ACETAMINOPHEN (PARACETAMOL) IN BOVINE URINE

Samantha Sasse\*<sup>(1)</sup>, Ariadni Geballa-Koukoura<sup>(1)</sup>, Toine Bovee<sup>(1)</sup>

<sup>1)</sup> Wageningen Food Safety Research (WFSR), part of Wageningen University & Research, The Netherlands

\*Corresponding author - E-mail: samantha.sasse@wur.nl

Paracetamol is a commonly used analgesic and antipyretic agent for humans worldwide. However, paracetamol overdoses or overuse can cause health issues, such as hepatotoxicity. As paracetamol is also used for the treatment of farm animals, it is essential to monitor these residues in animal-derived matrices at risk-based sites in order to minimize the intake of paracetamol through the food chain. In the present study, we have developed a novel carbon nanoparticle based indirect competitive lateral flow immunoassay (icLFIA) for the rapid detection of paracetamol in bovine urine. The developed icLFIA can detect paracetamol residues within 10 minutes, and its performance was validated according to Commission Implementing Regulation (EU) 2021/808, i.e. determination of the detection capability (CC $\beta$ ), specificity, robustness, and stability. The CC $\beta$  of the icLFIA for paracetamol in bovine urine is 5 mg/L and the icLFIA is proven to be selective and specific towards paracetamol in bovine urine, as no matrix interference and cross-reactivity to paracetamol-related substances, non-steroidal anti-inflammatory drugs and antibiotics were observed, except for high concentrations of orthocetamol. The icLFIA for paracetamol in bovine urine is robust to (small) variations in reading time, but it remains necessary to strictly use a dilution ratio of running buffer/bovine urine of 80/20. Moreover, the produced icLFIA are stable for at least 56 days when stored in closed aluminum bags with a silica desiccant at room temperature. In conclusion, the developed and validated icLFIA provides a rapid and cost-effective method for on-site monitoring of paracetamol abuse in cattle.

**Keywords:** paracetamol, animal-derived matrices, indirect competitive lateral flow immunoassay, carbon nanoparticles, on-site monitoring

**Acknowledgement:** This project was financially supported by the Dutch Ministry of Agriculture, Nature and Food Quality (project WOT-02-003-068 and WOT-HH-003-003).

### W8

#### **FAST SIMULTANEOUS DETERMINATION OF 23 VETERINARY DRUG RESIDUES IN FISH, POULTRY, AND RED MEAT BY LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY**

Ahmed Aljaber<sup>\*(1)</sup>, Saeed Alzahrani<sup>(1)</sup>

<sup>1)</sup> Saudi Food and Drug Authority (SFDA), Saudi Arabia

\*Corresponding author - E-mail: [asjaber@sFDA.gov.sa](mailto:asjaber@sFDA.gov.sa)

The misuses of veterinary drugs can result in the accumulation of residues in food of animal origin that can make its way to the final consumer. Herein we describe a simple method for the accurate determination of beta-lactams, quinolones, sulphonamides, and tetracyclines in fish, poultry, and red meat. No extraction cartridges were used; instead, the extraction process consisted of the addition of an organic solvent, shaking, centrifugation, and dilution. An extensive validation process demonstrated an excellent linearity ( $R^2 \geq 0.99$ ) for 23-drug residues. The recovery of drugs in different matrices at two concentration levels ( $n = 6$ ) was in the range of 82-119%. The method was proved to be repeatable and reproducible with intra/inter-day measurements (RSDs lower than 20%). The quantification limits of drug residues were in the range of 0.8 to 45.3 ug/kg, which is well below the maximum residue limits set by most regulatory authorities. This method was successfully applied to the routine analysis of 20 fish, poultry, and red meat samples ( $n = 60$ ).

**Keywords:** multi-residue, veterinary drugs, LC-MS/MS, red meat, antibiotics

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### W9

#### **DETERMINATION OF 14 AMINOGLYCOSIDES IN FOODSTUFFS BY LC-MS/MS USING MOLECULARLY IMPRINTED POLYMER SOLID PHASE EXTRACTION**

**Michel Arotçarena**\*<sup>(1)</sup>, Sami Bayoudh<sup>(1)</sup>, Kaynoush Naraghi<sup>(1)</sup>

<sup>1)</sup> AFFINISEP, France

\*Corresponding author - E-mail: michel.arotcarena@affinisep.com

Aminoglycosides are an important class of antibiotics frequently used to treat bacterial infections. They show an excellent activity against a broad range of Gram-positive and Gram-negative bacteria and were historically cornerstones of antimicrobial therapy for severe bacterial infection (Streptomycin was the first antibiotic used to successfully treat tuberculosis). In veterinary medicine, aminoglycosides are indicated to treat infections such as septicemias, digestive tract infections, respiratory and urinary infections in cattle, pigs, sheep, goats or horses. The presence of aminoglycosides in foodstuffs represents a risk to consumer health and is thus regulated through the enforcement of maximum residue limits (MRLs). Importantly, antimicrobial resistance is a major worldwide concern and a rational use of antibiotics including aminoglycosides is crucial to minimize it. Therefore, the development of analytical methods enabling to control the occurrence and the levels of aminoglycoside in food is of utmost importance.

The poster describes a screening method for the determination of 14 aminoglycosides in foodstuffs of animal origin by LC-MS/MS. The principle of the method includes an extraction under acidic aqueous conditions followed by a selective clean-up with molecularly imprinted polymer-solid phase extraction (MIP-SPE). Analytes are subsequently detected by ion pair reversed phase LC-MS/MS.

The method is intended to be fast and easy, applicable to a broad range of matrices covering raw materials, processed ingredients and finished products at levels that fit with MRLs established worldwide. Due to the occurrence of matrix effects that sometimes induce unpredictable variations in the response factor, a double extraction for each sample was found necessary: the first test portion is extracted as such, while the second one is spiked at the Screening Target Concentration (STC) which should be set at or below MRLs. Both extracts are processed, analyzed, and corresponding signals are compared against a cutoff value determined during the validation. This approach is very reliable as it takes into account the intrinsic matrix effect of each sample to be analyzed.

The STC value was set at 50 µg/kg for each aminoglycoside residue, fulfilling all regulatory limits worldwide. The method was validated according to the European Community Reference Laboratories Residues Guidelines giving false-negative and false-positive rates ≤3% for all compounds.

**Keywords:** aminoglycosides, SPE, molecularly imprinted polymers, sample preparation, LC-MS/MS

### W10

#### CONFIRMATION OF FIVE NITROFURAN METABOLITES BY LC-MS/MS IN VARIOUS FOOD MATRICES OF ANIMAL ORIGIN ACCORDING TO REGULATION (EU) 2021/808

**Pierre Guichard**\*<sup>(1)</sup>, **Sophie Gautier**<sup>(1)</sup>, **Dominique Hurtaud-Pessel**<sup>(2)</sup>, **Eric Verdon**<sup>(3)</sup>, **Laura Suaudeau**<sup>(4)</sup>

<sup>1)</sup> ANSES Fougères Laboratory, French National Agency for Food, Environment and Occupational Health & Safety, French and European Union Reference Laboratory for Veterinary Medicinal Product Residues and Pharmacologically Active Dye Residues in Food, 10B rue Claude Bourgelat CS 40608, 35306 Fougères, France

<sup>2)</sup> ANSES Fougères Laboratory, French National Agency for Food, Environment and Occupational Health & Safety, French and European Union Reference Laboratory for Veterinary Medicinal Product Residues and Pharmacologically Active Dye Residues in Food, 10B rue Claude Bourgelat CS 40608, 35306 Fougères, France

<sup>3)</sup> Anses, French Agency for Food, Environmental and Occupational Health & Safety, France

<sup>4)</sup> ANSES Fougères Laboratory, French National Agency for Food, Environment and Occupational Health & Safety, France

\*Corresponding author - E-mail: pierre.guichard@anses.fr

A liquid chromatography-tandem mass spectrometry method for the simultaneous confirmation of five nitrofurans metabolites, including the nifursol metabolite (DNSH), was developed and fully validated in accordance with the new Regulation (EU) 2021/808 in various foodstuffs of animal origin: muscle, fish flesh, eggs, casings, milk and honey. The five nitrofurans metabolites investigated were as follows: 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), 1-aminohydantoin (AHD), semicarbazide (SEM) and 3,5-dinitrosalicylic acid hydrazide (DNSH). The method takes into account the application of Regulation (EU) 1871/2019, which came into force in November 2022, lowering the reference point for action (RPA) to 0.50 µg.Kg<sup>-1</sup>.

The sample preparation includes a washing step, allowing to analyze only the fraction of protein-bound residues in order to establish whether the nitrofurazone marker residue (SEM) comes from a natural occurrence or from prohibited use. A two-fold liquid-liquid extraction was performed with ethyl acetate and purification step was added for eggs using iso-hexane and SPE was performed for milk and honey.

Capability of detection CC<sub>β</sub> for screening has been set at 0.10 or 0.25 µg.Kg<sup>-1</sup> depending on the analyte-matrix pair, with the exception of AHD and SEM in honey for which they are set at 0.75 and above 0.75 respectively due to low responses and numerous interferences despite the addition of a SPE purification step.

The validated data proved that the method is suitable for the confirmation of the five nitrofurans metabolites when implemented for official control in various foodstuffs of animal origin. Trueness ranged from 83.07% for SEM in aquaculture products at 0.25 µg.Kg<sup>-1</sup> to 112.7% for AHD in eggs at 0.10 µg.Kg<sup>-1</sup>. The maximum intra-laboratory reproducibility CV values obtained for muscle, aquaculture products, eggs, casings, milk and honey are respectively as follows: 11.3% for DNSH at 0.10 µg.Kg<sup>-1</sup>; 28.2% for SEM at 0.10 µg.Kg<sup>-1</sup>; 19.3% for AHD at 0.10 µg.Kg<sup>-1</sup>; 28.5% for SEM at 0.10 µg.Kg<sup>-1</sup>; 16.3% for AHD at 0.10 µg.Kg<sup>-1</sup> and 13.0% for AMOZ at 0.25 µg.Kg<sup>-1</sup>.

Limits of decision CC<sub>α</sub> ranged from 0,111 µg.Kg<sup>-1</sup> for DNSH in milk to 0,434 µg.Kg<sup>-1</sup> for SEM in aquaculture products, below 0.50 µg.kg<sup>-1</sup>, corresponding to the enforcing RPA recently adopted by the European Commission. Confirmatory analysis is not applicable for AHD and SEM in honey.

**Keywords:** nitrofurans metabolites, prohibited antimicrobials, veterinary residues, liquid chromatography/mass spectrometry (LC-MS/MS), foodstuffs of animal origin

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### W11

#### **A UHPLC-MS/MS METHOD FOR THE DETERMINATION OF ANTIPARASITIC DRUGS, INCLUDING AVERMECTINS, IN AVIAN EGGS, FEATURING A NOVEL STANDARD ADDITION APPROACH TO VALIDATION**

**Philip Mullen<sup>(1)</sup>, Cheryl Rainey<sup>(1)</sup>, Matthew Walker<sup>(1)</sup>, Anna Gadaj<sup>(1)</sup>**

<sup>1)</sup> *Chemical Surveillance Branch, Veterinary Sciences Division, Agri-Food and Biosciences Institute (AFBI), Belfast, UK, United Kingdom*

*\*Corresponding author - E-mail: philip.mullen@afbini.gov.uk*

A QuEChERS based extraction was developed to monitor the presence of 54 antiparasitic drugs, covering benzimidazoles, avermectins and flukicides, in avian egg, in which preblended egg samples are fortified with internal standards, followed by addition of 4:1 MeCN:H<sub>2</sub>O (v:v) and 4:1 MgSO<sub>4</sub>:NaCl (w:w). Following vortexing, the samples are centrifuged and the MeCN layer transferred for dSPE (C<sub>18</sub>), with an aliquot finally transferred to vials for analysis by UHPLC-MS/MS (Kinetex<sup>®</sup> Biphenyl 1.7µm, 50 × 2.1 mm with 0.5 mM ammonium formate in H<sub>2</sub>O and 0.5 mM formic acid in MeOH for mobile phases A & B respectively). The MS/MS conditions were selected to ensure the optimal response of avermectin and milbemycin compounds, which are known to be difficult to analyse on modern MS/MS platforms. Post column infusion of 0.2M ammonia solution was shown to have a significant impact on analyte sensitivity, in the range of -56 to 420% difference with and without infusion, with over 50% of analytes showing increased responses.

The method was validated as a semi-quantitative screening method with a screening target concentration (STC) of 5 µg kg<sup>-1</sup> for all analytes, except fipronil and its metabolites, at 1.25 µg kg<sup>-1</sup>, and all relevant criteria, as set out in CIR (EU) 2021/808, were fulfilled. A novel approach to confirmation, employing a standard addition technique, was demonstrated for all analytes with MRL values (abamectin, emamectin, fenbendazole, fipronil, flubendazole, fluralaner and phoxim). The developed method is currently in use for the screening and confirmation of antiparasitic drugs in the multiannual national control plan (MANCP) in Northern Ireland.

**Keywords:** QuEChERS, avermectins, standard addition, post-column infusion, antiparasitics

### W12

#### **A QUICK AND EASY LC-MS/MS METHOD FOR SEDATIVES AND TRANQUILIZERS IN KIDNEY VALIDATED UNDER REGULATION (EU) 2021/808**

Eva Munoz<sup>\*(1)</sup>, Meritxell Castello<sup>(1)</sup>, Josep Calderon<sup>(1)</sup>

<sup>1)</sup> *Agència de Salut Pública de Barcelona, Spain*

*\*Corresponding author - E-mail: emunozca@aspb.cat*

This study aims to establish a rapid, accurate, safe, inexpensive, and effective method that combines a simple extraction with acetonitrile, pre-concentration and injection into a liquid chromatography-tandem mass spectrometry (LC-MS/MS) determining simultaneously several sedatives and tranquilizers in kidney.

The substances analyzed were Carazolol, Azaperon, Azaperol, Xylazine, Acepromazine, Propiopromazine, Chlorpromazine, Nordiazepam, Oxazepam and Temazepam. The legislated LMR for those compounds that can be administrated are indicated in the regulated EU document 37/2010.

The performance of analytical methods for residues of pharmacologically active substances used in food-producing animals must be evaluated in accordance with Commission Implementing Regulation 2021/808. Thus, the selectivity, linearity, carry-over effects, decision limit for confirmation (CC $\alpha$ ), limit of quantification (LOQ), trueness, precision (repeatability and reproducibility), relative matrix effect, selectivity, stability and ruggedness were evaluated for the method validation.

The LOQs were 1  $\mu\text{g}/\text{kg}$  for all analytes and the CC $\alpha$  were 0.6  $\mu\text{g}/\text{kg}$  for forbidden substances. The coefficient of variation for reproducibility was 0.990. The method has been validated for the quantification in kidney of different animal species, showing no matrix effect.

The subsequent addition to the method of some other sedatives/tranquilizers has been able without method modifications, thus demonstrate its robustness.

This procedure can be used as confirmatory testing of sedatives and tranquilizers residues in kidney of different species and is therefore suitable for official control laboratories.

The method is currently being used in the Public Health Agency of Barcelona (ASPB) within official control programs and it has been included in the scope of the accreditation following ISO/IEC 17025 requirements.

**Keywords:** regulation (EU) 2021/808, sedatives, tranquilizers, LC/MS-MS, kidney

### W13

#### **RAPID SCREENING DETECTION OF DNSH (NIFURSOL) RESIDUES: A COST-EFFECTIVE SOLUTION FOR ENSURING EU COMPLIANCE**

Nicole Haveron<sup>(1)</sup>, Mark Squance<sup>(1)</sup>, Nicola Kane<sup>\*(1)</sup>, Morteza Afrasiabi<sup>(2)</sup>

<sup>1)</sup> Biorex Food Diagnostics, United Kingdom

<sup>2)</sup> Fortress Diagnostics, United Kingdom

\*Corresponding author - E-mail: nicola@biorexfooddiagnostics.com

Nifursol is a member of the broad-spectrum Nitrofurans class of antibiotics, which are used in food-producing animals but are banned in the EU due to their potential carcinogenic risks. Nifursol is rapidly metabolized upon ingestion. However, the protein-bound metabolites that form from the parent drug persists in tissues and remain stable even after cooking, making them easier to detect. As a result, the protein-bound metabolite 3,5-dinitrosalicylic acid hydrazide (DNSH) is a key marker for identifying the illegal use of Nifursol in the food industry.

Minimum performance limits (MRPL) for nitrofurans metabolites were replaced by Reference Points for Action (RPA) from 28 November 2022. Under Article 5 of Regulation (EU) 2019/1871 monitoring now must include Nifursol and its metabolite DNSH (3,5-dinitrosalicylic acid hydrazide) to a level of 0.5 µg/kg (ppb) to ensure compliance.

Utilising our advanced understanding of immunoassay techniques, including biological interactions, matrices, buffer compositions and sample preparation development; a highly sensitive ELISA kit for the detection of the Nifursol metabolite DNSH in prawn/ shrimp samples was developed. Metabolites can be extracted and quantified using acid hydrolysis and a rapid 30minutes derivatisation incubation, followed by solvent extraction from only a 1g sample. The test assay protocol can be completed in 45 minutes with all liquid ready to use kit reagents apart from the concentrate wash solution.

The calibration range of the developed assay is 0.05 to 4ppb. Excellent intra-assay precision was obtained with 9.5% and 8.6% at concentration levels of 0.125ppb and 0.5ppb respectively. Ready-to-use microtitre plate, standards, primary antibody and secondary antibody have been manufactured with 1 year shelf life. Prawn sample analysis has shown an LOD of 0.22ppb and a CCβ 0.25ppb which meets the new compliance regulations. Cross reactivity for Nifursol was 117%, whilst being highly specific with low detection of <0.1% shown for the Nitrofurans AHD, AOZ, AMOZ & SEM ensuring low level of false negatives if any of the other nitrofurans group was present.

The Nifursol (DNSH) ELISA kit developed exhibits good precision and sensitivity with a measuring range which allows for the detection of samples over a wide range of concentrations. The now commercially available kit offers a cost efficient testing option with ready-to-use reagents, a short 30-minute sample incubation time and a low detection capability for prawn / shrimp samples.

**Keywords:** nitrofurans, seafood, residues, immunoassay, compliance

### W14

#### ULTRA-HIGH SENSITIVITY QUANTIFICATION OF VETERINARY DRUG RESIDUES USING LC/MS WORKFLOWS

Jianru Stahl-Zeng\*<sup>(1)</sup>, Yoann Fillatre<sup>(1)</sup>, Gregoire Bonvin<sup>(2)</sup>, Michael Scherer<sup>(1)</sup>

<sup>1)</sup> SCIEX, Germany

<sup>2)</sup> Service de la Consommation et des Affaires Vétérinaires (SCAV), Switzerland

\*Corresponding author - E-mail: [jianru.stahl-zeng@sciex.com](mailto:jianru.stahl-zeng@sciex.com)

In this poster we will demonstrate a robust, sensitive and reproducible LC/MS method to identify and quantify veterinary drug in different animal by-products.

The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or intensive application. Therefore, these substances must be limited to mitigate negative consequences. One way to implement controls is to perform analytical testing of animal by-products. To limit these compounds within the food industry, it is important to achieve LOQ values that are as low as is reasonably possible

As regulations continue to tighten around food testing, it has become increasingly challenging to achieve the necessary levels of sensitivity during analysis while maintaining a high level of accuracy and precision.

Chromatographic separation was performed using a Phenomenex Kinetex Polar C18 (2.6  $\mu\text{m}$ , 100 x 2.1 mm). A triple quadrupole MS system was operated in scheduled multiple reaction monitoring (sMRM) mode using electrospray ionization (ESI) with fast positive and negative switching.

sMRM acquisition helps ensure that both quantifier and qualifier transitions can be measured to increase the specificity of the analysis without the need to compromise on data quality by reducing the number of data points across each peak

Within this method, LOQ values down to 0.005 ng/mL have been achieved while keeping high levels of accuracy and precision in standard solutions. When spiked into matrices, LOQs of 0.01  $\mu\text{g}/\text{kg}$  in pork and chicken and 0.005  $\mu\text{g}/\text{kg}$  in milk were achieved.

### W15

#### AN ALTERNATIVE STATISTICAL APPROACH FOR METHOD VALIDATION UNDER EU REGULATION 2021/808

Wendy Robinson<sup>(1)</sup>, Roy MacArthur<sup>(1)</sup>, Gary Dowsett<sup>(1)</sup>, Warwick Anderson<sup>(1)</sup>, Katherine Weddell<sup>(1)</sup>

<sup>1)</sup> Fera Science Limited, United Kingdom

\*Corresponding author - E-mail: wendy.robinson@fera.co.uk

The EU regulation 2021/808 for the analytical methods for pharmacologically active substances in food-producing animals requires extensive validation of methods to demonstrate that an analytical method complies with relevant performance characteristics. These characteristics include trueness, repeatability, precision, selectivity/specificity and others, depending on if a method is for confirmation or screening. For laboratories that carry out analyses for a vast range of matrices and species, conventional validation can be particularly time consuming and labour intensive. It may be beneficial for laboratories in these circumstances to look towards other validation approaches.

In this work, we have designed a statistical model which efficiently estimates trueness, precision, CC<sub>as</sub> and CC<sub>bs</sub> by modelling the relationship between true concentration and measurement uncertainty. The model consolidates the estimation of trueness and precision across a range of concentrations, rather than assessing them separately at individual concentrations. By applying this approach, we can use results that are more representative of methods in normal use and use all available results to provide estimates of trueness and precision at each level for which they are needed. Using all the results makes the estimates more reliable for a given total number of measurements. The model also aims to provide at least the same quality of information as a conventional validation approach. This is demonstrated by carrying out a parametric bootstrap which compares the uncertainty of each of the estimates provided by our approach with the uncertainty of the estimates produced using the standard approach. In using this statistical model we believe that time, labour and resource can be saved without compromising the quality of data generated for method validations under 808 regulation.

**Keywords:** veterinary drugs, residues, method validation, LCMS, statistics

### W16

#### DEVELOPMENT AND VALIDATION OF A MULTI-RESIDUE, MULTI-CLASS METHOD FOR EVALUATING DAIRY CATTLE EXPOSURE TO ANTIBIOTICS THROUGH FAECES ANALYSIS

Giulia Stephany<sup>(1)</sup>, Francesca Bovo<sup>(1)</sup>, Giovanni Sette<sup>(1)</sup>, Cristiana Benetti\*<sup>(1)</sup>

<sup>1)</sup> *Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

\**Corresponding author - E-mail: cbenetti@izsvenezie.it*

The use of antibiotics for therapeutic purposes can contribute to the selection, co-selection, cross-selection, persistence, and spread of mobile antibiotic resistance genes (MARGs) in exposed microbial communities. MARGs can be amplified and exchanged between microorganisms, propagate in farm environments and the gut microbiota of animals, and spread to the external environment (e.g., through the use of farm effluents as fertilizers) all of which can contribute to the introduction of resistance in human reservoirs.

The objective of this study was to develop and validate a multiclass quantitative method based on liquid chromatography-mass spectrometry (LC-MS) for the determination of 73 antibiotic residues from various classes (including sulfonamides, macrolides, lincosamides, tetracyclines, quinolones, pleuromutilins, phenicols, rifamycins, and diaminopyrimidine derivatives) in dairy cow faeces. This method aims to provide a tool for assessing the exposure of microbial communities on farms to antibiotic residues resulting from previous or current therapeutic treatments.

Various extraction and cleanup procedures were evaluated to optimize the performance for most analytes. Ultimately, a simple and rapid method using extraction with McIlvaine buffer (pH 4) and acetonitrile, without solid-phase extraction (SPE) purification, was selected.

Chromatographic separation was performed on a Poroshell 120 EC-C18 column (2.1 x 100 mm, I.D. 2.7 µm) using a gradient of aqueous solution with 0.1% formic acid and acetonitrile at a flow rate of 0.25 mL min<sup>-1</sup>.

The method was validated following the guidelines established by EU Regulation 2021/808 for confirmatory methods. Key performance characteristics assessed included: decision limit (CC<sub>α</sub>), trueness, repeatability, within-laboratory reproducibility, and specificity.

**Keywords:** multi-residue/multi-class method, antibiotics, faeces

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### W17

#### **APPLICATION OF LC-HRMS FOR OFFICIAL CONTROLS IN A NEW RANDOMISED SURVEILLANCE PLAN FOR VETERINARY MEDICINAL PRODUCT RESIDUES IN FOOD PRODUCTS IN FRANCE**

**Dominique Hurtaud-Pessel**\*<sup>(1)</sup>, **Pierrick Couedor**<sup>(1)</sup>, **Eric Verdon**<sup>(1)</sup>

<sup>1)</sup> *Anses, French Agency for Food, Environmental and Occupational Health & Safety, France*

\**Corresponding author - E-mail: dominique.pessel@anses.fr*

In order to verify the compliance with European Union legislation of the use of pharmacologically active substances as veterinary medicinal products (VMPs) or feed additives or other prohibited or unauthorized pharmacologically active substances, EU Member States are required to check food products of animal origin in their multi-annual national control plan. Since 2023, three types of plans have to be implemented, as lays down by regulations CIR (EU) 2022/1646 and CDR (EU) 2022/1644: Each Member State has to plan i) a national risk-based control plan for its domestic production ; ii) a national risk-based control plan for imported food products, and iii) a new randomized surveillance plan for its domestic production based on non-targeted marketed food products. This latter plan is intended to collect information useful to measure the fate of residues in marketed food products and to make further improvements in the risk-based targeted control plans within the Member State. Therefore for this surveillance plan have to be developed analytical methods capable of measuring low residue concentration below regulatory levels such as MRLs or MLs for authorised substances and also to identify substances that may be subject to misuse.

In France, a high-resolution mass spectrometry (HRMS) screening method has been developed to be applied to a portion of the 1,150 samples from the randomised surveillance plan for VMP residues. This method has been validated for different species of muscles, milks and eggs and performance characteristics as CC $\beta$  (capacity of detection), fidelity and trueness have been determined. Almost 200 VMP compounds from different veterinary classes are monitored in this method. Once the method had been validated, the question arose of how to implement it in routine production. Indeed, multi-residue methods involving more than 100 compounds can be challenging, particularly because of the recurrent preparation of stable solutions of standards for quality control and of the large volume of data to be acquired and processed. This is why it was important to find a way of applying this method routinely while limiting the technical workload. Quality controls and data processing have been designed to allow routine use of the method while guaranteeing reliability of the results and easy implementation. Finally, a decision tree to help the management of the surveillance plan samples has been proposed. This poster will explain the approach applied to samples from the French randomised surveillance plan.

**Keywords:** veterinary medicinal products, residue, LC-HRMS, surveillance, routine analysis

### W18

## VETERINARY MEDICINES: WHEN ONE ANTIBIOTIC MOLECULE HIDES ANOTHER!

**Celine Hedou**<sup>\*(1)</sup>, **Valerie Gaudin**<sup>(1)</sup>, **Melaine Bessiral**<sup>(1)</sup>, **Eric Verdon**<sup>(2)</sup>

<sup>1)</sup> ANSES, France

<sup>2)</sup> Anses, French Agency for Food, Environmental and Occupational Health & Safety, France

\*Corresponding author - E-mail: [celine.hedou@anses.fr](mailto:celine.hedou@anses.fr)

The certificate of analysis (CoA) provided by the manufacturer aims at characterizing each analytical standard. The lack of harmonization of CoA between manufacturers leads to significant data for reliable analysis being uneasy to determine, interpret, or are sometimes even absent. Moreover, although commercially available standards may sometimes be manufactured pure, they are often associated with salts, hydrate molecules, etc. Therefore, a team from the ANSES reference laboratory drafted an internal guidance to harmonize calculation of purity for chemical reference standards (<https://doi.org/10.5281/zenodo.1172022>).

However, a particular case drew our attention during the organization of an inter-laboratory proficiency test organised for the network of national reference laboratories (NRLs). Specifically, in a cow milk material to which was added neomycin sulfate (CAS # 1405-10-3), it turned out from the participants' results, the material was positive in neomycin, but also 7 out of 20 participants detected and confirmed the presence of paromomycin, which could appear as a "false-positive" result.

The CoA of the analytical standard released by the manufacturer did not mention paromomycin. However, observing the LC-MS/MS signal transitions and retention times, it was confirmed the paromomycin presence in the material. This PT provider's confirmation led to drafting a new version of the report to avoid penalizing the evaluated laboratories' performances.

To know the exact composition of an analytical standard, in addition to the CoA, one must refer to the European Pharmacopoeia (EP), or any other official Institutions. The EP highlights all stereoisomeric forms of neomycin that could show up in the analytical standard. In fact, it was concluded that when preparing a neomycin-fortified material or when developing a detection and/or confirmatory method, the analytical standard of neomycin B should preferably be used.

In official control residue analysis, the choice of analytical standard is a critical step in the development and validation of methods, as well as in the organization of inter-laboratory proficiency testing. In this regard, a standardization project is underway at the ISO (International Organization for Standardization), initiated by experts from the International Dairy Federation (IDF) (ISO/WD 24141 IDF 262). The objectives will address the lack of harmonization of certificates of analysis between manufacturers and to assist in their interpretation. This project will concern all users of manufactured analytical standards. This first time developed international standard for the interpretation of CoA should enable harmonization among all users and push analytical standards manufacturers to improve and harmonize CoA.

**Keywords:** analytical standard, certificate of analysis, harmonization

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### W19

#### EVALUATION OF SUITABLE PROCEDURAL CALIBRATION STANDARDS FOR THE MULTI-MATRICES QUANTIFICATION OF VETERINARY DRUGS BY LC-MS/MS

**Gregoire Bonvin\***<sup>(1)</sup>, Jenette Zwygart<sup>(1)</sup>, Philippe Jan<sup>(1)</sup>, Yoric Gagnebin<sup>(1)</sup>, Patrick Edder<sup>(1)</sup>, Didier Ortelli<sup>(1)</sup>

<sup>1)</sup> Food authority control and veterinary affairs of Geneva (SCAV), Switzerland

\*Corresponding author - E-mail: gregoire.bonvin@etat.ge.ch

Veterinary drugs are used on a worldwide scale for the treatment and prevention of disease in meat-producing animals. Therefore, controlling veterinary drugs residues remains an essential task for food control laboratories. LC-MS/MS is a method of choice to quantify numerous residues in different type of matrices. In general, the accuracy depends highly on the type of standards used for calibration. Procedural calibration standards, which relies on spiking a series of blank matrix with different amounts of analytes prior to extraction, provide generally very good accuracy due to compensation of matrix effects and losses during extraction. However, this calibration method is relatively restrictive, since the matrix of the procedural standard must to be identical to the samples. This lack of genericity can be a cumbersome for food control laboratories, where a wide range of matrices is analyzed on a daily basis.

This work proposed a methodology to evaluate the correlation between matrices in order to quantify accurately around 180 veterinary drugs and to select the most suitable matrix (or combination of matrices) for the preparation of generic procedural calibration standards. QC samples were prepared in four different matrices *i.e.* cattle, poultry, lamps, and swine and then quantified with procedural standard made up either of the same matrix, or of mixture (pool). The first set of results demonstrate that procedural standard based on poultry or lamps allow quantifying accurately between 80 to 90% of veterinary drugs, irrespective of matrices. The evaluation of procedural standards based on combination of different matrices is currently in progress, which may lead to a further improvement of the calibration performances.

**Keywords:** veterinary drugs, calibration standards, multi-matrices, LC-MS/MS

### W20

#### TRANSITION AND PERSISTENCE OF DOXYCYCLINE IN ORAL FLUID AND PLASMA AFTER PER OS ADMINISTRATION IN SWINE

**Ewelina Nowacka-Kozak**\*<sup>(1)</sup>, Anna Gajda<sup>(1)</sup>, Artur Jabłonski<sup>(2)</sup>, Małgorzata Gbylik-Sikorska<sup>(1)</sup>

<sup>1)</sup> National Veterinary Research Institute, Department of Pharmacology and Toxicology, Poland

<sup>2)</sup> Warsaw University of Life Sciences, Department of Pathology and Veterinary Diagnostics, Poland

\*Corresponding author - E-mail: ewelina.nowacka@piwet.pulawy.pl

Doxycycline, a member of the tetracycline group, is the most commonly used antibiotic in pig farming. Excessive antibiotic use in pigs may contribute to the risk of their residues in food and the development of bacterial resistance. Therefore, mandatory monitoring of antibiotics in food of animal origin is carried out as part of official EU monitoring programmes to ensure food safety and consumer health. Oral fluid analysis appears to be an alternative to post-mortem antibiotic analysis. The purpose of the research was to conduct an experiment in pigs to determine the concentration and depletion time of doxycycline in individual and pooled oral fluid and plasma after administration of veterinary medicines containing doxycycline per os to the animals. Doxycycline was administered in two doses - a therapeutic dose and a subtherapeutic dose, because tests of water collected from pig farms indicate the presence of trace amounts of this antibiotic, which would suggest illegal subtherapeutic administration for prophylactic purposes or inadequate cleaning of animal watering lines after treatment.

The analyses of doxycycline in oral fluid and plasma were performed using ultra high performance liquid chromatography with detection by triple quadrupole mass spectrometry (UHPLC-MS/MS). The developed methods allow simultaneous detection of 68 antibiotics from 10 groups (penicillins, cephalosporins, macrolides, tetracyclines, quinolones, sulfonamides, pleuromutilins, diaminopyrimidines, lincosamides, polypeptides) in the oral fluid and plasma of pigs. The time for the chromatographic analysis was set to 7 minutes.

The results indicate that doxycycline, regardless of dose, passes into the oral fluid and is still present on the day of tissue withdrawal (4 days). Doxycycline concentrations in individual oral fluid (therapeutic group - 11.3-18.3 µg/l on day 4; subtherapeutic group - 6.20-6.60 µg/l on day 4) and plasma (therapeutic group - 25.0-65.7 µg/l on day 4; subtherapeutic group - 21.4-38.2 µg/l on day 4) differed significantly. The concentrations of doxycycline detected in both groups of animals were significantly higher in the pooled oral fluid (therapeutic group - 820 µg/l after 24 hours) than in the individual oral fluids (therapeutic group - 116-146 µg/l after 24 hours).

Oral fluid analysis will allow the non-invasive detection of off-label antibiotic use before slaughter. The solutions presented will make it possible to assess the presence of undesirable substances just before the slaughter of animals. Such analysis can significantly reduce the losses caused by the need to dispose of meat when antibiotics are detected in tissues.

**Keywords:** doxycycline, oral fluid, UHPLC-MS/MS, non-invasive control, pigs

### W21

#### TO LOOK AT CHEMICAL EXPOSURE IN A BROADER CONTEXT: MULTIDIRECTIONAL ANALYSIS OF CONTAMINANTS AND FEED ADDITIVES IN ANIMAL FEED AND WATER

Ewelina Patyra<sup>(1)</sup>, Małgorzata Warenik-Bany<sup>(2)</sup>, Anna Gajda\*<sup>(3)</sup>, Ewelina Nowacka-Kozak<sup>(3)</sup>, Tomasz Kiljanek<sup>(3)</sup>, Katarzyna Pietruszka<sup>(3)</sup>, Konrad Pietruk<sup>(3)</sup>, Agnieszka Nawrocka<sup>(3)</sup>, Ewelina Kowalczyk<sup>(1)</sup>, Aleksandra Grelik<sup>(1)</sup>, Monika Przeniosło-Siwczyńska<sup>(1)</sup>, Krzysztof Kwiatek<sup>(1)</sup>, Jolanta G. Rola<sup>(4)</sup>, Sebastian Maszewski<sup>(2)</sup>, Szczepan Mikołajczyk<sup>(2)</sup>, Magdalena Gemba<sup>(2)</sup>, Wojciech Pietron<sup>(2)</sup>, Piotr Jedziniak<sup>(3)</sup>

<sup>1)</sup> National Veterinary Research Institute, Department of Feed Hygiene, Poland

<sup>2)</sup> National Veterinary Research Institute, Department of Radiobiology, Poland

<sup>3)</sup> National Veterinary Research Institute, Department of Pharmacology and Toxicology, Poland

<sup>4)</sup> National Veterinary Research Institute, Department of Hygiene of Milk and Milk Products, Poland

\*Corresponding author - E-mail: [anna.gajda@piwet.pulawy.pl](mailto:anna.gajda@piwet.pulawy.pl)

Modern food and feed safety control systems focus on testing compounds and specific groups of compounds in the context of requirements imposed by international law. This approach, however economically justifiable, results in the loss of a broader view of the problem of the presence of several contaminants in the samples tested. This is particularly important when considering the huge number of compounds required for testing. This is also relevant when considering the huge number of compounds required for testing, which cannot be determined simultaneously despite the development of analytical techniques (e.g. mass spectrometry). This is due to the differences in physical and chemical properties and above all to the very low limits imposed by legislation. These requirements necessitate specific sample preparation and instrumental analysis conditions adapted to the group of compounds in question.

In order to verify the levels of contamination of feed in Poland, tests were undertaken on 64 feed samples and 48 water samples fed to swine. Samples of feed were analysed for the following analytes: mycotoxins, plant toxins, toxic nuclei, pesticides, coccidiostats, antibiotics, dioxins, polybrominated diphenyl ethers, per- and polyfluoroalkyl substances, radioactive contaminants. A wide range of analytical techniques were used in the study: ICP-MS, ASA, HPLC-FLD, GC-ECD, GC-MS/MS, UPLC-MS/MS, HRGC-HRMS.

Feed samples analysed showed no exceedances of the regulatory maximum limits for mycotoxins, heavy metals (Pb, Cd, Ar, Hg) and do not pose a toxicological risk to the animals fed them. The PCDD/PCDF, PCDD/PCDF/dl-PCB and ndl-PCB levels were about 8%, 6% and 0.5% of the maximum allowable levels, respectively. The analysed samples of compound feed were practically free of PBDEs, with the content of tested compounds not exceeding 0.63 µg/kg. Perfluorinated compounds were not detected in 96% of the samples analysed. The detected caesium radioisotope concentrations in 95 % of the samples tested were below the Minimum Detectable Activity. Antibiotics were confirmed in two feed samples (tylosin at approximately 90 mg/kg feed and doxycycline at 0.36 mg/kg). Coccidiostats was found in 6 out of 64 samples of pig feed mixtures, in five samples the determined amounts of nicarbazin, narasin, salinomycin and monensin were below the maximum limits (ML) for these compounds in feed. A non-compliant result was obtained for one sample only, with salinomycin found in the sample at 5.87 mg/kg feed. Tropane alkaloids was detected in the feed samples, which were determined in nine feed samples. The determined concentrations of atropine/scopolamine ranged from 1.4 to 40.25 µg/kg. For ergot alkaloids, their presence was confirmed in 7 feed samples, the determined concentrations for these compounds ranged from 12.1 to 73.8 µg/kg.

The results show a low level of contamination of feed with contaminants and confirm the advantages of multidirectional sample testing.

**Keywords:** food safety, feed safety, water analysis, pigs

### W22

## THE INFLUENCE OF HEAT TREATMENTS ON THE RESIDUES OF SEVEN ANTIBACTERIALS IN BEEF MEAT

**Anna Gajda**<sup>(1)</sup>, **Magdalena Bilecka**<sup>(1)</sup>, **Natalia Mizak**<sup>(1)</sup>

<sup>1)</sup> *National Veterinary Research Institute, Poland*

*\*Corresponding author - E-mail: [anna.gajda@piwet.pulawy.pl](mailto:anna.gajda@piwet.pulawy.pl)*

The irrational and improper use of antibiotics in livestock can lead to the accumulation of their residues in animal tissues and products. Generally, the residues are estimated on uncooked meat, but most animal products are usually processed before consumption and undergo different heat treatment. Cooking procedures are often related to reduction of drug residues in food. However, depending on the time-temperature combinations, as well as chemical structure and properties of drugs, the susceptibility to degradation by heating can be various. The aim of this study was to determine the effect of different cooking methods under different time, on seven antibacterials, most commonly detected in bovine tissues: neomycin, dihydrostreptomycin, penicillin G, oxytetracycline, tylmicosin, tulathromycin and marbofloxacin. Antibiotics-free beef muscle was divided into 7 portions of 200g, each spiked with tested antibiotic on the level of 2 x MRL (maximum residue limit). After homogeneity analysis, 15 g balls of meat were prepared, intended for heat treatment. The first treatment included boiling for 5, 15 and 30 min. The second one involved frying of meat in a pan with oil and without oil for 5, 10 and 20 min. and the third one placing samples into microwave for 0.5, 1 and 2 min. Antibiotics analyses in raw and cooked material were assayed by liquid chromatography - tandem mass spectrometry (LC-MS/MS). In all presented cooking methods, a decrease of measured sample weights was observed, with the greatest decline in case of microwaving. The most significant reduction for penicillin G was observed, for all kinds of cooking. A considerable reduction in the concentrations of oxytetracycline, tylmicosin and tulathromycin was found, especially during boiling and frying with oil for 30 min. For marbofloxacin, a slight reduction during boiling and microwaving was recorded, however the marbofloxacin concentration was increased at frying. Neomycin and dihydrostreptomycin were stable for heat treatment, and due to the water evaporation from the meat and related weight reduction, much higher concentrations in cooked material were determined than in raw samples. The results of this study show that cooking processes do not guarantee complete degradation of antibacterial drug residues, even in some cases the final concentration in cooked product can be greater.

**Keywords:** residues, antibacterials, heat treatments, beef

### W23

## SIMULTANEOUS SCREENING OF 118+ VETERINARY DRUGS IN MILK AND MUSCLE USING UNTARGETED LC-HRMS ANALYSIS

**Tina Angerer<sup>(1)</sup>, Stina Söderlund<sup>(1)</sup>, Martina Degerman<sup>(1)</sup>, Samantha Madawala<sup>(1)</sup>, Petter Englund<sup>(1)</sup>**

<sup>1)</sup> Swedish Food Agency, Sweden

\*Corresponding author - E-mail: [tina.angerer@slv.se](mailto:tina.angerer@slv.se)

This is a multi-residue multi-class screening method, based on high-resolution mass spectrometry (HRMS), for the research of pharmacological, veterinary substances in foodstuffs of animal origin. The method is intended for screening i.e. qualitative analysis and quantitative determination is possible for most compounds included in the method. The mass analysis is performed untargeted, allowing for the identification of analytes of interest even at a later date. The method performance parameters for each compound have been determined in validation studies in muscle and milk. It was originally developed at European reference laboratory ANSES (France) and adapted for use in the national control program at the Swedish Food Agency.

21 (bovine) milk or (beef/pork/poultry) muscle samples are spiked, prior to extraction, with 118 veterinary drug compounds (antibiotics, NSAIDs, sedatives and coccidiostats) corresponding to a concentration of 0-200 µg/kg + deuterated internal standard compounds. In total 99 extracts per sample matrix are separated via HPLC using a RP-C18-column and analysed via high resolution mass spectrometry using a Q-Exactive Orbitrap (Thermo) in FS/v-DIA mode, (R = 70000 FWHM,  $\Delta m \pm 3$  ppm (max), m/z 120-1200). Data analysis is performed using a database in Tracefinder (Thermo) including the 118 compounds with various adducts, retention times and 2-5 fragments for each compound for verification.

Out of 118 studied veterinary drugs, 115 in milk and 103 in meat were reliably detectable (false negative rate <5%) and 43 (meat)/ 60 (milk) analytes fulfilled the requirements for a quantitative screening analysis according to EU Commission Regulation (EU) 2021/808 [1].

With this newly implemented method we were able to detect various classes of veterinary drugs including B1-a antibiotics, B1-b anthelmintics, B1-c sedatives, B1-d NSAIDs, and B2 coccidiostats, within one extraction and analysis procedure. With few exceptions detection was as sensitive as with dedicated single drug class methods. In the future this one method could replace multiple screening methods, saving time and resources. Additionally, the generated untargeted datasets allow for scanning of future compounds of interest with high confidence of correct identification due to the use of high mass resolution and the recording of MS<sup>2</sup> spectra.

[1] Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC.

**Keywords:** veterinary drugs, HRMS, untargeted MS analysis, EU, HPLC

### W24

## THE COST ACTION BESAFEBEEHONEY: A PROMISING NETWORK TO PROMOTE THE SAFETY OF BEES AND HONEY AND THE SGL'S ENGAGEMENT

**Maria Strouthou**<sup>(1)</sup>, **Christopher Papachrysostomou**<sup>(1)</sup>, **M Leite**<sup>(2)</sup>, **Andreia Freitas**\*<sup>(3)</sup>

<sup>1)</sup> *Veterinary Drug Residues Lab, State General Laboratory (SGL), Cyprus*

<sup>2)</sup> *Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Rua dos Lágidos, Lugar da Madalena, REQUIMTE/LAQV, R. D. Manuel II, Apartado, 55142, Porto, Portugal, Portugal*

<sup>3)</sup> *Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Rua dos Lágidos, Lugar da Madalena, 4485-655, Vila do Conde, Portugal, REQUIMTE/LAQV, R. D. Manuel II, Apartado, 55142, Porto, Portugal, Portugal*

\*Corresponding author - E-mail: [andreia.freitas@iniav.pt](mailto:andreia.freitas@iniav.pt)

BeSafeBeeHoney is a network established to implement the COST Action "Beekeeping Products Valorization and Biomonitoring for the Safety of Bees and Honey" (CA22105), with the goal of promoting the safety and protection of both bees and honey. Honey has been valued since ancient times for its health benefits. However, the increasing presence of contaminants and the accumulation of harmful chemicals in honeybee products poses a risk to both bee and human health and highlights the need for more research.

To address these challenges and support honeybee colonies, the BeSafeBeehoney network brings together experts from diverse scientific fields, including chemistry, nutrition, ecology, veterinary medicine, beekeeping, agricultural engineering, economics, and policy. Its goal is to generate and share innovative research to protect bee health and promote sustainable beekeeping. BeSafeBeeHoney goes beyond traditional scientific collaborations by engaging non-scientific stakeholders, with a focus on the European Green Deal and ensuring the accomplishment of the "Farm to Fork" strategy in a "One Health" approach.

The State General Laboratory of Cyprus, as member of this action, promotes healthier, sustainable honey while addressing the presence of veterinary drug residues in honey. Its Veterinary Drug Residues Lab implements LC-MS/MS methods to detect antibiotics in honey, ensuring reliable official control of potential treatments in honeybees. Non-compliant honey samples highlight the need for control measures and responsible veterinary medicine use, encouraging good beekeeping practices among beekeepers.

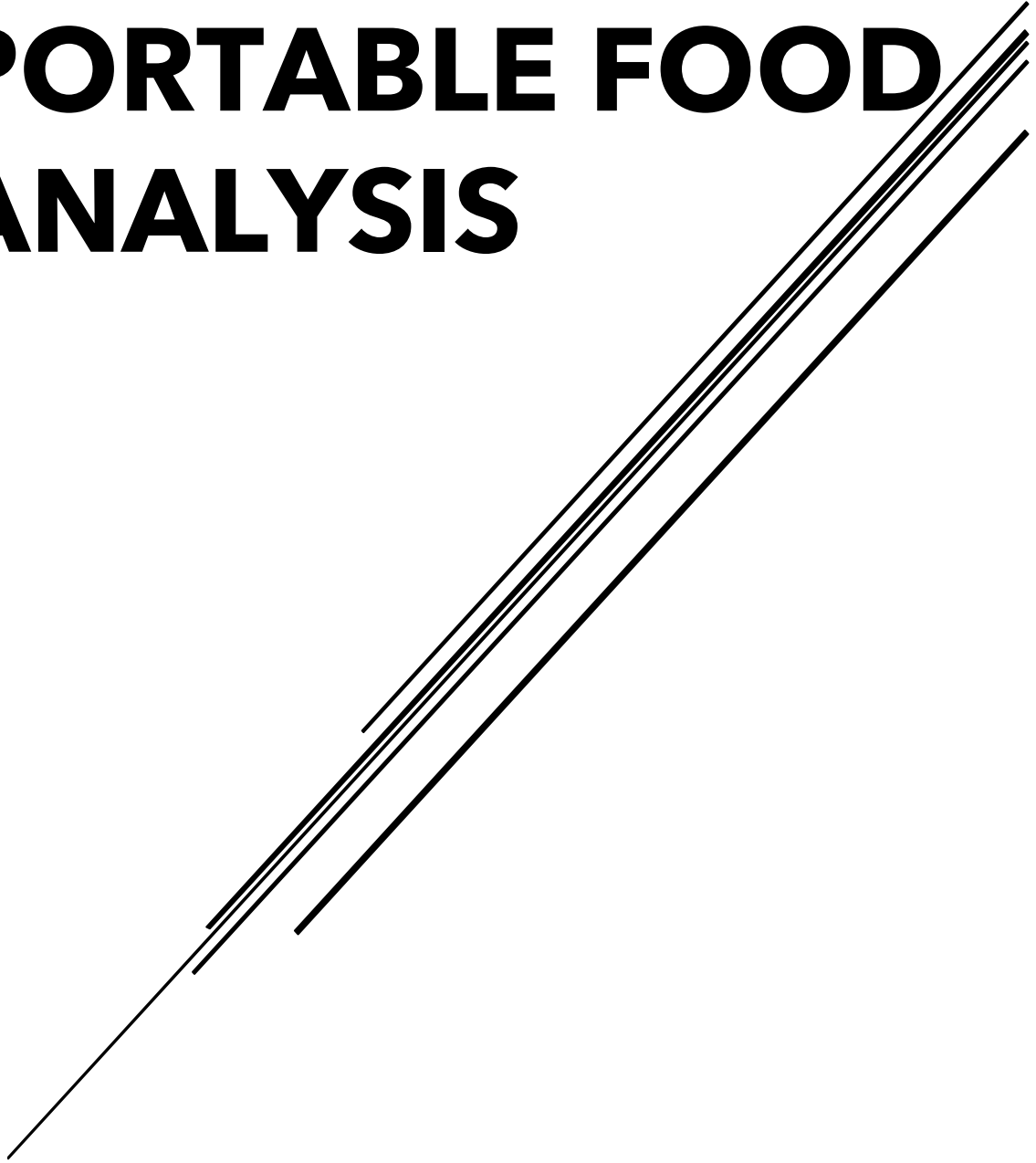
[1] Memorandum of understanding CA22105, available at <https://www.cost.eu/actions/CA22105/>.

**Keywords:** BeSafeBeeHoney, veterinary drug, antibiotics

**Acknowledgement:** *This presentation is based upon work from COST Action CA22105 - BeSafeBeeHoney, supported by COST Association (European Cooperation in Science and Technology).*



# **SENSORS & PORTABLE FOOD ANALYSIS**



### X1

#### ADVANCED INFRARED SPECTROSCOPIC TECHNOLOGIES: TOWARDS ON-SITE ANALYSIS OF MYCOTOXINS IN CEREALS

**Polina Fomina**<sup>\*(1)</sup>, Antoni Femenias<sup>(1)</sup>, Miriam Aledda<sup>(2)</sup>, Stephan Freitag<sup>(3)</sup>, Michael Sulyok<sup>(3)</sup>, Achim Kohler<sup>(2)</sup>, Rudolf Krska<sup>(3)</sup>, Boris Mizaikoff<sup>(1)</sup>

<sup>1)</sup> *Ulm University, Germany*

<sup>2)</sup> *Norwegian University of Life Sciences, Norway*

<sup>3)</sup> *University of Natural Resources and Life Sciences, Austria*

*\*Corresponding author - E-mail: polina.fomina@uni-ulm.de*

Mycotoxin contamination in cereals is recognized as a global food safety concern. One of the most common mycotoxins in grains is deoxynivalenol (DON), a secondary metabolite of fungi *F. graminearum* and *F. culmorum*. Exposure to DON can result in adverse health effects for both humans and animals. Hence, it is crucial to provide robust analytical techniques for the on-site detection of relevant DON contaminations preventing health issues and economic loss.

In the current study, we introduce advanced infrared spectroscopic technologies for DON detection. A handheld mid-fidelity (MI-FI) spectrometer was developed proving the scalability of IR spectroscopy towards on-site analysis. Exemplarily, 26 wheat samples with DON concentrations between blank and 10600 µg/kg were extracted with ethanol:water (30:70) and subsequently analyzed. For differentiating high vs. low DON-contaminated wheat according to the European Union threshold (1250 µg/kg), sparse partial least squares discriminant analysis (SPLS-DA) has been applied. The obtained accuracy of cross-validation was 84.6%.

This study underscores the potential of advanced infrared spectroscopic technologies serving as an eco-friendly approach for on-site mycotoxin analysis in cereals and other commodities.

**Acknowledgement:** *This work was supported by the EU Horizon 2020 project PHOTONFOOD [#101016444] which is part of the PHOTONICS PUBLIC PRIVATE PARTNERSHIP.*

### X2

#### **AUTOMATED AND SENSITIVE ALLERGEN DETECTION WITH A PORTABLE MICROFLUIDIC PLATFORM INTEGRATING SAMPLE PREPARATION AND A SMART IMMUNOASSAY**

**Maxime Gavage**<sup>(1)</sup>, **Bastian Breiner**<sup>(2)</sup>, **Daniel Kainz**<sup>(2)</sup>, **Stefan Wagner**<sup>(2)</sup>, **Serhat Sahakalkan**<sup>(2)</sup>, **Riccardo Marega**<sup>(1)</sup>

<sup>1)</sup> CER Groupe, Belgium

<sup>2)</sup> Hahn-Schickard, Germany

\*Corresponding author - E-mail: m.gavage@cergroupe.be

Point-of-care diagnostic systems are increasingly gaining attention in healthcare, environmental monitoring, and the agro-food sectors due to their ability to bypass traditional laboratories, enabling rapid and on-site analysis that saves time and money. Centrifugal microfluidics offers an easy-to-use and portable platform by performing precise and accurate liquid handling without the need for pumps or tubing.

In this context, our aim is to demonstrate that integrating portable sample preparation strategies with immunological assays can lead to innovative technological solutions capable of lowering on-site detection limits. We selected soybean, a common dietary protein source and major food allergen, as a model for developing a prototype centrifugal microfluidic cartridge.

We developed and optimized a denaturing extraction protocol for complex and processed food matrices, which is not directly compatible with traditional immunoassays. In parallel, microfluidic operations were designed, integrated, and tested using thermoformed prototypes before transitioning to injection-moulding. These operations included the clean-up of the denatured extracted sample, the capture of the analyte on fluorescent microbeads functionalized with specific antibodies, and the lateral flow immunochromatographic assay. An injection-moulding tool was subsequently developed, with simulations optimizing the injection process.

Special attention was given to the functionalization of the fluorescent beads with in-house developed anti-soybean antibodies. Several regioselective modification strategies were considered to prevent bead aggregation due to cross-linking and interference with the antigen-binding regions. These optimizations increased the immunoreactive fraction, thereby improving overall detection sensitivity and reducing biologicals consumption.

Final testing of the cartridge with pre-stored functionalized fluorescent beads demonstrated its potential in simplifying sample preparation and detecting allergens in complex food matrices. The system achieved sensitivities of 0.01 ppm soybean protein in buffer and 4 ppm in various incurred matrices.

In conclusion, this research represents a significant advancement in microfluidic-based allergen detection, establishing a foundation for portable assays with broader applications in food safety monitoring and beyond.

**Keywords:** smart immunoassay, food allergen, microfluidic cartridge, point-of-care diagnostic system, antibody functionalization

**Acknowledgement:** *The research that yielded these results was funded by the Public Service of Wallonia through the Cornet Program, contract n°2010272 THESEUS.*

### X3

#### ENZYME-FREE AMPLIFICATION DETECTION OF NOROVIRUS (NOV) RNA USING HYBRIDIZATION CHAIN REACTION AND GOLD NANOPARTICLES

**Prabir Kumar Kulabhusan**<sup>\*(1)</sup>, **Andrey Ipatov**<sup>(1)</sup>, **Marta Prado**<sup>(2)</sup>

<sup>1)</sup> Food Quality and Safety Research Group, INL-International Iberian Nanotechnology Laboratory, Portugal

<sup>2)</sup> Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Veterinary Science, Campus Terra. University of Santiago de Compostela, Spain

\*Corresponding author - E-mail: prabir.kulabhusan@inl.int

Human Norovirus (NoV) is one of the primary causes of viral gastroenteritis and food-borne diseases, worldwide. The frequency of outbreaks highlights the importance of timely and efficient detection methods to mitigate the spread in the food supply chain. Currently, real-time PCR is the gold standard method for the detection of NoV. However, the technique is tedious, time-consuming, and demands expensive enzymes to amplify the NoV RNA.

We aim to develop a novel enzyme-free hybridization chain reaction amplification method for the specific and sensitive detection of NoV RNA. The amplification method coupled with gold nanoparticles (AuNPs) will provide a colorimetric detection platform for the NoV. For this, we designed two catalytic hairpin (H1 and H2) probes using the Nupack software. NoV RNA was used as the initiator to trigger a cascade hybridization reaction. The amplification was confirmed using polyacrylamide gel electrophoresis. Further, the cascade hybridization reaction was confirmed by an increase in the fluorescent intensity observed in the presence of the target RNA when compared to the control. The temperature and concentrations of the hairpins were also determined for both H1 and H2, which was sufficient to carry out the reaction.

The salt-induced aggregation of AuNPs will be employed to develop the colorimetric detection platform. For this, AuNPs were synthesized using citrate reduction method and characterized using different analytical techniques. In principle, in the absence of the target RNA, the hairpin probes stabilize the AuNPs and effectively prevent them from salt induced aggregation (Purple colour). However, in the presence of the target, the amplification occurs and the AuNPs undergo aggregation (Blue colour). As a result, a purple to blue colour variation will be recorded in different concentrations of the target RNAs. The developed assay will also be tested using the spiked samples. Moreover, the sensitivity of this detection platform will be compared to that of enzyme-mediated AuNPs-based colorimetric assay. The method developed will be highly selective and specific to detect the RNA, offering a rapid, cost-effective, and naked eye visual detection method for NoV.

**Keywords:** norovirus, biosensor, food safety, nanoparticles, enzyme-free amplification method

**Acknowledgement:** P.K.K acknowledges funding from the European Union's Horizon 2020 research and innovation programme via CHAMPIONS Project under the Marie Skłodowska-Curie grant agreement No. 101066736

### X4

#### A SENSITIVE AND FAST TETRODOTOXIN LATERAL FLOW BIOSENSOR BY ANTIBODY-APTAMER SANDWICH ASSAY

Ulises Guillermo Díaz Avello<sup>(1)</sup>, Vasso Skouridou<sup>(1)</sup>, Monica Campas<sup>(2)</sup>, Ciara Kathleen O'Sullivan<sup>\*(1)</sup>

<sup>1)</sup> *Universitat Rovira i Virgili, Spain*

<sup>2)</sup> *Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Spain*

*\*Corresponding author - E-mail: ciara.osullivan@urv.cat*

Tetrodotoxin (TTX) is a small and highly toxic marine compound related to increasing algal blooming events. Marine organisms accumulate TTX via the food chain and, as cooking does not destroy TTX, human intoxication cases are reported frequently. TTX blocks sodium voltage-gated channels impeding nerve function and causing severe symptoms. Furthermore, no antidotes are available and the early detection of TTX in contaminated products is crucial to reduce intoxications. However, the detection of TTX is complicated. Analytical techniques require extensive and costly processes to overcome matrix effects or hold low sensitivity thresholds limiting their field application. Lateral flow assays (LFAs) are fast and robust analytical tests in a paper-based cost-efficient format, which provide equipment-free signal readout. LFAs typically use a pair of bioreceptors named capture bioreceptor and detection bioreceptor for analyte detection. A capture bioreceptor is immobilized on a nitrocellulose membrane, and detection bioreceptor is conjugated with a signal transducer enabling naked eye detection. Bioreceptors interact with the specific target analyte and can provide a signal-off or signal-on result. Competitive assays are based on a competition between sample target in solution and immobilized target for binding to the detection bioreceptor and result in a signal-off event when target is present in sample. On the other hand, sandwich assays produce a signal-on result after binding of both bioreceptors to different regions of the target analyte, thus providing a more sensitive and robust detection system. However, due to difficulties in establishing a sandwich with small targets, competitive assays are commonly employed. The past decade has seen an increase in the use of aptamers for small molecule detection. These molecules are single stranded oligonucleotides with similar binding features as antibodies but exhibiting higher stability, as well as easier and more cost-efficient production. In our previous work we selected a TTX binding aptamer and combined it with an anti-TTX mouse IgG antibody to establish a microplate antibody-aptamer sandwich-type assay for TTX detection. In this work, we developed an LFA strip test for fast and equipment-free TTX detection exploiting the antibody-aptamer sandwich assay. The test achieved a visual limit of detection of 0.3 ng/mL TTX in less than 20 minutes. The stability and specificity of the test were also demonstrated to be very high. Finally, its performance was validated with contaminated puffer fish samples. The test was able to detect TTX far below the safety limits of 2 mg TTX/kg fish tissue established in Japan, with absence of both matrix effects and cross-reactivity with other marine toxins. This TTX LFA test is the first sandwich-assay for TTX and significantly improves detection limits compared to current commercially available tests or other antibody-based competitive LFA tests published in the literature.

**Keywords:** puffer fish, food poisoning, small molecules detection, sandwich-type assay, lateral flow assay

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### X5

#### MINIATURIZED DEVICE FOR POINT-OF-CARE TESTING OF FOODBORNE PATHOGENS

**Ana Costa-Ribeiro**\*<sup>(1)</sup>, **Sofia Araujo Pereira**<sup>(1)</sup>, **Alexandre Lamas**<sup>(2)</sup>, **Alejandro Garrido-Maestu**<sup>(3)</sup>, **Marta Prado**<sup>(2)</sup>, **Adrian Sanchez-Visedo**<sup>(1)</sup>

<sup>1)</sup> International Iberian Nanotechnology Laboratory, Portugal

<sup>2)</sup> Food Hygiene, Inspection and Control Laboratory (Lhica), Department of Analytical Chemistry, Nutrition and Bromatology, Veterinary School, Campus Terra, University of Santiago de Compostela (USC), Spain

<sup>3)</sup> Institute of Marine Research Consejo Superior de Investigaciones Científicas, Spain

\*Corresponding author - E-mail: ana.c.ribeiro@inl.int

*Salmonella* spp. are among the most prevalent and widespread foodborne pathogens in the world. *S. Enteritidis* and *S. Typhimurium* are the most frequently reported serovars, with poultry and eggs being the main source of disease, but many other serovars also frequently cause illness in humans and are therefore a significant public health concern [1]. Consequently, the demand for methods that allow rapid detection is increasing. Molecular methods have become an essential method in areas such as human health, quality and food safety, since traditional methods are time-consuming, expensive and require specialized equipment and staff training [2]. Miniaturized Point-Of-Care (POC) testing devices offer a revolutionary alternative, providing fast, accurate and on-the-spot detection of pathogens. Isothermal DNA amplification techniques, such as loop-mediated isothermal amplification (LAMP), is an alternative technique to the gold standard technique, PCR, as it does not require thermal cycles and can be easily integrated into these microfluidic devices [3]. To obtain a faster method for the detection of *Salmonella* spp, a new microfluidic device has been developed that can integrate all the necessary steps for bacterial DNA extraction, purification and subsequent DNA amplification (LAMP). The matrix used for this project was the soil present in chicken coops, since it has been described that chickens is one of the main sources of *Salmonella* outbreaks. DNA extraction begins by lysing bacterial cell walls with a combination of lysozyme and proteinase K. The DNA is then purified using silica beads, which are immobilized inside the device and capture the DNA on their surface. After elution, the DNA is amplified using Real-Time Loop-Mediated Isothermal Amplification (RT-LAMP) and colorimetric LAMP, enabling rapid and straightforward detection. The results obtained with the soil samples spiked with the *Salmonella* spp. demonstrated that it is possible to obtain LAMP- amplifiable DNA with comparable quality to that of the commercial kit. When implementing the real-time fluorescence approach, positive results were obtained in 20 min, while with the colorimetric LAMP a longer incubation time was needed, 45 min for more clear color discrimination. In summary the protocol and device reported herein have the potential to provide a simple to operate approach, for reliable pathogen detection in decentralized setups with low infrastructure.

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[2] Byron BS, Charles Y, Lee-Ann J, Tortorello M Lou. Sample preparation: The forgotten beginning. *J Food Prot [Internet].* 2009;72(8):1774-89.

[3] Jiang Y, Zou S, Cao X. Rapid and ultra-sensitive detection of foodborne pathogens by using miniaturized microfluidic devices: A review. *Analytical Methods.* 2016;8(37):6668-81.

**Keywords:** microfluidic device, foodborne pathogen, point of care detection, DNA amplification, LAMP

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### X6

#### NOVEL BIO-SENSING TECHNOLOGY FOR RAPID AND COST-EFFECTIVE ANALYSES OF BOAR TAINT COMPOUNDS AT THE POINT OF TEST

Olena Doran<sup>(1)</sup>, Adrian Crew<sup>(1)</sup>, Kelly Westmacott<sup>(1)</sup>, John Hart<sup>(1)</sup>

<sup>1)</sup> University of the West of England, United Kingdom

\*Corresponding author - E-mail: olena.doran@uwe.ac.uk

Boar taint is the unfavourable taste and aroma of meat from some entire male pigs which is due to an excessive accumulation of the two naturally occurring compounds, skatole and androstenone in adipose tissue. Boar taint is one of the main meat quality defects which can be prevented by surgical castration of pigs. However, surgical castration has been banned in an increasing number of countries due to animal welfare concern. Therefore, the international meat industry faces a challenge of detecting boar taint and preventing the entry of tainted carcasses into the food chain. The available methods for detecting skatole and androstenone are based on high resolution gas chromatography (GC) or similar approaches, which are expensive, time-consuming, require specialised equipment and cannot be conducted at the point of test. Innovative bio-sensing technology developed and patented at the University of the West of England, Bristol combines two low-cost screen-printed carbon electrode (SPCEs) into a dual electrode detection system for rapid simultaneous measurement of androstenone and skatole directly in adipose tissue. The measurements were performed by two electrochemical techniques which have previously been successfully used by the authors for a variety of agri-food applications. Skatole measurement was conducted by the differential pulse voltammetry technique with a plain SPCE; androstenone measurement was done using chronoamperometry with an enzyme-based bio-sensor combining hydroxysteroid dehydrogenase and a SPCE modified with the electrocatalyst Meldolas Blue. The solution and electrochemical conditions were optimised to obtain the best selectivity and sensitivity for detecting and quantification of the two boar taint compounds. The bio-sensors were evaluated against a GC-based method using porcine tissue samples spiked with skatole and androstenone. This was followed by an *in-situ* evaluation of the bio-sensors on 56 carcasses in a commercial abattoir processing line. There was a strong correlation between the results obtained with the novel bio-sensing technology and the GC analysis ( $R^2 > 0.95$ ). The results of these analyses also indicated that animal weight, which is used in some countries as a way to predict the occurrence of boar taint, was not a reliable predictor of boar taint in the sample cohort studied (boars, mature boars, sows and gilts). This highlights the importance of detection of boar taint by direct analyses rather than by prediction. The bio-sensors for skatole and androstenone allow to undertake simultaneous analyses of the boar taint compounds in less than 2 minutes with a possibility to decrease the analyses time further. The novel bio-sensing technology for rapid, simple and low-cost analyses of skatole and androstenone can be applied for boar taint analyses either as a stationary or a portable device.

**Keywords:** sensors, skatole, androstenone, boar taint

**Acknowledgement:** This research was funded by the Biotechnology and Biological Sciences Research Council and by the Agriculture and Horticulture Development Board. The authors thank the University of the West of England, Bristol, JSR Genetics Ltd and Biosciences Knowledge Transfer Network for their support, and Gwent Electronic Materials Ltd for supplying the screen-printed carbon sensors.

### X7

#### APPLICATION OF FLUORESCENCE SPECTROSCOPY TO DETECT COLD CHAIN DISRUPTIONS DURING STORAGE OF MODIFIED-AIR PACKAGED MINCED PORK

Johannes Schlosser<sup>(1)</sup>, Martin Mühling<sup>(2)</sup>, Heinar Schmidt\*<sup>(3)</sup>

<sup>1)</sup> Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Fritz-Hornschuch-Str. 9, 95326 Kulmbach, Germany, Germany

<sup>2)</sup> Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Meat, E.-C.-Baumann-Str. 20, 95326 Kulmbach, Germany, Germany

<sup>3)</sup> Chair of Bioanalytical Sciences and Food Analysis, University of Bayreuth, Fritz-Hornschuch-Str. 9, 95326, Kulmbach, Germany, Germany

\*Corresponding author - E-mail: heinar.schmidt@uni-bayreuth.de

Cold chain (CC) management is essential to maintain the quality of perishable foods. Especially for minced meat, legal requirements oblige manufacturers and retailers to store the product continually at max. 2 °C. In this context, it is of interest to what extent a shorter or longer CC disruption may impair product quality and whether this can be detected using a non-invasive inspection of the product.

While there is an ample literature documenting the correlation of bacterial plate counts with fluorescence spectra of meat during storage, only few authors have focused on the correlation of the fluorescence spectra with the duration of the storage. Given that the latter correlation is linked to biochemical changes in the meat matrix, a storage at elevated temperatures should lead to an accelerated ageing of the product.

The aim of this work was to show that a CC interruption can be detected in modified-air packaged (MAP) minced pork based on the correlation of the fluorescence spectra with storage time. To this end, minced MAP pork batches (n = 12) were stored at 2 °C over a period of 17 days. Spectra were measured regularly on 8 or 9 days with 3 packages per day with a hand-held fluorescence device to build a partial-least squares regression model (PLSR) with, in total, 296 calibration samples. For the CC disruptions, independent minced MAP pork batches (n = 5) were stored at 2 °C. For four batches, half of the batch served as control. The remainder was stored temporarily at 14 °C on day 2 of storage for 6 h (n = 3) or 12 h (n = 3).

As a result, the PLSR model predicted samples with CC disruption systematically older than the control samples. This effect was apparent already at the first day after the disruption. This is noteworthy because, at that time of storage at 2 °C, bacteria are in general, still in the lag phase. Control samples were predicted with the correct storage time ( $R^2 = 0.94 - 0.95$ , RMSEP = 1.3 - 1.5 d). For the 6 h disruption, the bias was not significant (0.8 d), whereas this effect was significant for the 12 h disruption (2.4 d).

This work demonstrates the feasibility to use fluorescence spectroscopy to detect a disruption early in the cold chain and already one day after the transient increase of the storage temperature.

**Keywords:** cold chain disruption, fluorescence, minced pork, MAP



### X8

#### FLOWSENSE: SINGLE AND MULTIPLEX ON-SITE TESTING FOR DETECTION OF ANTIBIOTICS AND MYCOTOXINS

**Mark Squance**<sup>(1)</sup>, **Kielan Toal**<sup>(1)</sup>, **Nicola Kane**\*<sup>(1)</sup>, **Morteza Afrasiabi**<sup>(2)</sup>

<sup>1)</sup> *Biorex Food Diagnostics, United Kingdom*

<sup>2)</sup> *Fortress Diagnostics, United Kingdom*

\*Corresponding author - E-mail: [nicola@biorexfooddiagnostics.com](mailto:nicola@biorexfooddiagnostics.com)

Testing honey for antibiotic residues is an important process to ensure that it meets the required safety standards for human consumption. Antibiotic residues have long been detected in commercial honey, arising from direct beekeeping practices or environmental contamination. This raises concerns about potential health risks and the impact on antibiotic resistance. Consequently, honey is now subject to stringent regulations by global authorities, with the E.U. regularly updating guidelines on permissible levels.

Mycotoxins are toxic substances produced by certain moulds, which can develop in various crops like cereals, nuts, fruits, and spices, particularly when stored improperly. These toxins pose a significant threat to human and animal health due to their carcinogenic properties. In response to this issue, regulatory bodies worldwide have set limits on mycotoxin levels in food products. The list of regulated mycotoxins and the affected food types is continually revised, underscoring the importance of ongoing testing.

Currently, to screen a sample for multiple antibiotics or mycotoxins, producers primarily rely on costly confirmatory analysis such as liquid chromatography-mass spectrometry (LC-MS) testing. As a result, there is a strong demand in these industries for rapid, cost-effective residue screening methods that enable the simultaneous detection of multiple contaminants and allowing for the efficient monitoring of raw materials.

At Biorex Food Diagnostics, a range of single and multi-analyte diagnostic platforms using lateral flow microarray immunoassay are being developed. This range (FlowSense) includes devices that can be used for the screening of various antibiotics in honey and mycotoxins in grains and cereals. These lateral flow assays utilise a cost-effective half-stick format, incorporating a freeze-dried conjugate and a straightforward user protocol. The detection process is facilitated by simple extraction protocols, predominantly green-based, with a focus on a universal sample preparation approach for mycotoxin analysis. The lateral flow competitive principle employed allows for quantification of the target analyte based on the intensity of the signal observed.

A test reader has also been incorporated into the reporting methodology to allow for testing in various environments, from field operations to different areas within food production facilities. The reader also provides semi-quantitative and quantitative results and reduces visual interpretation errors across single and multi-analyte tests.

The expectation is that these test devices, combined with the integration of a user-friendly reader, will positively impact the monitoring of antibiotic levels in honey and mycotoxin levels across grains and cereals. The high sensitivity, specificity, and rapid turnaround time (10 minutes) offered by the FlowSense testing range make it an invaluable tool for users at all levels.

**Keywords:** lateral flow, honey, antibiotics, mycotoxins, grains

### X9

#### RECEPTOMIX: A CUSTOMIZABLE MULTIPLEX RECEPTOR BIOCHIP FOR THE ANALYSIS OF FLAVOUR AND HEALTH PROPERTIES OF FOOD INGREDIENTS

**Lorenzo Corsico**<sup>\*(1)</sup>, **Maurice Henquet**<sup>(2)</sup>, **Aria Samimi**<sup>(1)</sup>, **Margriet Roelse**<sup>(2)</sup>, **Maarten Jongmsma**<sup>(2)</sup>

<sup>1)</sup> *InsectSense BV, The Netherlands*

<sup>2)</sup> *Wageningen University & Research, The Netherlands*

*\*Corresponding author - E-mail: lorenzo@insectsense.com*

G-protein coupled receptors (GPCRs) are important targets for various food components. The members of this large family of membrane proteins are involved in virtually every physiological process. By analysing the interactions between ingredients and receptors, food scientists can determine the flavour and health potential of particular food products. ReceptomiX is a powerful novel biosensor platform based on the receptomics technology developed at Wageningen Research (WR). Previous research by WR has already demonstrated that the technology works with various human taste and health-related GPCRs and ion channels, as well as insect olfactory receptors for the detection of volatile compounds. In this regard, the bioactivity of functional ingredients and flavour molecules has been analysed for a range of different receptors, including sweet, umami, and bitter receptors (TAS1R and TAS2R), various hormonal receptors (e.g. GLP1R, cholecystokinin), as well as dopamine, serotonin, and free fatty acid receptors. This allows for the analysis of complex extracts or pure compounds and their interactions with tailored sets of relevant receptors. Additionally, the technology can be used for the identification and quantification of relevant bioactivities. The implementation of ReceptomiX holds the potential to revolutionize the food and nutraceutical industries, offering more efficient and effective methods of bioactivity discovery and product development. This information can also be used to optimize product formulations and improve bioactivity.

**Keywords:** GPCR, receptomics, bioactivity, food, health

### X10

#### ENHANCING ESCHERICHIA COLI DETECTION: INTEGRATING HYBRID PMMA/PAPER MICROFLUIDIC DEVICE FOR DNA PURIFICATION, AMPLIFICATION AND COLORIMETRIC DETECTION

Sofia Araujo Pereira<sup>(1)</sup>, Ana Costa-Ribeiro<sup>(1)</sup>, Rui Campos<sup>(1)</sup>, Andrey Ipatov<sup>(1)</sup>, Adrián Sanchez-Visedo\*<sup>(1)</sup>

<sup>1)</sup> *International Iberian Nanotechnology Laboratory, Portugal*

\*Corresponding author - E-mail: [adrian.visedo@inl.int](mailto:adrian.visedo@inl.int)

*Escherichia coli* (*E. coli*), a major foodborne pathogen, poses a serious public health threat, causing illnesses that range from gastroenteritis to severe life-threatening infections. Shiga toxin-producing *E. coli* is of particular concern due to the severity of infections and the frequent outbreaks associated with contaminated food sources, such as ready-to-eat salads. Rapid and accurate detection of pathogenic strains is crucial for effective treatment, outbreak control, and ensuring food safety. Although conventional culture-based methods offer reliable pathogen detection, their time-consuming and resource-intensive nature drive the demand for alternative approaches.

Addressing the need for rapid *E. coli* detection in ready-to-eat salads, this study presents a novel microfluidic hybrid PMMA/paper microfluidic device that integrates bacterial DNA purification and amplification, through Loop-mediated Isothermal Amplification (LAMP). The LAMP technique is highly suitable for point-of-care diagnosis given its rapidity, sensitivity, and ability to bypass the need for complex thermal cycling.

To streamline the workflow within the device, bacterial cells first undergo off-chip lysis using lysozyme and proteinase K. The released DNA is then transferred to the microfluidic device for on-chip purification via a filter paper-based column, followed by amplification using both real-time and colorimetric LAMP methods.

Evaluations using *E. coli*-spiked salad samples demonstrated the device's efficacy in extracting high-quality, LAMP-amplifiable DNA from complex food matrices. The on-chip extracted DNA quality was comparable to that obtained using a commercially available DNA extraction kit (RTP® Pathogen Kit, INVITEK Diagnostics). Real-time fluorescence LAMP yielded positive results within 25 to 55 minutes, depending on the initial *E. coli* concentration. Colorimetric LAMP, while requiring a longer incubation time (45 to 75 minutes) for accurate colour discrimination, also provided straightforward visual confirmation of *E. coli*, suitable for on-site use.

This integrated microfluidic device offers a promising platform for rapid, accurate, on-site detection of *E. coli* in food, with the potential to revolutionize bacterial diagnosis and to significantly enhance food safety and improve public health outcomes.

**Keywords:** *Escherichia coli*, microfluidics, loop-mediated isothermal amplification, point-of-care diagnostics, ready-to-eat salads

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### X11

#### RAPID AND PORTABLE PYRROLIZIDINE ALKALOID SCREENING IN OREGANO USING SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)

**Aristeidis S. Tsagkaris**<sup>(1)</sup>, **Ondrej Brabenec**<sup>(1)</sup>, **Cinzia Cafarella**<sup>(1)</sup>, **Jana Pulkrabova**<sup>(1)</sup>, **Jana Hajslova**<sup>(1)</sup>

<sup>1)</sup> *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague, Czech Republic*

*\*Corresponding author - E-mail: tsagkara@vscht.cz*

Pyrrrolizidine alkaloids (PAs) and their derived N-oxides (PANOs) are toxins naturally produced by plants when they are exposed to stress factors. Importantly, several harmful health effects have been noticed upon dietary exposure, e.g., pneumotoxic, genotoxic and carcinogenic effects. Considering PA/PANO toxic potential, maximum levels (MLs) were recently imposed by the European Commission (EC Regulation 2020/2040) including 21 compounds in certain food commodities [1]. This indicates the need to deliver analytical methods to control the presence of these compounds along the food chain. Up to date, chromatographic analysis has been the most widely used approach for PA/PANO determination in food providing excellent analytical performance and even isomer separation, in some cases. Nevertheless, instrumental analysis can be mostly performed in the lab after applying multistep sample preparation protocols. To face this challenge, we are developing a fully portable method for the rapid screening of PAs/PANOs based on surface enhanced Raman spectroscopy (SERS). The monitored spectra of senecionine, lycopsamine and europine-N-oxide were not specific (due to similarities in molecule structure), which is not a problem considering that the MLs were established for the sum of 21 compounds. Calibration curves of individual standards and mixtures were tested and the spectroscopic signals were proportional to analyte concentrations (0.39-50 µg/mL,  $r^2 > 0.95$ ). In addition, the impact of nanoparticle colloidal solutions (both gold and silver) was evaluated and proved to be significant on signal acquisition. To investigate the method applicability in food matrices, oregano was selected (ML equal to 1 mg/kg) and a rapid (less than 10 min) and fully portable sample preparation protocol was performed. Currently, we are working on improving performance characteristics with main goal to avoid false negative results. A low false negative rate (< 5%, Decision 2002/657/EC) is necessary and considering that SERS is aimed to be used as a screening tool, avoiding false negative results will be of outmost importance. Overall, SERS demonstrated unique analytical features and, according to our knowledge, this is the first effort reported to use it in PAs/PANOs screening in food.

[1] Fuente-Ballesteros A., Brabenec O., Tsagkaris A.S., Ares A.M., Hajslova J., Bernal J.: Comprehensive overview of the analytical methods for determining pyrrolizidine alkaloids and their derived oxides in foods. *Journal of Food Composition and Analysis* (2024), 125:105758.

**Keywords:** plant secondary metabolites, point-of-need, nanoparticles, vibrational spectroscopy

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### X12

#### NEXTGENMICROFLUIDICS: HIGHLY-INTEGRATED, MODULAR LAB-ON-A-CARTRIDGE DEVICES TOWARDS MULTIPLEXED CONTAMINANT/ANALYTE DETECTION FOR FOOD SAFETY AND QUALITY MONITORING

**George Tsekenis**<sup>\*(1)</sup>, **Nikitas Melios**<sup>(1)</sup>, **Joerg Nestler**<sup>(2)</sup>, **Jan Kafka**<sup>(3)</sup>, **Mirko Lohse**<sup>(4)</sup>, **Willi Weigel**<sup>(5)</sup>, **Claude Leiner**<sup>(6)</sup>, **Martin Smolka**<sup>(6)</sup>, **Pakapreud Khumwan**<sup>(7)</sup>, **Jenny Graunitz**<sup>(8)</sup>, **Anja Haase**<sup>(6)</sup>, **Jan Hesse**<sup>(6)</sup>

<sup>1)</sup> Biomedical Research Foundation of the Academy of Athens, Greece

<sup>2)</sup> BiFlow Systems GmbH, Germany

<sup>3)</sup> Inmold, Denmark

<sup>4)</sup> Micro Resist Technologies, Germany

<sup>5)</sup> Scienion, Germany

<sup>6)</sup> Joanneum Research, Austria

<sup>7)</sup> Joanneum, Austria

<sup>8)</sup> Biflow Systems GmbH, Germany

\*Corresponding author - E-mail: gtsekenis@bioacademy.gr

Current and emerging challenges to food safety, due to the presence of new toxins and rising antibiotic resistance, coupled with environmental change and shifting consumer habits and preferences have intensified the need for low-cost and reliable diagnostic tests that can be widely implemented at the Point-of-Need. In an attempt to address this demand, and within the context of the H2020 NextGenMicrofluidics project, we have focused on the development of portable devices that combine microfluidics-based cartridges made by injection-molding with structured sensor foils produced on a large scale and cost-efficiently by roll-to-roll (R2R) procedures. The modular design of the cartridges, consisting of reaction chambers and reservoirs carefully-selected from a design library, allows for a plethora of biochemical and molecular assays to be undertaken, while detection multiplexity is achieved through the utilization of probe microarrays, spotted onto appropriately-functionalized thermoplast polymer sensor foils. The latter also serve as a waveguiding element, where sensitive TIRF (total internal reflection fluorescence) readout is realized. Furthermore, liquids are moved within the cartridge and/or heated by integrated electrochemically-driven hydrogel micropumps and heating elements, both of which are made by printing nano-enabled carbon-based conductive inks that render PCB-based fluidic actuation obsolete. To showcase the capabilities of these devices in food safety and quality monitoring, multiplexed detection of antibiotics and Aflatoxin M1 in milk is demonstrated. More specifically, selective detection of oxytetracycline and AFM1 is achieved with the use of aptamers and their employment into assays developed and optimized in solution prior to their transfer onto the sensor surface and integration into a microarray format. Contaminant quantification in milk samples results in an increase in the fluorescence intensity due to the target-induced displacement of a quencher-modified strands complementary to the fluorophore-modified aptamers that are immobilized onto the sensor foil. The elegant combination of the aforementioned advances and innovations in assay development, sensor foil fabrication and biofunctionalization, cartridge design and fluidics actuation act as a paradigm shift in the development of portable biosensing platforms and will significantly aid towards securing food safety and quality in the challenging times to come.

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### X13

#### VASTLY IMPROVED LEAD TIME FOR APTASENSOR DEVELOPMENT THROUGH THE UNCONVENTIONAL USE OF CLASSICAL BIOPHYSICAL TECHNIQUES IN APTAMER SEQUENCE SELECTION, OPTIMIZATION AND ASSAY INTEGRATION

**George Tsekenis**\*<sup>(1)</sup>

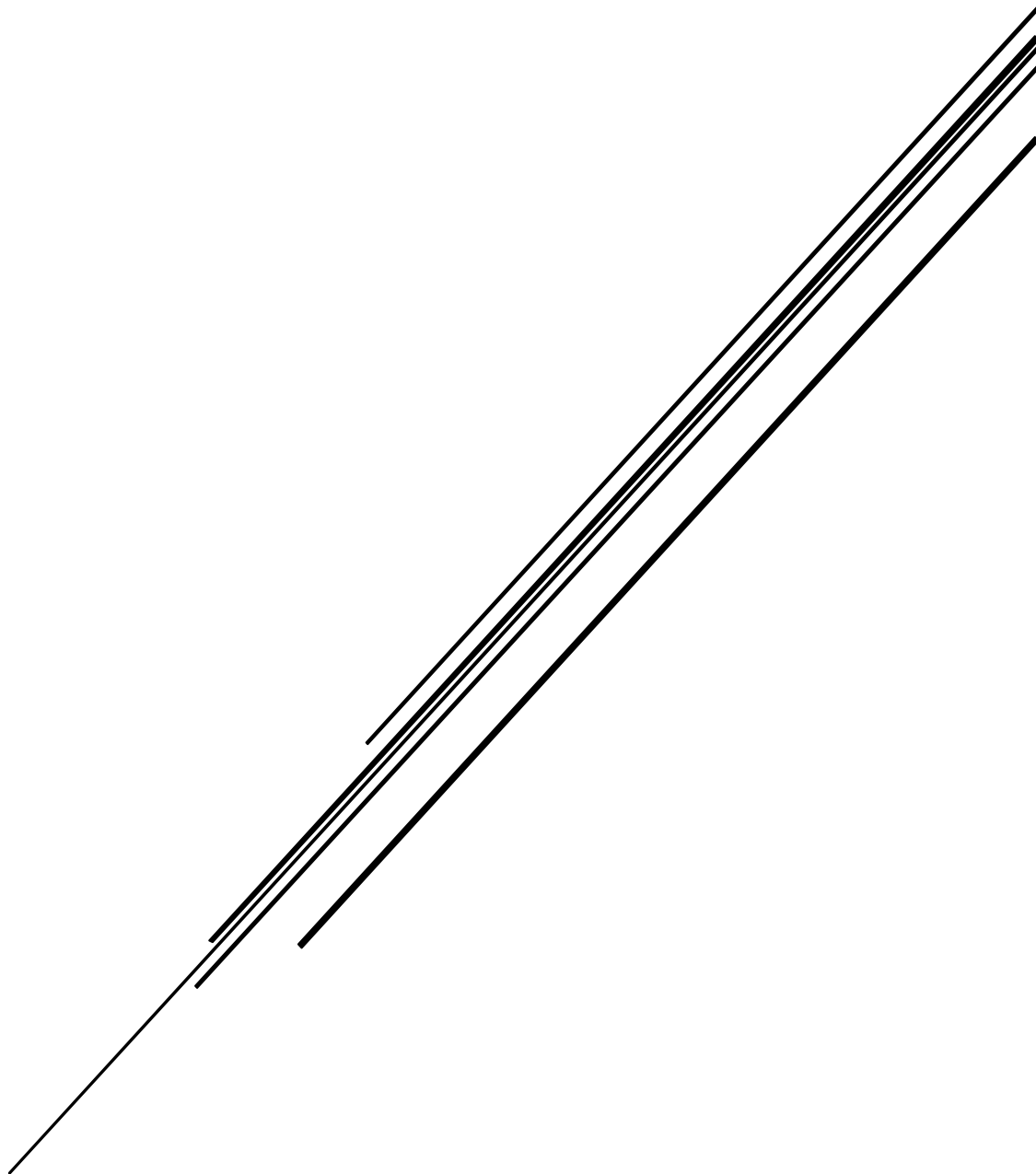
<sup>1)</sup> Biomedical Research Foundation of the Academy of Athens, Greece

\*Corresponding author - E-mail: gtsekenis@bioacademy.gr

Development of aptamer-based sensors for the detection of food contaminants invariably demands choices to be made on the aptamer sequence to be employed, which is especially true in the case of multiple published sequences being available, as well as on the need to further engineer a sequence to improve on its affinity against the target analyte. Aptamer sequence aside, one is faced with a series of dilemmas with regards to the buffering system to be employed and considerations of the best way to integrate an aptamer into an assay so that the presence of a contaminant in a sample is sensitively and selectively quantified, taking into account the signal transduction principle the sensor under development relies upon. Finding the right answer or choosing the best option for the aforementioned critical decision-making points during the time course of an aptamer-based sensing platform is no trivial task, and often requires the employment of multiple techniques and several time-consuming experimental runs to be set up. Herein, an alternative approach towards the selection and optimization of an aptamer sequence followed by its integration into a sensor-compatible assay is presented that relies on the unconventional use of classical biophysical techniques, namely UV-Vis Absorption, Circular Dichroism and Fluorescence (Polarization). More specifically, the characteristic absorbance spectrum which most of the compounds relevant to the food industry display in the UV region along with the observation that association with an aptamer results in its case-specific disappearance were exploited to evaluate the strength as well as the mode of interaction of aptamers with representative contaminants from the mycotoxin, antibiotics, and pesticide families of food contaminants. Data on absorption acquired with mole ratio and melting UV spectroscopy measurements were complemented with results obtained via circular dichroism and induced circular dichroism as well as fluorescence and fluorescence polarization spectroscopies and were collectively employed to simultaneously extract quantitative as well as qualitative information on the interacting partners. Similarly, the same techniques were employed towards the fast screening of candidate aptamer sequences' binding affinity for a target analyte, the optimization of both sample pre-treatment and analysis measurement parameters and the design and validation of strategies appropriate to the downstream integration of aptamer-based assays into sensors and complete analysis systems. Lastly, analysis of the collected spectra was made possible by the development and deployment of an advanced spectral processing algorithm capable of estimating the contribution of the aptamer to the recorded UV-Vis/CD spectra and distinguish it from that of the analyte as well as the spectral shifts due to the analyte-induced structural rearrangements of the aptamer.

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# INDEX-AUTHORS



## INDEX-AUTHORS

### A

Aalizadeh R.....	244
Abbondandolo V.....	360, 604
Abdullajeva E.....	507
Abdykarimov B.....	380
Abete M. C.....	330
Abia W.....	261
Abidar A.....	447
Abouelenein D.....	93, 339, 499
Abraham A.....	149
Abu-Al-Halaweh L.....	231
Abuhamrh T.....	453
Abuin S.....	320
Aburatani K.....	220
Aceto M.....	429
Ackerl R.....	175
Adamczyk I.....	356
Adams S.....	368, 369, 370, 387, 610
Adil N.....	443
Adiletta G.....	207
Afrasiabi M.....	652, 672
Agamy N.....	591
Ahmed Ethmane K.....	269
Aiello M. L.....	192
Aigner A. N.....	136
Ait Elkassia A.....	447
Akbar A.....	237
Akiyama H.....	638
Alajami R.....	347
Alappat J.....	360, 617
Albalawi A.....	237, 251
Albendea P.....	60, 96, 128
Albergamo A.....	414, 470
Albert O.....	175
Alborali G. L.....	205, 264
Aledda M.....	665
Alevra A.....	569
AlFayez M.....	317
Aliano-Gonzalez M. J.....	479
Alimzhanova M.....	379, 380
Aljaber A.....	647
Aljardan A.....	534
Alkhidhr M.....	240
Allan I.....	176
Allen A.....	583
Allen Lopes P.....	600
Almalki H.....	240
Almanna S.....	347
Almasoud Y.....	426
Almeida A.....	296
Almubayedh S.....	237, 251
Alnabati K.....	347
Alo G.....	286
Aloisi I.....	165, 620, 624
Alqahtani D.....	347
Alrowais F.....	240
Alsalem T.....	237, 347
Alsaqabi A.....	240
Alshehail K.....	240
Alvarez M.....	320
Alvarez-Romero M.....	626
Alzahrani S.....	647
Amador-Luna V. M.....	290
Amato A.....	418
America T.....	144
Amlund H.....	93, 344, 499, 500
Amodio M. L.....	514
Amortegui J. C. E.....	88, 624
Anastassiades M.....	87
Andelkovic V.....	412
Anderson W.....	654
Andre A.....	573
Andronache J.....	153, 155
Angeli E.....	225
Angeli L.....	407
Angerer T.....	662
Anlanger M.....	168
Anselmo A.....	242
Antignac J. P.....	72
Anumol T.....	305
Aparicio L.....	600
Aparicio-Muriana M. del M.....	152, 550
Apicella A.....	514
Apolloni S.....	427
Appiah E. A.....	301
Araujo Pereira S.....	669, 674
Ardizzone M.....	179
Arena K.....	141, 167, 197
Arlorio M.....	76, 119, 169, 291, 313
Armini V.....	292, 546
Arnaboldi S.....	222, 553
Arotçarena M.....	371, 372, 648
Arrizabalaga-Larranaga A.....	644
Artigues M.....	398
Ashraf J. Z.....	514
Austin S.....	181
Avolio R.....	330
Azokpota P.....	571
Azzollini D.....	175

### B

Bae E.....	118
Baessmann C.....	120, 238, 245, 246, 270, 349, 350, 611, 623
Baeten V.....	239, 242
Bagnulo E.....	298, 310, 311
Bahlmann A.....	561
Bajoub A.....	447
Baker-Austin C.....	547
Ballabio D.....	98
Ballesteros-Gomez A.....	209
Ballin N.....	83, 119
Balogh F.....	305
Bals S.....	588, 589
Balzani N.....	199
Bandinelli L.....	495
Bandong G.....	274
Baniya A.....	600
Bankoglu E. E.....	362
Baptista-Silva S.....	295
Barberis M.....	227
Barbero G. F.....	332, 479
Barbieri S.....	199, 447
Barbosa-Pereira L.....	134
Bard E.....	252
Barknowitz G.....	373, 517
Barók T.....	538
Barragan F.....	635
Barreto Crespo M. T.....	321, 322
Bartejsova I.....	645
Bartok T.....	566
Barton K.....	382
Basidi N.....	447
Bates T. L.....	365
Batik A.....	645
Battaglia A.....	199
Battaglia F.....	199



## INDEX-AUTHORS

Battaglia M.	319
Bauer N.	100, 593
Baune M. C.	174
Bauwens G.	60, 96, 99
Bavaro S. L.	297
Bayen S.	119, 258, 305
Bayoudh S.	371, 372, 648
Beauchamp J.	454
Beccaria M.	171, 427, 462, 466
Bechynska K.	275, 518
Becker A.	436
Becker E.	513, 564, 603
Becker R.	176, 345
Begic M.	424, 425
Begolli M.	569
Behner A.	111, 558, 575
Behnke T.	360, 509, 513, 564, 603, 617
Beij E.	137
Bellahoues O.	97
Bella-Velidou D.	548
Bellini F.	617
Belmonte-Sanchez J. R.	539
Benameur O.	394
Bendini A.	199, 447
Benedetto N.	133
Benes F.	198, 234, 401, 455
Benetti C.	655
Beneventi E.	175
Benismail N.	176
Bento da Silva A.	107
Berardi G.	80, 488
Bergaentzle M.	252
Bergamaschi L.	176
Bergkamp W.	594, 619
Bertasi B.	222, 553
Bertolassi R.	222
Bertolino M.	312, 314
Bertuola D.	363, 516
Bessiral M.	657
Bester E.	176
Bhattarai B.	507
Biagi M.	399
Bianchi F.	98, 205, 264
Bianchi M.	223
Biancotto G.	223, 262, 562
Biazik E.	293
Bicchi C.	147, 310, 311, 530
Bidmon C.	517
Biedermann M.	176
Bikaki M.	58
Bilamjian S.	305
Bilandzic N.	358, 359
Bilecka M.	661
Bilgesoy S.	436
Bin Helel B.	240
Binda G.	492
Binici B.	176
Binova Z.	198, 401, 455
Birse N.	51, 106, 119, 121, 138, 152, 158, 161, 443
Bitskinashvili K.	216, 217, 243
Blokland M.	144
Bo S.	529
Bochentyn B.	504
Bochud M.	471
Bodenbender L.	170
Bohni N.	176
Bojarowska A.	480
Bøkevoll A.	196
Bolbos A.	459
Bonciolini A.	249
Bönick J.	415
Bonnave M.	192
Bonomini M. G.	229, 230
Bontempo L.	416
Bonvin G.	653, 658
Bordiga M.	76, 313, 429
Borel P.	348
Borges S.	296
Borreani G.	528
Bortolini C.	311
Bosch S.	221
Boschello G.	604
Bosco G.	418
Bosma S.	137
Botana A.	544, 590
Botana L.	328, 544, 590, 609
Botoran O.	276, 300
Botta C.	523
Botta G.	427
Böttcher J.	564
Bottex B.	179
Bouakache S.	219
Boubetra A.	219, 255
Bourdeau M.	189
Bourgos P.	304
Bovee T.	137, 139, 646
Bovo F.	655
Brabenec O.	579, 580, 582, 675
Bradley E.	518
Bradley K.	547
Bräkling S.	309, 366, 611
Brandl W.	360
Brandsch T.	176
Brandscher T.	63, 357, 557, 612
Brandsma S.	350
Brankov M.	412
Brankovic Lazic I.	424, 425
Brasil H.	446
Braunersreuther T.	265
Breiner B.	666
Breitling-Utzmann C.	100, 593
Breitruck K.	420
Brigante F.	416
Brito E.	107
Brockmeyer J.	55
Brockmeyer J.	119
Brodkorb A.	143
Broer W.	625
Broeren M.	644
Bronze M. R.	107, 599, 600
Bruehl L.	176
Bruggeman C.	83
Brüggemann D.	265
Bruhn T.	362
Bruni R.	78, 390, 399
Brunkhorst J.	52
Bruno F.	528
Bruschweiler B.	471
Bryła M.	578
Bucek J.	283
Bucur D.	572
Buczowski A.	513, 603
Bueno C.	554
Buettner A.	615
Bugey A.	515
Buhrman M.	274
Buller R.	573
Buono A.	297
Bürki-Thurnherr T.	190
Burkon A.	584
Bursic V.	585, 586
Burt O.	368

## INDEX-AUTHORS

Bustos Gaspar F.....	321, 322
Bustos J.....	134
Butt C.....	353, 556

### C

Caballero-Casero N.....	166, 209, 325, 508
Cabrices O.....	634
Caccia N.....	523
Cacciola F.....	141
Cafarella C.....	197, 675
Cahova M.....	455
Calcinai L.....	156
Calderon J.....	320, 398, 651
Caligiani A.....	229, 230, 391
Calopek B.....	358, 359
Calucci V.....	133
Calvillo J. L.....	635
Câmara Costa V.....	494
Cambria A.....	419
Camel V.....	206
Cameron H.....	490, 583
Campaniello M.....	488
Campas M.....	668
Campmajo G.....	78, 390, 399, 526, 531, 549
Campos R.....	674
Cangini M.....	553
Cano-Sancho G.....	475
Cao A.....	328, 590, 609
Cao C.....	352
Cappellin L.....	383
Caprai E.....	565
Capraro E.....	183
Caputo L.....	527
Caratti A.....	147, 210, 427, 528, 529, 530
Carbonell-Rozas L.....	65, 165, 539
Cardenia V.....	249, 312
Cardini J.....	191
Caredda M.....	150
Careri M.....	98, 205, 264
Carini C.....	169, 291
Cariou R.....	134, 355
Carl P.....	537
Carmo C.....	599
Carmona-Molero R.....	550
Carnaroglio D.....	427
Carra F.....	169, 313
Carrasco J.....	360, 617
Carrera C.....	421, 479
Carvalho Leonardo I.....	321, 322
Carvalho M.....	436, 614
Casadei E.....	199
Casale M.....	80
Casarotto M.....	360
Casetta C.....	530
Cassap M.....	62, 501, 502
Castello M.....	398, 651
Castineira-Landeira A.....	644
Castro D.....	113
Castro-Puyana M.....	287, 288, 289, 290
Catalan V.....	456
Catania S.....	203
Cattapan F.....	360
Cattellani D.....	536
Cavallero M.....	541
Cavallo C.....	247, 418
Cavazza A.....	205
Cavazzini A.....	462
Cavlovic K.....	335, 641
Cazzaro S.....	604
Cecchi L.....	313

Ceroni S.....	203
Cetin Karasu M.....	440, 441
Cevoli C.....	199
Ceznerova E.....	56, 309, 366, 611
Chagnon M. C.....	189
Chahal S.....	119, 305
Chailleux A.....	329
Chakraborty A.....	360
Chan J.....	333
Chan S. H.....	142, 411
Chandelier A.....	570
Charvatova M.....	645
Chene R.....	371
Chetschik I.....	311, 573
Chew Y. F.....	411
Cheyns K.....	176, 495
Chiappinelli A.....	488
Chilaka C. A.....	138
Chin Y. S.....	333
Chinchilla N.....	479
Choi E.....	422
Christophe O.....	307
Chrpova J.....	558
Chullipallyalil K.....	382
Chung T.....	162
Ciano S.....	589
Cichna-Markl M.....	136, 153, 155
Cifte N. E.....	233
Cifuentes A.....	294
Cimetiere N.....	410
Cimini A.....	524
Cimino N.....	53
Cincera I.....	530
Cinta-Pinzarú S.....	117
Ciriaco L.....	292, 546
Cirlini M.....	390, 526, 531
Ciucure C. T.....	300
Ciulu M.....	150
Cladiere M.....	206
Clayton-Cuch D.....	93, 344, 500
Clement F.....	97
Cochran R.....	384
Cocolin L.....	529
Coisson J. D.....	76, 291, 429
Cojocariu C.....	386
Colombo R.....	227, 279
Connolly P.....	557, 612
Conrad A.....	568
Consonni V.....	98
Conte E.....	604
Conway C.....	283
Cordero C.....	147, 171, 210, 298, 311, 427, 466, 528, 529, 530
Corradini M. G.....	119
Corsico L.....	673
Cortejade A.....	371
Costa-Ribeiro A.....	669, 674
Costinel D.....	276
Cottet Fontannaz C.....	405
Couedor P.....	656
Couto Oliveira H. J.....	429
Couvreur B.....	192
Cozar B.....	117
Cramer B.....	578
Cravotto G. C.....	291
Crew A.....	670
Cristea G.....	151
Cruz I.....	295
Cseko G.....	566
Cubadda F.....	588
Cucco G.....	314

## INDEX-AUTHORS

Cuthbertson D. ....	305
Cutillas V. ....	637
Cutroneo S. ....	156, 174, 532
Cvetnic L. ....	358
Czarnowska-Kujawska M. ....	444, 458, 483, 484, 485
Czeh A. ....	566, 567
Czerwenka C. ....	505
Czmorik Ludvickova E. ....	631

### D

D'Amico S. ....	281, 282
D'ambra K. ....	230
D'Auria G. ....	143
Dahiot S. ....	360, 604
Dalipi R. ....	80
Dall'Asta C. ....	75, 78, 156, 174, 390, 399, 469, 526, 531, 532, 536, 541, 542, 549
Dalvit C. ....	223
Damaskinos C. ....	277
Danaher M. ....	572
Dante M. ....	604
Darge S. ....	318
Darwish A. ....	591
Dasenaki M. ....	244, 623
Datukishvili N. ....	216, 217, 243
David M. ....	117, 151
Davies G. ....	632
Dayon L. ....	271
De Angelis E. ....	85, 172, 409, 527
De Back R. ....	218
De Bellis F. ....	527
de Boer J. ....	187
De Boevre M. ....	540
De Castro Alves L. ....	328, 609
De Clercq E. ....	192
De Dominicis E. ....	360, 604, 617
De Jong A. ....	144
De Keersmaecker S. ....	280
De Leoz L. ....	305
De Llanos R. ....	602
De Loose M. ....	214, 215, 221
De Pablo A. ....	456
De Saeger S. ....	540
De Sesmaisons Lecarre A. ....	175
De Vries E. ....	552
Debetencourt H. ....	77
Debode F. ....	215
DeBord D. ....	377
Decruyenaere V. ....	460
Degerman M. ....	662
Dehareng F. ....	307
Dehelean A. ....	151
DeJager L. ....	183
Del Olmo-Iruela M. ....	550
Del Vecchio L. ....	526
Delgado-Povedano M. del M. ....	193
Dellaflora L. ....	548
Demaegdt H. ....	589
Deng R. ....	360
Dervilly G. ....	72, 123, 126, 189, 193, 341, 355
Desbrow C. ....	632
Descamps B. ....	405
Devakishen M. ....	370
Dhanji-Rapkova M. ....	547
Di Bella G. ....	247, 394, 414, 419, 470, 481, 597
di Marco Pisciotano I. ....	551
Di Mario M. ....	99, 510
Di Rocco M. ....	572
Diallo T. ....	204
Diamantidou D. ....	598

Díaz Avello U. G. ....	668
Diaz J. ....	398
Diaz T. ....	176
Diaz-Diaz R. ....	272
Dionis S. M. ....	272
Dirks C. ....	139
Disca V. ....	169, 198, 291, 313, 429
Djinovic-Stojanovic J. ....	423, 424, 425
Djordjevic V. ....	423, 424, 425
Dobrovolny S. ....	136, 153, 155
Dokic M. ....	358, 359
Dolezal M. ....	392, 434
Dolezalova T. ....	111, 563, 575, 576, 627
Domig K. J. ....	136
Dominguez-Rodriguez G. ....	287, 290, 294
Donini L. M. ....	524
Donnelly C. ....	489, 490, 583, 584
Doran K. N. ....	396
Doran O. ....	670
Douny C. ....	571
Dowd S. ....	387
Downs M. ....	184
Dowsett G. ....	654
Drabova L. ....	486, 487, 498
Drakopoulou S. ....	120, 270, 433, 623
Dreolin N. ....	368, 536
Drigkopoulou I. ....	304
Drole J. ....	109
Du W. X. ....	340
Duarte Marques I. ....	608
Dubois L. ....	337, 463
Dubois M. ....	84, 215, 271
Dudek-Salisbury W. ....	413
Duedahl-Olesen L. ....	93, 339, 507, 595
Dugo P. ....	141, 167, 197
Dusek J. ....	487
Dutta A. ....	406
Dvorakova D. ....	188, 334, 472, 628
Dzuman Z. ....	111, 188, 334, 558, 574, 575, 576, 580, 582, 631

### E

Easter E. ....	52
Eckardt M. ....	520, 521
Edder P. ....	82, 269, 515, 658
Edgington A. ....	632
Eggermont D. ....	438
Ehrat M. ....	58
Eigenmann P. ....	82
El Amraoui Aarab C. ....	192
El Hajj O. ....	383
El Hawari K. ....	640
El Khallabi H. ....	373
El Morabit. M. ....	460
Eljarrat E. ....	506
El-Khatib A. ....	561
Elliott C. 106, 119, 121, 138, 152, 159, 161, 261, 443, 555	
Ermas A. H. ....	251
Engel E. ....	102, 348, 523
English C. ....	413
Englund P. ....	662
Eppe G. ....	349
Erdely V. ....	336
Eseller K. ....	118
Espada Bellido E. ....	332, 421, 479, 496
Espourteille F. ....	365
Esslinger S. ....	318, 415
Estines L. ....	499
Eugster P. ....	397
Evangelatos S. ....	304

## INDEX-AUTHORS

Ewers M.....97  
Eylenbosch D.....570

### F

F. McGrath T.....106  
Fabrile M. P.....205, 264  
Facchinetti G.....367  
Fæste C.....83  
Faggio C.....470  
Fakih A.....102, 523  
Falappa S.....203  
Faldyna M.....645  
Fanesi B.....241  
Farah S.....206  
Faraon F.....360, 604  
Fasan S.....604  
Fauhl-Hassek C.....115, 259  
Faux A. M.....570  
Fedrizzi G.....565  
Felsmann D.....266  
Femenias A.....665  
Ferguson L.....384  
Fernandez Barbero G.....421  
Fernandez Dumont A.....81, 175  
Fernandez N.....599, 600  
Fernandez Pierna J. A.....239  
Fernandez-Alba A. R.....86  
Fernandez-Arribas J.....506  
Fernandez-Barbero G.....496  
Ferranti P.....143  
Ferrara D.....171, 427, 466  
Ferrari L.....562  
Ferreira A.....599  
Ferreira H.....321, 322  
Ferrentino G.....407  
Ferrero F.....528  
Ferri I.....207  
Ferrocino I.....529  
Fessler B.....338  
Feuerstein M. L.....190  
Feyissa A. H.....595  
Fico M.....634  
Fifeik M.....89  
Figueroa Sn. M. C.....346  
Filatova M.....315, 401  
Filipovic M.....412  
Fillatre Y.....625, 653  
Fina A.....147, 210, 427, 528, 530  
Fischer L.....79  
Focant J. F.....99  
Foddy H.....386  
Fomina P.....665  
Foulos D.....225  
Fragniere Rime C.....471  
Fraiture M. A.....215, 280  
Frank N.....397  
Freimüller Leischfeld S.....573  
Freitag S.....110, 168, 665  
Freitas A.....663  
Frera M.....617  
Frey R.....525  
Freyova M.....129  
Fromberg A.....92, 598, 605  
Fuchs J.....260  
Fuchsbauer N.....102  
Fumagalli F.....588  
Fundikira S.....540  
Füreder J.....190  
Furey A.....382  
Furey S.....253

Fusari P.....286

### G

Gabler A. M.....415  
Gadaj A.....650  
Gaddiseur R.....221  
Gagnebin Y.....658  
Gago Martínez A.....113  
Gajda A.....659, 660, 661  
Galamini C.....314  
Galaverna G.....526, 541  
Gallardo-Ramos J. A.....475  
Gallego L.....456  
Gallego L. M.....456  
Gallina A.....562  
Gallina Toschi T.....199, 447  
Gamiz-Gracia L.....475, 626  
Ganci A.....64, 384, 385, 614  
Garcia M. A.....508  
Garcia M. C.....287, 288, 289, 464, 465  
Garcia Ruiz E.....175, 228  
Garcia-Campana A. M.....193, 475, 550, 626  
Garcia-Cansino L.....508  
Garnier J. F.....257, 384, 385, 614  
Garrido F.....86  
Garrido Frenich A.....165, 539, 626  
Garrido-Maestu A.....669  
Garzoli S.....207  
Gasco A.....134  
Gaudin V.....657  
Gauriat B.....384, 385, 614  
Gautier S.....649  
Gavage M.....214, 221, 666  
Gazdag Z.....566  
Gazza L.....541  
Gbylik-Sikorska M.....659  
Geana E. I.....300  
Geballa-Koukoula A.....646  
Gebauer J.....645  
Gelbmann W.....175  
Gembal M.....660  
Genova G.....530  
Gensberger-Reigl S.....146, 154  
Genualdi S.....183  
Gerardi C.....297  
Germain C.....372  
Germini A.....81, 175, 228  
Gerssen A.....139  
Geschwender D.....147, 210  
Ghadri M.....335  
Ghidini R.....248  
Ghidini S.....205, 248, 264  
Ghirardello D.....312, 319  
Giaccone D.....528  
Giacobbe S.....481  
Gialouris P. L.....244, 263, 433, 439  
Gibson A.....183  
Gikas E.....270  
Gillard N.....84, 135, 214, 215, 218, 221  
Gillerat F.....515  
Giomo A.....199  
Giordano M.....312, 319  
Giovando S.....319  
Giovannozzi A. M.....286  
Giovinazzo G.....297  
Giraldez J.....113  
Giusti A. M.....524  
Gjoni H.....399  
Glicerina V.....249  
Glomb M.....259

## INDEX-AUTHORS

Glymenaki M. ....	175, 228
Gobbo A. ....	280
Godin B. ....	570
Godri D. ....	377
Gofflot S. ....	570, 571
Goitre I. ....	529
Gokcen T. ....	176
Gomersall V. ....	487
Gomez Ruiz J. A. ....	327
Gomez-Mejia E. ....	289
Gonçalves F. L. ....	599
Gonçalves L. ....	107
Gonzalez P. ....	360
Goran G. V. ....	299
Gordon D. ....	369
Gorse A. ....	219, 255
Gorska A. ....	60, 96
Gosciny S. ....	99, 195
Gotsens S. ....	398
Gould D. ....	610
Gouley J. ....	372
Gozzi M. ....	78, 390, 541
Gramblicka T. ....	472, 473
Granitzer S. ....	190
Grassi G. ....	198
Graunitz J. ....	676
Grausgruber H. ....	168
Gravador R. ....	555
Grcic N. ....	412
Greer B. ....	555
Grelik A. ....	660
Grembecka M. ....	480, 504
Greunwald M. ....	363
Grffin A. ....	374
Grgic D. ....	548
Grieco P. ....	292
Griffin A. ....	183
Griffiths M. ....	396
Grigoletto I. ....	199
Gritti E. ....	604
Groschke M. ....	176
Gru Y. ....	410
Grudlewska-Buda K. ....	356
Grüning A. ....	388
Gruszecka D. ....	637
Grzetic Martens J. ....	91, 551, 552
Guardiola F. ....	239
Gude T. ....	186
Guerin T. ....	204
Guerrini F. ....	553
Guglielmetti A. ....	311
Guichard P. ....	640, 649
Guignard C. ....	307
Gulia G. ....	268
Gundacker C. ....	190
Guner San S. ....	441
Gutmann R. ....	408
Guyon F. ....	117
<b>H</b>	
Haar M. ....	339
Haase And. ....	588
Haase Anj. ....	676
Haase H. ....	543
Habedank F. ....	63
Hajslova J. ....	60, 73, 101, 103, 111, 133, 197, 198, 202, 234, 273, 275, 315, 401, 432, 445, 452, 455, 518, 563, 574, 579, 580, 582, 627, 629, 630, 631, 636, 675
Hakme E. ....	88, 507, 595, 616
Hamed I. ....	149
Hammel Y. A. ....	97
Han J. ....	60, 627
Hancock P. ....	610, 622
Hann S. ....	79, 119
Hanner R. ....	119
Hansen K. ....	402
Hanusch J. ....	176
Harasztia M. ....	567
Harden S. ....	400
Harris M. ....	231
Hart J. ....	670
Hategan A. R. ....	117, 151
Haughey S. ....	161, 261, 443, 555
Hauns J. ....	90
Haupt M. ....	176
Hauschildt C. ....	176, 345
Haveron N. ....	652
Haverson D. ....	547
Hayes C. ....	632
He X. ....	340
Heath D. ....	109
Heath E. ....	109
Hecht K. ....	573
Heckle S. ....	231
Hedou C. ....	657
Heinzen H. ....	116
Held A. ....	605
Hellwig M. ....	104, 235
Helm I. ....	176
Hemmerle L. ....	397
Henquet M. ....	673
Henrottin J. ....	84
Hentschel A. ....	617
Hepner J. ....	413
Hernandez Olivas E. ....	143
Hernandez-Mesa M. ....	193, 475, 626
Herter S. O. ....	543
Hesse J. ....	676
Hettwer K. ....	284
Hilkert A. ....	267
Hill S. ....	176
Himmelsbach M. ....	194
Hiramatsu Y. ....	637
Hird S. ....	622
Ho J. ....	370
Ho N. H. ....	515
Hochegger A. ....	511
Hochegger R. ....	136, 153, 155
Hodkovicova N. ....	645
Hoek E. ....	354
Hollebrands B. ....	173
Holst Laursen K. ....	119
Holzapfel J. ....	520
Hong Y. ....	121, 152
Hood-Nowotny R. ....	79
Hopley C. ....	176
Horemans B. ....	602
Horvath A. K. ....	566
Horvath L. ....	538
Houbraken J. ....	551
Houdkova M. ....	406
Hoyer C. ....	545, 561
Hradecka B. ....	101, 103, 445
Hrbek V. ....	73, 103, 275, 392, 518, 631
Hren S. ....	176
Hudobivnik M. ....	109
Huet A. C. ....	84, 214, 218, 221
Humpf H. U. ....	578
Hurtaud-Pessel D. ....	329, 640, 649, 656
Hutterli M. ....	383
Hutzler C. ....	561

## INDEX-AUTHORS

Hwang H. J. .... 442

### I

Iammarino M. .... 80, 488  
Ianieri A. .... 205, 248, 264  
Ibanez E. .... 290, 294  
Ichikawa C. .... 637  
Idialu O. .... 622  
Iko Afe O. H. .... 571  
Ilhan E. .... 441  
Iliopoulou S. .... 569  
Incarnato L. .... 514  
Ingendoh A. .... 309, 349, 366, 611  
Ionete R. E. .... 276, 300  
Iordache A. M. .... 300  
Ipatov A. .... 667, 674  
Islam M. .... 116, 149  
Izzo L. .... 292, 546

### J

J. Sloth J. .... 93, 191, 339  
Jablonski A. .... 659  
Jaegerova T. .... 133, 452  
Jähne M. .... 284  
Jahnke A. .... 430, 431  
Jamin E. .... 77  
Jan P. .... 658  
Jandric Z. .... 79  
Jankovic S. .... 424, 425  
Janssen H. G. .... 74, 173  
Jaouhari Y. .... 313  
Jaremko M. .... 251  
Jarosova L. .... 645  
Jasinska-Melon E. .... 450, 467, 476, 606  
Jaus A. .... 420, 471  
Jeannot C. .... 123, 341  
Jedziniak P. .... 660  
Jellema T. .... 552  
Jira W. .... 265  
Jiros P. .... 463  
John C. .... 335, 404, 560, 641  
Joly L. .... 192  
Jonard C. .... 570, 571  
Jones E. .... 353  
Jones N. .... 337, 463  
Jongsma M. .... 673  
Jorga S. .... 383  
Jotanovic A. .... 176  
Juarez V. C. .... 616  
Jucewicz K. .... 504  
Juchelka D. .... 267  
Jung C. .... 345  
Jung E. S. .... 442  
Jungen M. .... 250

### K

Kacjan Marsic N. .... 109  
Kadac-Czapska K. .... 480, 504  
Kafka J. .... 676  
Kainz D. .... 666  
Kakoulides E. .... 176  
Kaliszczak K. .... 356  
Kalli S. .... 91, 551, 552  
Kaltner F. .... 581  
Kaminski M. .... 601  
Kane N. .... 652, 672

Kappenstein O. .... 362, 428, 512, 545  
Kapsali T. .... 225  
Karagiannidis A. .... 623  
Karanikolopoulou S. .... 263  
Karboune S. .... 258  
Karchkhadze K. .... 243  
Karoonuthaisiri N. .... 157  
Kass G. .... 175  
Kastanek P. .... 455  
Kaurinovic J. .... 358  
Kause R. .... 326, 354  
Kelly S. .... 116, 149, 490  
Kenjeric L. .... 140  
Khalefa A. .... 337  
Khan Z. .... 632  
Kharoshka A. .... 432  
Khoo J. .... 142, 411  
Khumwan P. .... 676  
Kiefer C. .... 148  
Kildea S. .... 572  
Kiljanek T. .... 660  
Kim H. W. .... 442  
Kim J. H. .... 442  
Kim S. A. .... 442  
Kimanya M. .... 540  
Kittler K. .... 89  
Kjellmann L. .... 344  
Klampfl C. W. .... 194  
Klee S. .... 309, 366, 611  
Klein L. M. .... 581  
Klepacka J. .... 444, 458, 483, 484, 485  
Klusmann L. .... 430  
Kobayash M. .... 638  
Koberidze T. .... 216, 217  
Koch E. .... 362  
Koch M. .... 513, 543, 603  
Kocourek V. .... 630, 631, 636  
Koelme J. .... 377  
Kohler A. .... 665  
Kokkinofa R. .... 277  
Kokolakis S. .... 623  
Kokoska L. .... 406  
Kolamunna A. T. .... 253  
Kolovou M. A. .... 375  
Kolrep F. .... 428  
Kondjoyan N. .... 102, 523  
Konetzki J. .... 509, 564  
Kong P. W. R. .... 643  
Konstantinovci B. .... 585  
Koops A. .... 83, 213  
Koplik R. .... 498  
Kopp M. .... 584  
Koricnac V. .... 423  
Körner X. A. .... 428  
Kornfeind S. .... 79  
Kosek V. .... 73, 105, 275, 518  
Koss A. .... 383  
Kostakis M. G. .... 186  
Koster S. .... 97  
Kosubova P. .... 633  
Kouloura E. .... 175  
Koupa A. .... 433  
Kourimsky T. .... 101, 103  
Koutroumpi S. .... 478  
Kovacic A. .... 109  
Kowalczyk E. .... 660  
Kraft V. .... 323  
Krätschmer K. .... 364, 594  
Krenz O. .... 260  
Kriek W. .... 617  
Kristalli E. .... 102, 176

## INDEX-AUTHORS

Kritikou A.	263
Krnjaja O.	424
Krska R.	110, 114, 140, 159, 168, 559, 665
Krüger B.	235
Krygier B.	450, 467
Krystalli E.	478
Kuah K. X.	370
Kuckova S.	145
Kuhlbusch N.	267
Kuklya A.	362
Kulabhusan P. K.	667
Kulas S.	560
Kulik A.	293
Kupfer A.	146
Kurata T.	220, 224
Kurbis C.	430, 431
Kusznierowicz B.	482
Kutasy E.	301
Kutateladze T.	216, 217, 243
Kutscher D.	268, 364, 501
Kwiatek K.	660
Kwon R. H.	442
Kyselka J.	103, 234

## L

La Tella R.	167
Laczko A. O.	567
Ladak A.	268, 364
Ladreyt A.	381
Lagana Vinci R.	141, 167
Laganaro M.	81, 175, 228
Lakatos I.	567
Lalor F.	253
Lam Y. Y. N.	162
Lamas A.	669
Lambertini F.	85
Lamonaca A.	172, 409, 527
Lamoree M.	71
Lamote A.	218
Landova P.	633
Landsteiner N.	641
Langasco I.	150
Langianese M.	488
Laporda S.	569
Lappas P.	304
Lara F. J.	550
Latado H.	271, 405
Lau T. L. T.	162
Laurentie M.	640
Lazdiņa L.	127, 331
Le Bizec B.	72, 123, 126, 189, 193, 341, 355
Le Roux E.	206
Leao J. M.	113
Leblois J.	307
Ledenko I.	292, 546
Ledent M.	192
Lee D.	232
Lee G.	435
Lee H.	52, 353, 556
Lee K. G.	200, 232, 308, 422, 435
Lee K. S.	442
Lee L. M.	142, 411
Lee W.	308
Leeman D.	583
Lehmann L.	260
Leifels T.	336
Leiner C.	676
Leite M.	663
Leitner E.	58, 95, 511
Leito I.	176

Lela L.	133
Lemmink I.	137
Leonards P.	350
Leprovost J.	255
Lestido-Cardama A.	134
Leurs F.	144
Leva L.	645
Li B.	411
Li G.	164
Li H.	142, 411
Li Q.	266, 267
Li Y.	106, 161
Libenska L.	486, 487
Liberto E.	210, 298, 310, 311, 314, 427, 528, 529, 530
Licht Cederberg T.	176
Lim H. Y.	411
Lim I.	333
Lindekamp N.	393, 596
Liotta L.	247, 418
Lioupi A.	387
Lippl M.	168
Litrenta F.	247, 418, 481, 597
Liu B.	338
Liu H.	176
Liu J.	164
Liu L.	258
Liu X.	352
Liwara D.	350
Lliberia J. L.	398
Lo Fiego D. P.	230
Lo Turco V.	247, 394, 414, 419, 481, 597
Lobsiger S.	420
Locatelli M.	76, 291, 298
Lodder H.	632
Loepz Flores J.	618
Loeschner K.	339
Logan N.	106, 161, 352, 443
Lohse M.	676
Lointier M.	116, 252
Lolli V.	391
Lombardi S.	292, 546
Lombroni C.	407
Lommatzsch M.	130, 520
Lommen A.	619
Lopez A. R.	332, 479, 492
Lopez C.	590
Lopez Castillo J. G.	332
Lopez D.	382, 557, 612
Lopez J.	349, 351, 611
Lopreiato V.	418
Loredana Tarraran L.	297
Löschner K.	93, 191
Losio M. N.	222
Losso K.	245
Louckova A.	197, 234
Louka S.	277
Lourenço S.	600
Louzao M. C.	328, 609
Loyola-Sepulveda R.	346
Łubiech K.	482
Lucci P.	241
Luch A.	362, 512
Lücken J.	641
Luparelli A.	85, 172, 409, 527
Lüth A.	430, 431
Lynch P.	59

## M

MacArthur R.	654
MacDonald S.	583

## INDEX-AUTHORS

Macorps N. ....	126	McLachlan N. ....	489, 584
Madawala S. ....	662	McManus B. ....	231, 338
Madikizela L. ....	342, 343	McMillan D. ....	374, 625
Madode E. Y. ....	571	Mdimagh A. ....	258
Maenz J. S. ....	335, 641	Mecha E. ....	107
Maeshima N. ....	638	Mechtler K. ....	168
Maestroni B. ....	149	Medugno M. ....	264
Maffezzoni C. ....	98	Meijer M. ....	551
Magani M. ....	175	Meikopoulos T. ....	642
Magdas D. A. ....	117, 151	Meledina A. ....	145
Maggiore A. ....	179	Melios N. ....	676
Maglione B. ....	292	Menasco D. ....	632
Mahlik S. ....	504	Mencin M. ....	457
Mailleux M. ....	84	Mendes V. ....	175, 228
Maino M. L. ....	449	Mendoza A. ....	346
Majer H. ....	612	Menegon V. ....	604
Makkai E. ....	566	Mentana A. ....	488
Makni Y. ....	204	Meras V. ....	569
Malachova A. ....	114, 140	Mercier F. ....	102, 523
Malegori C. ....	80	Merkel S. ....	512
Mallia S. ....	420	Mert B. ....	440
Maly M. ....	198, 401, 455	Mertin N. ....	431
Malz A. ....	323	Miedico O. ....	80
Mannaing E. ....	489, 583	Miescher Schwenninger S. ....	573
Mannina L. ....	207, 294, 524	Mihailova A. ....	116, 119, 149
Manning E. ....	584	Mihalache O. ....	75, 156, 174, 469, 542
Mansy F. ....	218	Mihalache O. ....	131
Mänz J. S. ....	404	Mikołajczyk S. ....	660
Manzinello C. ....	254	Mikulic Petkovsek M. ....	457
Mara A. ....	150, 241	Milbert S. ....	260
Maragou N. ....	623	Milella L. ....	133
Marc R. ....	448, 449, 459	Miller Branco Ferraz F. ....	446
Marchal K. ....	280	Milligan C. ....	489, 490, 583
Marega R. ....	666	Mimrova K. ....	432
Marian (Toca) G. ....	256	Minelli G. ....	230
Marilleau E. ....	410	Mingione S. ....	199
Marina M. L. ....	287, 288, 289, 290, 464, 465, 508	Minkoumba Sonfack G. ....	565
Marincas M. O. ....	448	Miricioiu M. G. ....	276, 300
Marin-Kuan M. ....	185, 271, 405	Mischler S. ....	573
Marin-Saez J. ....	475, 626	Mizaikoff B. ....	665
Marki L. ....	420	Mizak N. ....	661
Marko D. ....	548, 568	Mladenovic Drinic S. ....	412
Martin D. ....	376	Moche H. ....	189
Martin L. ....	176	Mojska H. ....	450, 467, 476, 606
Martin Ortiz D. ....	618	Mokhtari N. ....	447
Martin R. ....	351	Mol H. ....	91, 131, 165, 608, 619, 620, 624
Martin-Alberca C. ....	602	Moles L. ....	372
Martinello M. ....	621	Moliterni V. M. C. ....	526
Martinez M. M. ....	514	Monaci L. ....	55, 85, 172, 409, 527
Martinez-Urtaza J. ....	547	Mondello L. ....	141, 167, 197
Martingo M. ....	295	Montalvo D. ....	495
Martocchia M. ....	429	Montgomery H. ....	261
Martysiak-Zurowska D. ....	482	Monticelli D. ....	332, 492, 493
Masin R. ....	562	Montoya A. ....	176
Masquelier J. ....	280, 535	Moore I. ....	353, 373
Massaro A. ....	203, 238, 262	Moreau S. ....	388, 638
Mast J. ....	495, 588, 589	Morello S. ....	223
Mastovska K. ....	131, 180	Moreno T. ....	506
Maszewski S. ....	361, 660	Moresi M. ....	524
Mateos Bernal R. M. ....	421	Moretti A. ....	549
Matijevec L. ....	175	Moriceau J. ....	614
Mattarozzi M. ....	98, 205, 264	Morozova K. ....	407
Matteini P. ....	360, 617	Moskowitz M. ....	611
Mattiazzo A. ....	360	Mostoles D. ....	241
Mauracher A. ....	408	Mouly P. ....	500
Mausser A. ....	154	Mraz P. ....	629, 630, 631, 636
McAuliffe M. ....	382	Mueller H. ....	349
McCleary B. ....	181	Mühling M. ....	671
McCullagh M. ....	387	Mulder P. ....	91, 551, 552, 577
McKenzie-Coe A. ....	377	Mullen P. ....	650
McKillen C. ....	518	Müller N. ....	573



## INDEX-AUTHORS

Müller T. M.	260
Mülow-Stollin U.	89
Mumtaz W.	458, 484
Muniz-Bustamante L.	166, 325
Munoz E.	398, 651
Munoz Gonzalez A.	228
Muresan C.	448, 449, 459
Muriana M. A.	121
Mutinelli F.	254, 621
Muzyka A.	615
Myhra T.	196

### N

Na H.	442
Naels L.	257, 502
Nanou A.	478
Naraghi K.	371, 372, 648
Nardelli V.	488
Narduzzi L.	193
Narvaez A.	193
Nastou E.	244, 263, 270, 433, 439
Nava V.	414, 418, 597
Nawrocka A.	660
Ndlovu M.	360
Ndungu K.	176
Negri A.	248
Nekic T.	359
Nessler F.	189
Nestler J.	676
Ng C.	643
Ng D.	370
Nguyenova C. M.	392, 434
Nichani K.	284
Nicolas M.	371
Nicotra S. B.	239
Nieddu E.	604
Niederkofler I.	155
Niehaus E. M.	365, 366
Nielen M. W. F.	74
Niemeijer R.	52, 67
Nieschalke K.	89
Nijssen R.	326
Nikiforov V.	386
Niklas A.	93, 176, 344
Ninga E.	88, 613, 624
Ninidze T.	217, 243
Nishi T.	220, 224
Nitride C.	143, 409
Nordhorn I.	120, 238, 245, 246, 270, 350
Noriega Fernandez E.	81, 175
Noviello S.	223, 562
Novkovic S.	586
Nowacka-Kozak E.	659, 660
Ntantasios A.	225, 569
Ntemou A.	304
Ntoupis T.	569
Nuin Garciarena I.	175, 228
Nunez O.	150, 241
Nyandi M. S.	451

### O

O'Sullivan C. K.	668
Oane Z. M.	196
Obkircher M.	420
Ogrinc N.	94, 109
Oliveira E.	126
Oliveri P.	80
Olluy N.	495

Omer E.	97
Omura Lund Y.	339
Orndrova K.	633
Oppermann U.	323, 388, 519, 637
Ordornez L.	396
Organtini K.	368
Ortega Caneda E.	332, 421, 479, 496
Ortelli D.	82, 269, 515, 658
Ortiz D.	351
Orval R.	215
Osko J.	480
Ostermann A. I.	545
Oughad A.	447
Oveland E.	196
Owens A.	637
Oztop M. H.	233, 440, 441

### P

Pacetti D.	241
Padilla Gonzalez F.	326, 577
Padioleau A.	355
Pakade V.	342
Paladi A.	381
Palma Lovillo M.	332, 421, 479, 496
Palma-Manrique R. M.	288, 289
Pan D.	517
Panto S.	337, 463
Paolillo A.	292, 546
Paolillo M.	279
Papachrysostomou C.	663
Papageorgiou G.	225, 569
Papayianni E. I.	277
Papelis D.	120, 270
Papetti A.	227, 279
Papin M.	348
Papuc C.	299
Pardi F.	438
Parinet J.	123, 204, 341, 376
Parizek O.	188, 334, 472, 473, 474
Parizkova D.	334, 474, 628
Park Y. H.	442
Parry R.	632
Paseiro-Losada P.	134
Pasti L.	462
Pastorelli A.	83
Paszczyk B.	444, 483, 485
Pati S.	514
Patin A.	271, 405
Patyra E.	660
Pavon V.	320
Pavoni E.	222, 553
Pedersen M.	642
Pedrini Brandão G.	494
Pellegrini S.	367
Pellegrino G.	310
Pellicciotti S.	565
Pellicorio C.	360, 604, 617
Peloso M.	565
Peltier F.	99, 510
Penaranda E.	456
Penin A.	113
Pepo P.	451
Perez J. M.	635
Perez M. A.	349
Perinello G.	360
Perini M.	247
Perkons I.	127, 331
Perna A.	286
Petchkongkaew A.	106, 160
Petcu C. D.	299

## INDEX-AUTHORS

Petronijevic R.....	423
Petrova S. M.....	196
Pezzolesi L. ....	553
Pfister M. ....	259
Pham Y. L. ....	454
Pianezze S. ....	247
Piazza C. ....	360
Picicci I.....	549
Piergiovanni M.....	98, 205, 264
Pierret G.....	135
Pierzgalski A. ....	578
Pietron W. ....	660
Pietruk K. ....	660
Pietruszka K. ....	660
Piletta-Zanin A. ....	82
Pillai S. ....	556
Pilo M. I. ....	150
Pilolli R. ....	85, 172, 409, 527
Pineiro Y. ....	328, 609
Pintado M.....	295, 296
Pintar M. ....	109
Pinto C.....	370, 400
Pirna A. ....	117
Piro R. ....	238
Pirova H.....	101, 445
Pischetsrieder M.....	146, 154
Piscitelli A. ....	143
Pissard A. ....	242
Pistocchi R.....	553
Pittois D.....	307
Piva M. ....	604
Planche C.....	348
Platel A. ....	189
Plumb R. ....	387
Pöchlhammer M. ....	568
Pollastro F. ....	198
Pollitt K. ....	377
Polzer J. ....	89
Ponti J. ....	588
Ponzo V. ....	529
Popov M.....	585
Poquet L.....	397
Porretti M. ....	470
Portesi C.....	94, 125, 176, 286, 330
Pospisil O.....	202, 273
Pospisilova V. ....	383
Potenziani G. ....	249
Potocnik D. ....	109
Potorti A. G. ....	247, 394, 414, 419, 597
Poulsen M. E.....	88, 613, 616, 624
Poustka J. ....	392, 434
Powel D. ....	53
Powell D.....	437
PP H. ....	556
Prado M.....	667, 669
Prandi B.....	174, 229
Precup G.....	175, 228
Predescu N. C. ....	299
Preza-Mayo-Kataki D.....	244, 439
Prizio I. ....	565
Prochazkova Z. ....	498
Provopoulou E. ....	304
Prusova N.....	111, 563, 574, 575, 576
Przeniosła-Siwczyńska M.....	660
Pschierer J. ....	557, 612
Pühringer K.....	155
Pulkabova J. ....	73, 103, 129, 188, 334, 472, 473, 474, 486, 487, 628, 675
Purcaro G.....	60, 96, 99, 128, 171, 427, 438, 462, 466
Puscas R. ....	151
Puvaca N. ....	586

Puype F. ....	519
---------------	-----

## Q

Quendera A. P. ....	321, 322
Quinn B.....	152
Quintieri L.....	527

## R

R. Brandao P.....	107
Ragni L. ....	222, 553
Rainer M.....	245
Rainey C.....	650
Rainville P. ....	400
Rajan S. ....	556
Rajwa B. ....	118
Rakov D.....	337
Ramanzini E.....	447
Ramm B.....	363
Ramos M.....	206
Rampler E.....	281, 282
Randazzo C. L. ....	418
Rando R. ....	597
Rantsiou K.....	523
Rapeanu G.....	256
Raposo-García S.....	328, 609
Ratel J.....	102, 523
Rathamann C. ....	66
Rathke M.....	560
Rathmann C. ....	336, 368, 369
Rautenberg T.....	596
Recchia S. ....	492, 493
Rechidi-Sidhoum N.....	394
Redaelli M.....	360, 604
Reding R. ....	307
Rega B.....	206
Reichel M.....	335, 404, 560, 641
Reichenbach S. E.....	147, 210
Reil D.....	505
Reiter E.....	168, 281, 282
Rennie E.....	377
Rentz P. ....	116, 252
Revello Chion A. ....	528
Rey V. ....	544
Reyes Diaz D. ....	600
Riboni N.....	98, 205, 264
Richards L. ....	632
Richter A. ....	415
Riedl J. ....	259
Riemenschneider C. ....	90, 125, 176
Rigano F.....	167, 197
Righetti L.....	112, 165, 577
Rinaldi M.....	169
Riou J. ....	471
Ritchie J. ....	547
Rivas J. ....	328, 609
Robert C. ....	135
Robert S.....	360, 617
Roberts D.....	268
Robinson J. P. ....	118
Robinson W.....	654
Rodrigues A. M.....	599, 600
Rodriguez Bernaldo de Quiros A. ....	134
Rodriguez Fernandez P.....	175
Rodriguez-Santos L.....	328, 609
Roelse M.....	673
Rogeboz P.....	271, 405
Roguszewska M.....	176
Rohn S.....	148, 170, 393, 596

## INDEX-AUTHORS

Rola J. G.	660
Roldan-Torres R.	175, 228
Rolli E.	541
Rollo E.	110, 114, 536
Roloff A.	362
Romaniello F.	125, 176, 286, 297, 330
Romano E.	294
Romanotto A.	363, 402, 516
Romero A.	239
Romero-Gonzalez R.	165, 539
Romnee J. M.	460
Römpf A.	265
Roncoroni G.	492
Ronewa R.	343
Roosens N.	215, 280
Rosa A.	296
Rosell M.	239
Rosi F.	360
Ross E.	622
Rossi A.	175
Rossi A. M.	94, 176, 286, 330
Rossini C.	199
Rost H.	538
Roussef F.	255
Rovelli D.	531, 532
Rovellini P.	286
Rovira M.	239
Rubas O.	580
Rubini S.	553
Rubino M.	604
Rubio Marin C.	464, 465
Rubio S.	166, 209, 325, 508
Rufolf R.	525
Ruggeri F. S.	74
Rühmer T.	155
Ruin N.	466
Ruiz Perez S.	357
Ruiz-Lopez A.	496
Rund K.	90, 125
Russel H.	261
Russo P.	207
Ryparova Kvirencova J.	631

## S

Sachse B.	393
Säger S.	520, 521
Sahakalkan S.	666
Saito I.	638
Sakai M.	220, 224
Salamanca O. M.	346
Salcedo N.	421
Salentijn G. IJ.	137
Salomone S.	198
Samardzic N.	585
Sambblas D.	320
Samimi A.	673
Sammarco G.	360, 617
Sanchez-Visedo A.	669, 674
Sanchis V.	475
Sandjong Sayon D.	102
Sandru C.	300
Sangiorgi E.	80
Sanna G.	150, 241
Santana G.	494
Santanta-Diepa G.	272
Santimaria D.	515
Santrucek J.	145
Sao-Jose C.	321, 322
Sarvan I.	362
Sasano M.	638

Sasse S.	644, 646
Saurina J.	150, 241
Savage A.	183
Savvidou C.	277
Scali F.	205, 264
Scalisi M.	418
Scampicchio M.	407
Scantamburlo B.	360
Schaechtele A.	90, 125, 176, 364
Schäfer B.	393
Schamann A.	110, 114
Schandl S.	155
Scharmann F.	417
Scheibelreiter J.	155
Schelker R.	176
Scherer M.	374, 554, 653
Scherl A.	82, 269
Schiavone C.	125, 176, 286, 330
Schincaglia A.	462
Schittko S.	641
Schlachter S.	281, 282
Schleicher S.	560
Schlittenbauer L.	336
Schlosser J.	671
Schmidt H.	671
Schmidt R.	512
Schmiedeken L.	335
Schmitz J.	274
Schmolka B.	505
Schneider B.	350
Schneider S.	318
Schoener A.	231
Schooten F. J.	375
Schott K.	79
Schreiber A.	373, 517
Schreiter P.	335
Schulz E.	513, 603
Schulzova V.	432
Schumacher D. M.	430
Schusterova D.	627, 629, 630, 631
Schwaar T.	537
Schwarz S.	148
Schwerdtle T.	259
Scippo M. L.	571
Sebelova K.	129, 202, 273, 629
Sedak M.	358, 359
Seeber C.	559
Seele Y.	545
Sehovic D.	595
Seidel A. K.	521
Seifert S. T.	260
Sejeroe-Olsen B.	605
Sendon R.	134
Senior A.	632
Sentellas S.	150, 241
Serneels F.	192
Serrano N.	360
Servadei I.	553
Sette G.	655
Setti M.	447
Severin I.	189
Sgro B.	394, 481
Shao M.	258
Shegunova P.	605
Shen P.	333
Shi R.	333
Shi Y.	164
Shin S.	118
Siciliani L.	588, 589
Siegmund B.	132, 155
Sigl K.	190

## INDEX-AUTHORS

Sijm D. ....	375
Silveira Rodrigues R. ....	494
Simankova A. ....	498
Simat T. ....	520, 521
Simon K. ....	284
Singer F. ....	174
Singh U. ....	251
Siniscalco S. ....	59
Sinkiewicz-Darol E. ....	356, 482
Sirajo M. ....	303
Sitzmann W. ....	235
Skoupa K. ....	645
Skouridou V. ....	668
Skowron K. ....	356
Skrastiņa A. ....	127
Slade S. ....	387
Sladek Z. ....	645
Slimani K. ....	329
Smirnova T. ....	145
Smith A. ....	228
Smith E. ....	59
Smith T. ....	632
Smits N. ....	83, 144, 213
Smolka M. ....	676
Socolsky C. ....	360
Söderlund S. ....	662
Sogbossi Gbetokpanou C. ....	571
Soldesti R. ....	248
Soler A. ....	239
Soler S. ....	320
Solomun Kolanovic B. ....	358, 359
Solovyev P. ....	416
Sölter S. ....	560
Sommerfeld F. ....	415
Sonntag K. ....	202
Sopel M. ....	91
Soto-Rosina P. ....	539
Soultani G. ....	433
Spanò I. M. ....	419, 470
Spano M. ....	207, 294, 524
Spano N. ....	150
Spanu D. ....	492, 493
Spieker M. ....	431
Spirovic-Trifunovic B. ....	586
Sprecher H. ....	420
Squance M. ....	652, 672
Squara S. ....	210
Sram R. J. ....	487
Srdic J. ....	412
Stahl-Zeng J. ....	373, 374, 517, 554, 625, 653
Stambula I. ....	602
Stara L. ....	445
Stassi J. ....	274
Stastny K. ....	645
Stefanelli M. ....	634
Stefanovic S. ....	423, 425
Stefanuto P. H. ....	99
Stehnova K. ....	275, 628
Steinberg J. ....	52
Steiner D. ....	538
Steininger-Mairinger T. ....	119
Stelben P. ....	377
Steliopoulos P. ....	417
Stell A. ....	338
Stella R. ....	223, 262, 562
Stenzel A. ....	454
Stephany G. ....	655
Sterk S. ....	644
Stevens D. ....	610
Stevens F. ....	460
Steuerink M. ....	602
Stoebener N. ....	266
Stojanovic T. ....	585
Stolper O. ....	635
Stow S. ....	377
Straka D. ....	145
Stranska M. ....	111, 455, 558, 563, 574, 575, 576
Stratakos A. C. ....	396
Strathmann F. ....	377, 634
Stratton J. ....	583
Straub L. ....	577
Strnad I. ....	281, 282
Strocchi G. ....	310
Strojniak L. ....	109
Strouthou M. ....	663
Strzelec M. ....	176
Stupak M. ....	60, 73, 131, 315, 401, 415
Stützer J. ....	146
Su Z. ....	164
Suarez-Torres R. S. ....	272
Suaudeau L. ....	649
Sudwischer P. ....	235
Suleiman R. ....	540
Sulyok M. ....	140, 538, 559, 665
Sulzer P. ....	408
Suman M. ....	69, 110, 114, 119, 238, 536
Sunkara M. ....	183
Surekar B. ....	501
Suriano F. ....	175
Svobodova V. ....	188, 334, 472
Sy P. ....	430
Sykorova H. ....	452
Syrgabek Y. ....	379
Szekeres A. ....	538
Szemethy L. R. ....	567
Szörenyi T. ....	567

## T

Tadjine F. ....	89
Taillandier J. F. ....	640
Takahashi M. ....	224
Tam C. ....	340
Tanaka D. ....	220
Tao Q. ....	147, 210
Tapparo G. ....	314
Taracena-Zepeda O. R. ....	496
Tarapoulouzi M. ....	277
Tas O. ....	441
Tata A. ....	203, 238, 262
Taverne W. ....	178
Taverniers I. ....	221
Tedeschi T. ....	156, 174, 532
Teixeira Alves M. ....	547
Teixeira Pinto de Castro M. L. ....	296
Teo W. Y. D. ....	411
Terjung N. ....	174
Termopoli V. ....	98
Terpinc P. ....	457
Tesfamariam K. ....	540
Thalhamer B. ....	194
Theodoridis G. ....	387
Theurillat X. ....	124, 374
Thibert V. ....	64, 384, 385, 436, 614
Thomaidis N. ....	120, 244, 263, 270, 433, 439, 623
Thomas D. ....	614
Thomas F. ....	77
Thomopoulos R. ....	102
Tian L. ....	119, 305
Tienstra M. ....	620, 624
Tietz T. ....	362, 512
Tigyi P. D. ....	567

## INDEX-AUTHORS

Tilocca F.....	150
Tirard A.....	219, 255
Titera D.....	580
Toal K.....	672
Toffoletto S.....	604
Tomaiuolo M.....	80
Tomaniova M.....	518
Tomasko J.....	103, 129, 188, 474
Tomiki M.....	220, 224
Topaloglu E.....	595
Torrents B.....	102
Torres-Cobos B.....	239
Torrijos R.....	75, 156, 469, 531, 542
Tosnerova K.....	645
Tramseur A.....	583
Trapani F.....	147, 210, 528, 529, 530
Travaglia F.....	76, 169, 291
Travnickova M.....	558
Trbovic D.....	423
Tres A.....	239
Treyer A.....	593
Triesch N.....	393, 596
Trimmel M.....	282
Trinanes J.....	547
Trivedi E.....	622
Troyer C.....	79
Tsagkaris A.....	101, 197, 334, 675
Tsamba L.....	77
Tsekenis G.....	676, 677
Tsochatzis E.....	514
Tsoureki D.....	523
Tucci R.....	199
Tura M.....	447
Turla E.....	175
Turner A.....	547
Tuthorn M.....	62, 266, 267
Twaruzek M.....	356, 482, 578
Tzioni E.....	277

## U

Ugay C.....	555
Uhlig S.....	284
Uhr L.....	430
Umbricht G.....	420
Undas A.....	74, 619
Usanase G.....	371
Uttl L.....	518, 629, 630, 636

## V

Vackova P.....	636
Vaclavik L.....	182, 274
Valente G.....	360
Valli E.....	199, 447
Valtuna Martinez S.....	175
Van Bavel B.....	176
Van Camp C.....	535
Van de Schans M.....	139
Van der Meer M.....	192
Van der Velden B.....	70
Van Dijk B.....	326, 354
Van Dongen K.....	354
Van Hassel W. H. R.....	535
Van Hoeck E.....	99, 176, 192, 510, 589
Van Laere Y.....	280
Van Leeuwen S.....	122, 326, 354, 375
Van Loco J.....	192, 195
Van Nieuwenhuysen T.....	280
Van Poucke C.....	83, 214, 215, 221

Van Ruth S.....	119, 163
Van Vlierberghe K.....	214
Van Wichelen J.....	535
Van Zwynsvoorde F.....	221
Vanneste K.....	280
Varela J.....	97
Varenina I.....	358, 359
Varga E.....	548, 568
Varga I.....	358, 359
Varga M.....	538
Varra M. O.....	205, 248, 264
Vatazevic A.....	585, 586
Vaz Pato M. C.....	107
Vazquez-Loureiro P.....	134
Vehar A.....	109
Venegas S.....	154
Ventura C.....	599
Verdon E.....	640, 649, 656, 657
Verdu A.....	410
Verleysen E.....	588, 589
Vermeulen P.....	570
Verstraete F.....	208
Ververis E.....	175, 228
Verza P.....	360
Vetter M.....	309
Veys P.....	242
Vezse M.....	54
Vichi S.....	239
Vida A. P.....	567
Vidick N.....	192
Vilarino N.....	328, 609
Vincent C.....	371
Viscusi P.....	391
Vishnepolsky B.....	216, 217, 243
Vitali C.....	74
Vitova E.....	563
Vittadello A.....	360
Vlachou C.....	149
Volkov A.....	579
von Gotz N.....	471
von Leliwa S.....	512
Voorhuijzen M.....	83, 213
Voß A.....	596
Vranic D.....	423, 424, 425
Vrasadis Y.....	181
Vrbos D.....	228
Vroljik M.....	375
Vukadinovic J.....	412
Vukovic G.....	585, 586

## W

Wagner M.....	110
Wagner S.....	666
Walford L.....	489
Walker Ma.....	650
Walker Mi.....	83
Warenik-Bany M.....	361, 660
Warner N.....	268, 364
Warth B.....	190
Watanabe J.....	638
Watkin B.....	497
Watzinger A.....	79
Weber W.....	378, 637
Weckend L.....	259
Weddell K.....	654
Wee C. D.....	442
Weidner K.....	431
Weigel S.....	393, 561, 596
Weigel W.....	676
Weil D.....	377

## INDEX-AUTHORS

Weitzel Dias Carneiro M. T. ....	494
Weller P. ....	148, 170, 201, 525
Wenzel K. ....	603
Werner D. ....	116, 252
Westmacott K. ....	670
Westphal Y. ....	83, 144, 213
Whyatt K. ....	369
Wieman J. ....	608
Wildbacher M. ....	155
Willemsen M. ....	620, 624
Williams L. ....	632
Williams P. ....	261
Winkel M. ....	83
Winkler S. ....	525
Wissmueller S. ....	584
Wissmuller S. ....	61
Wiszumirska K. ....	176
Wojciechowska P. ....	176
Wolf J. C. ....	283
Wu D. ....	106, 161
Wunsche K. ....	275
Wushensky J. ....	634
Wustrack F. ....	259
Wyns I. ....	510

### X

Xia J. ....	119
Xiao C. ....	192

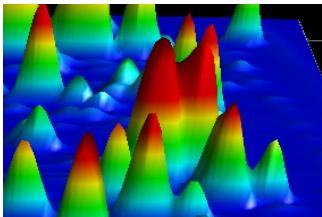
### Y

Yakova K. ....	155
----------------	-----

Yamamoto M. ....	430
Yang J. ....	400
York J. ....	357
Yu D. ....	142, 411
Yu W. ....	333

### Z

Zacometti C. ....	203, 238, 262
Zahrae El-Mossaid F. ....	447
Zamariola G. ....	228
Zampieri L. ....	604
Zanardi E. ....	205, 248, 264
Zanella S. ....	621
Zantedeschi S. ....	447
Zbinden M. ....	176
Zelinkova Z. ....	602
Zellmer S. ....	362
Zeppa G. ....	312, 319
Zhang W. H. ....	192
Zianni R. ....	488
Ziegler J. ....	430
Zimmerli P. ....	82, 269
Zitek A. ....	79
Zomer P. ....	608, 619
Zübner I. ....	601
Zuilhof H. ....	137, 577
Zuliani T. ....	176
Zwahlen J. ....	271
Zwickel T. ....	90
Zwygart J. ....	658



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