BOOK OF ABSTRACTS

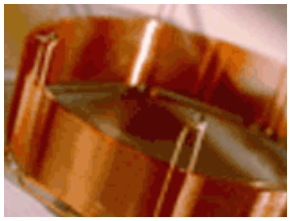
10th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS

September 6–9, 2022

Prague, Czech Republic

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



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

WAGENINGEN UNIVERSITY & RESEARCH

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10th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

September 6–9, 2022

Prague • Czech Republic

Don Giovanni 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

MINISTRY OF AGRICULTURE OF THE CZECH REPUBLIC

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RAFA 2022 is held under auspices of the Minister of Agriculture of the Czech Republic, Zdeněk Nekula, and the Capital City of Prague.

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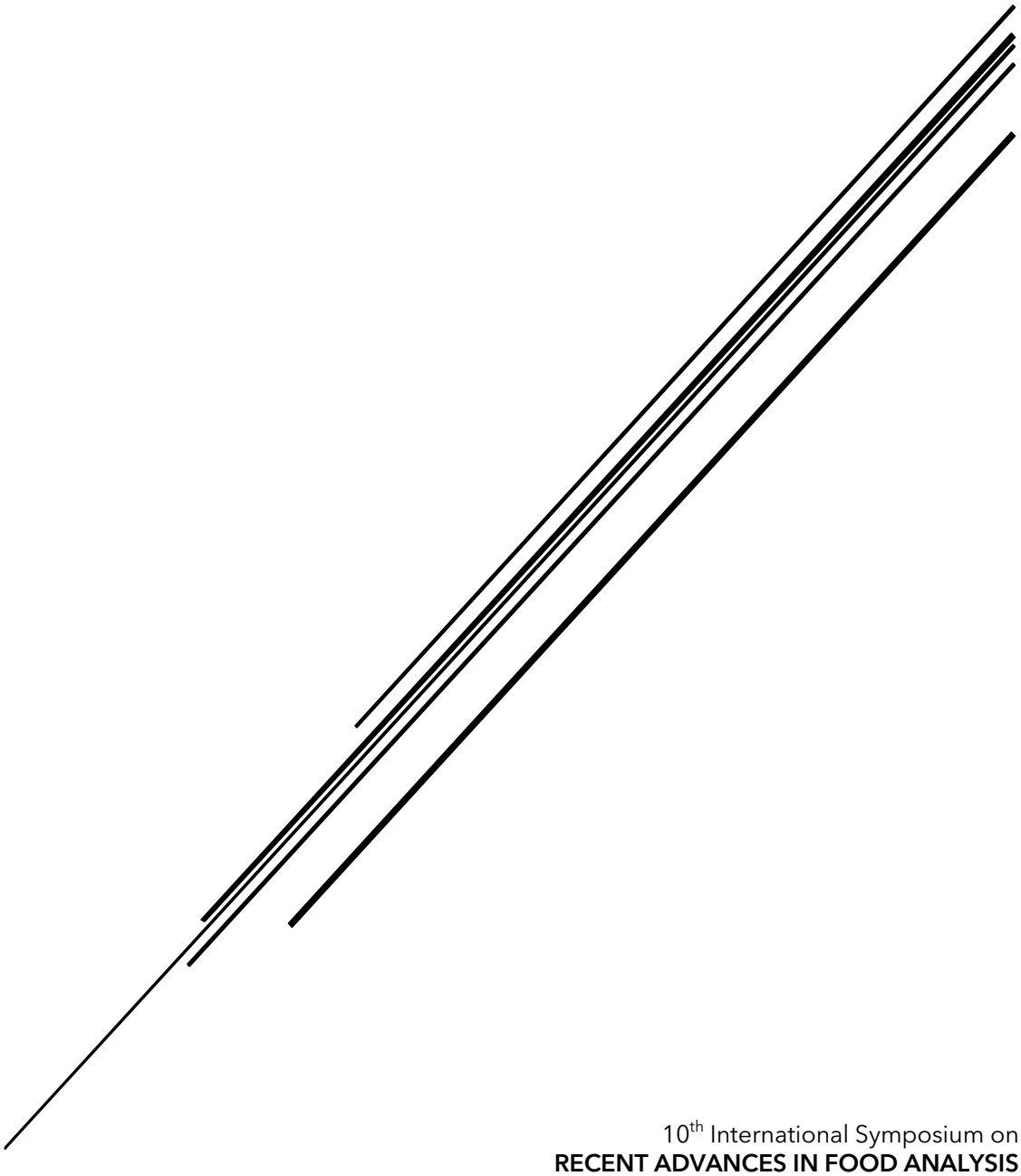

10th International Symposium on
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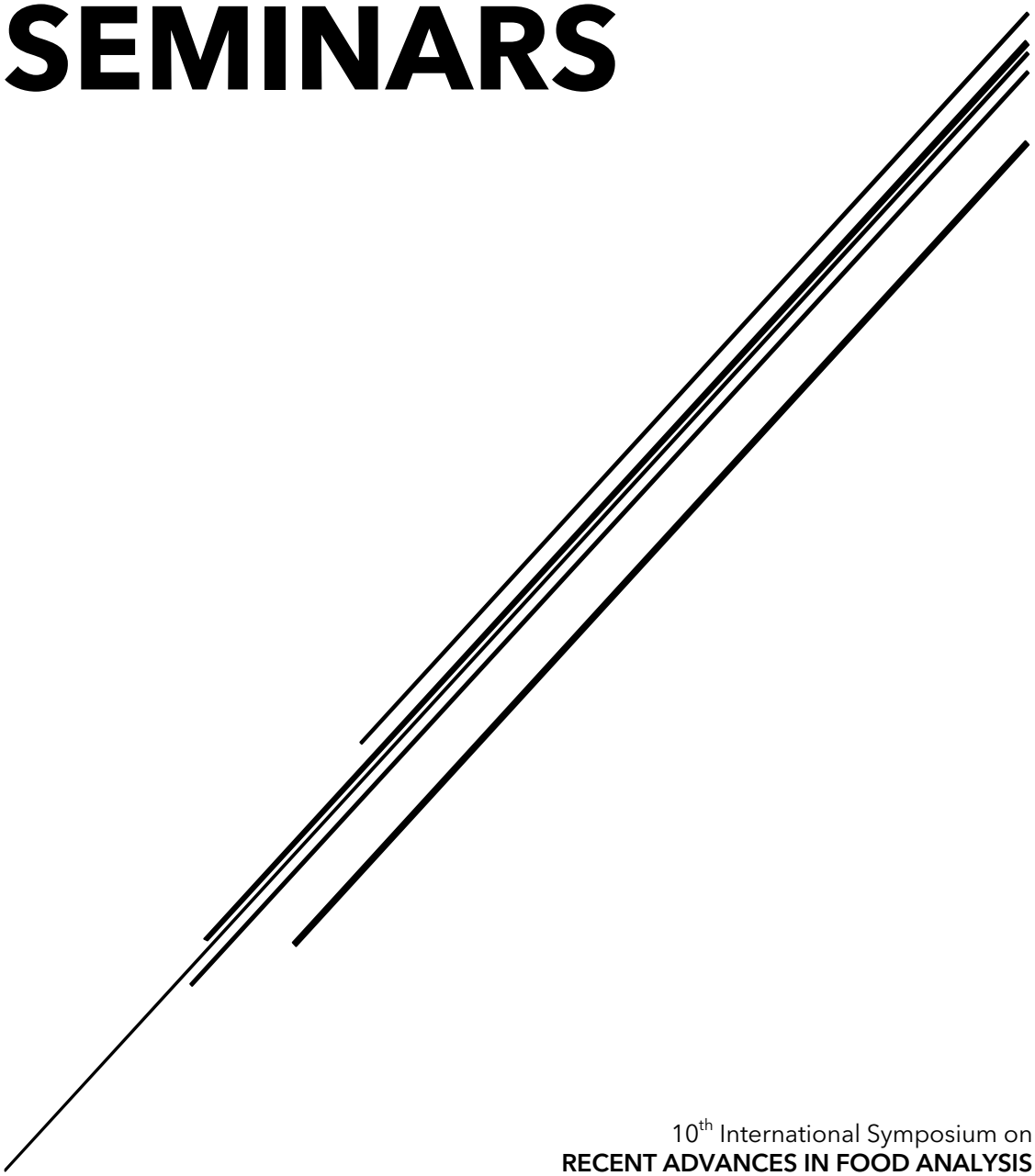
VENDOR SEMINARS



10th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, September 6–9, 2022

September 6, 2022 (12:15–13:00)

VENDOR SEMINAR:

Improvements in LC-MS/MS Analysis of Anionic Polar Pesticides in Fruits and Vegetables

Improvements in LC-MS/MS Analysis of Anionic Polar Pesticides in Fruits and Vegetables

Jörg Baute

Business Development Manager Food/Environmental Europe, Phenomenex

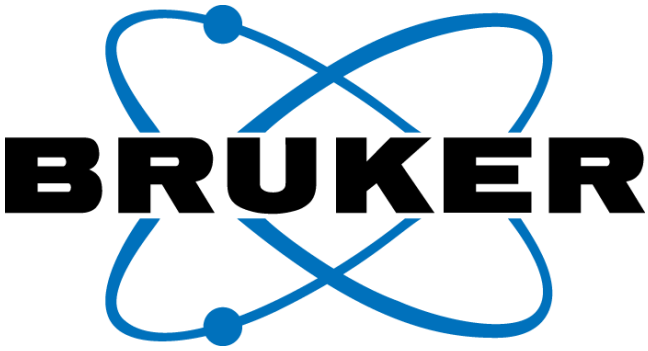
Many polar pesticides used in conventional agriculture are difficult to retain on standard C18 reversed phase HPLC columns. In addition, polar pesticides are a very diverse group of analytes which is hard to be analyzed using one standard method to identify and quantify all of them. This forces food testing laboratories analyzing samples multiple times using different methods to identify and quantify all the relevant polar pesticides in each food sample.

The purpose of the study, I will present, was the development of a fast and robust method for the LC-MS/MS determination and quantification of several common anionic polar pesticides in fruits and vegetables after sample preparation using the QuPPe method. As the samples we tested were from plant origin, we followed the QuPPe-PO-Method suggested by the EU Reference Laboratories for Residues of Pesticides–Single Residue Methods (EURL-SRM). In the frame of the study, we evaluated the effect of sample dilution, injection solvent, injection volume, and concentration of formic acid in the mobile phase on the reduction of matrix effects affecting the recovery and quantification of anionic polar pesticides including phosphonic acid, fosetyl, chlorate, perchlorate, Glyphosate, and AMPA. Presented is the resulting method allowing the identification and quantification of a variety of polar anionic pesticides including a separation of phosphonic acid from phosphoric acid in water samples.

Keywords: polar pesticides, LC-MS/MS, QuPPe

Acknowledgement: Luigi Margarucci, Phenomenex Italy, Castel Maggiore, Italy; Pietro Azzione and Marco Loperfido at EuroQualitylab S.r.l., Gioia del Colle, Italy

September 6, 2022 (12:15–13:00)



VENDOR SEMINAR:

New Developments in Fast Food Testing

DART and MALDI for rapid direct analysis

Dr. Carsten Baessman,

Bruker Daltonics GmbH & Co. KG, Bremen, Germany

As the consumer community continues moving towards understanding more about food quality and its source, plus greater awareness by analysts in developing 'greener' methods, rapid direct analysis of samples is gaining importance in meeting these criteria.

To achieve these goals, 'chromatography free' methods have been developed using DART technology from IonSense, MALDI mass spectrometry and real-time CI TOF from TOFWERK. All methods offer much shorter analytical times meaning less consumable costs and higher throughput of samples.

The benefits of all the above technologies will be explained using examples of DART coupled to triple quadrupoles, high resolution MS and ion mobility MS systems, MALDI-TOF and chemical ionization MS systems. The analyses shown will include wine analysis including quantitation of antioxidants, pesticide analysis, authenticity of olive oil, feta cheese, yoghurt and beef plus flavour analysis.

Real-time CI-TOF and ecTOF for food and flavour analysis

Marleen Vetter, Ph.D.

TOFWERK, Thun, Switzerland

TOFWERK, a strategic partner of Bruker, offers time-of-flight (TOF) mass spectrometers for applications that demand exceptional speed and sensitivity, delivering innovative solutions for chemical analysis. After a quick company overview of TOFWERK, the seminar highlights two product lines for food analysis applications: The Vocus CI-TOF and the ecTOF.

The Vocus is a real-time chemical ionization mass spectrometer (CI-TOF) that directly samples, quantifies, and characterizes volatile and semi-volatile organic and inorganic compounds in complex mixtures in real time, without the need of chromatography. It delivers sub-ppt limits of detection for a diverse range of compound classes. The rugged, compact design offers a field

deployable system for even the most difficult environments. The Vocus offers robust solutions for food and flavor analysis, including the Vocus Cork analyzer which demonstrates a fast, real-time application in monitoring and quality control applications.

Furthermore, the newly developed ecTOF is presented. The uniqueness of this mass spectrometer is the parallel operation of an electron ionization (EI) and a chemical ionization (CI) source. By directly coupling a single gas chromatograph (GC) to both ionization sources, target and suspect screening analysis is improved as well as effective non-target analysis rendered possible. In addition, the ecTOF can be run in real time and GC sampling in parallel, bridging the gap between real time and conventional GC applications. Example applications for the food and fragrance market are discussed. By using the concurrently generated structural as well as accurate mass molecular ion information, the identification of compounds of interest is improved, highlighting the potential for the ecTOF during product development and quality control, as well as a tool for authenticity concerns.

September 7, 2022 (7:45-8:30)

SHIMADZU
Excellence in Science

VENDOR SEMINAR:

The Quality of Food Characterized by Gas Chromatography

The quality of food characterized by gas chromatography

Erich Leitner

Head of the Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, Austria

It is written in the European General Food Law that "European citizens need to have access to safe and wholesome food of highest standards."(https://ec.europa.eu/food/horizontal-topics/general-food-law_en). To ensure these requirements different principles and procedure are in action to monitor the whole production chain of food and feed production. It also ensures a high level of protection of human life and consumers interests in relations to food but also ensures the effective functioning of the internal market. Last but not least the sensory perspective (hedonic quality) is very important for the acceptance or rejection of a product by consumers.

In this presentation I try to explain different aspects of food quality and safety and the requirements for analytical methods.

Gas chromatography is a technique which can provide useful information on

- Characterization of the main ingredients (mainly fats, but also carbohydrates and proteins)
- Aroma active compounds
- Undesired organic residues and contaminants

Several examples will demonstrate the applicability and the potential of various sample preparation methods in combination with one and two dimensional gas chromatography.

September 7, 2022 (7:45–8:30)

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

VENDOR SEMINAR:

Make It Your Analysis with Waters' Solutions for Food Quality Control!

Increase Workflow Efficiency by Automation, Latest UPLC Innovations and a Compact Highly Selective Detector

The connected laboratory for LC and LC-MS workflow automation

Janitha De-Alwis

Waters Corporation

As contract testing labs face increasing demands, modern laboratories have been quick to embrace automation as a critical component to streamlining analytical workflows. The automation of routine and complex sample preparation helps minimize variability, improve traceability, and simplify method transfer.

Andrew+ is an intelligent robot that allows scientists to develop reproducible, traceable, and easily implemented sample preparation protocols through highly repeatable pipetting. When using OneLab, a cloud-native software environment, experiments can be intuitively designed, reproducibly executed, and tracked through a rapidly evolving ecosystem of connected devices and accessories.

In this workshop we will present findings from the extensive evaluation of Andrew+ in a routine food analysis lab at Eurofins Nutrition Analysis Center (Des Moines, IA US). The performance of the robot was found to be consistent with rigorous requirements in accuracy and precision in sample preparation. The OneLab software will be shown in a live demonstration the intuitive and the performance of Andrew+ will be demonstrated in a video.

Organic Acids Analysis - Ensuring the quality and authenticity of beverage products

Cecile Pinto

Waters Corporation

Organic acids can occur naturally or are added to beverages as acidulants, flavourings and preservatives. Waters' chromatography solutions enable beverage testing laboratories to efficiently analyze organic acids, ensuring products are authentic and maintain a consistent quality and flavour profile.

The chromatography of organic acids can be impacted by interactions between target analytes and the metal surfaces of the chromatographic flow path. With Waters MaxPeak High Performance Surfaces technology, the ACQUITY Premier Solution is designed to eliminate the unpredictability of analyte losses due to metal interactions. It removes the need for lengthy passivation and conditioning and reduces the complexity of mobile phase as well as method set-up to increase laboratory productivity.

In this workshop we will present a method for the analysis of 14 organic acids. Achieving baseline separation of analytes while avoiding co-elution of matrix interference can be challenging. The use of ACQUITY QDa Mass Detector instead of an optical detector provides increased selectivity in a compact design. The use of selected ion recording (SIR) acquisition can reduce the impact of co-eluting matrix components, allowing for less complex chromatograms, lower detection limits and greater method flexibility to test a range of different beverage products.

September 7, 2022 (13:30–14:15)

RESTEK
Pure Chromatography

VENDOR SEMINAR:

Tipps and Tricks to Quantify Emerging Toxins and Process Contaminants

Alternarias, Ergots and other major mycotoxins simultaneously in various food matrices - Furan and Alkyfurans, utilizing SPME Arrow increase sensitivity

Jan Pschierer[(1)], Jana Hepner[(2)]

[(1)] Restek GmbH, Bad Homburg, Germany
[(2)] Restek Corporation, Bellefonte, USA

Food Safety Laboratories have to face a broad variety of different contaminants in different matrices, with Pesticides as a prominent and complex example.

Next to these, mycotoxins have seen some major changes in regulations within Europe. Ergot Alkaloids are regulated since this year, and Alternaria Toxins have gained some publicity due to contaminated Tomato Purees' with effect on upcoming regulations.

Several screening (or multi compound) methods for a large combination of mycotoxins are published, but most often using non-MS-detector friendly and column challenging conditions.

Jan Pschierer will present a screening method for a variety of regulated mycotoxins, including Alternaria Toxins and all 6 nowadays EU-regulated Ergot Alkaloids and their Epimers. He utilizes a Biphenyl phase chemistry and a generic easy to use sample preparation to reach and exceed regulatory LOQ's and LOD's.

Jana Hepner will focus on process contaminants, which are widely discussed in the Food Safety regulatory bodies at the moment. Next to Acrylamide, Furan and its methylated and ethylated derivates are formed during heat driven processes like baking, grilling, toasting. Especially Baby Food and Infant formulas are of common interest as well as food consumed in large quantities, like coffee.

Methods reported for the analysis of these volatile organic compounds include static headspace (HS) and solid phase microextraction (SPME) in combination with GC-MS. The use of SPME for the analysis of these process contaminant has demonstrated improved method sensitivity and higher S/N for some of the alkylfurans.

However, the fragility of traditional SPME fibers can be a concern. In her presentation, Jana Hepner will report about a HS-SPME-GC-MS method for the analysis of furans and alkylfurans in baby formula and coffee using an SPME Arrow. The SPME Arrow geometry allows for a much better mechanical robustness of the extraction device and enhanced method sensitivity.

At the end of this vendor seminar, Restek will invite you for a traditional Prague Beer Tasting.

Keywords: Ergot Alkaloids, Alternaria Toxins, Alkylfurans, SPME Arrow

September 7, 2022 (13:30–14:15)

VENDOR SEMINAR:

Implementing New GC-MS and LC-MS Technologies to Stay Ahead with Your Food Safety Analysis from Pesticides to PFAS and Microplastics

Implementing new GC-MS and LC-MS technologies to stay ahead with your food safety analysis from pesticides to PFAS and microplastics

Dominic Roberts[(1)], Frans Schoutsen[(2)]

[(1)] Product Marketing Manager GC and GC-MS, Thermo Fisher Scientific
[(2)] Sales Support Specialist LSMS, Thermo Fisher Scientific

The first half of the presentation will focus on the capabilities of the new GC-MS/MS system from Thermo Scientific, which was launched in March 2022. The system design was based on direct customer feedback in order to overcome challenges faced by analytical testing laboratories. The system provides excellent sensitivity to meet the strictest regulatory limits and has modular GC design for increased flexibility in system configuration, while including unique maintenance options and compatibility with online automated clean-up to minimize instrument downtime and increase sample throughput. An overview of applications on the new GC-MS/MS with data examples will be presented, including the analysis of pesticides in baby food and the determination of ethylene oxide in food. We will also explore how pyrolysis -GC high resolution accurate mass MS can overcome challenges associated with the analysis of microplastics in food.

The second part of the lecture will focus on the development of a sensitive PFAS (Per- and Polyfluoroalkyl Substances) screening and quantitation method in pork muscle meat utilizing LC-Orbitrap and a novel cloud hosted application called myLibrary™ Enterprise, which allows users to easily extract spectra and create fit-for-purpose MS/MS spectral libraries. Curation is done directly in the application, and the final library can be exported for use directly in Thermo Scientific™ TraceFinder™ Software with mzVault. Details of the method will also be presented in terms of calibration, limits of quantitation, recovery, and identification according to SANTE guidance and MS/MS spectral matching.

At the conclusion of the presentation our application experts will answer any questions in a live Q and A.

September 7, 2022 (13:30–14:15)

VENDOR SEMINAR:

Recent Advances in a Well-Established Analytical Method – The Next Generation of Enzymatic Food Analysis

Enzymatic food analysis is probably one of the oldest biochemical methods in food and feed testing. Enzymes have been used in analytical methods since the 1950's. Initially used for clinical applications, analytical methods based on enzymes found their way into food and feed analysis too. In 1975 the company Boehringer Mannheim (now part of Roche Diagnostics) developed the first enzymatic assays for food testing. Thanks to the high specificity, sensitivity and ease of use of this method compared to other, physicochemical methods, the enzymatic tests were soon adopted as official methods or reference methods for various applications in dairy, juices and wine. R-Biopharm got the worldwide exclusive distribution rights for these products in 2000 and we successfully developed this market further. By now the "Yellow Line" is a well-known and most used brand in food analysis.

Yet, after almost 50 years, we think it is time for the next generation enzymatic assays: Enzytec™ Liquid – or as we like to call it: The "Yellow Line 2.0". We listened carefully to our customers on how we could improve a gold standard method and we developed this new product line for enzymatic analysis.

Part 1: Enzytec™ Liquid: Use cases of ethanol and citric acid determination of the ready to use and liquid stable enzymatic premium reagents

Steffen Passig

Product Manager enzymatic food analysis, R-Biopharm AG, Germany

Explore with us the application areas of citric acid analysis in food. Learn more about our world novelty, the first liquid-stable citric acid test and the advantages of this assay, especially in the automation of modern enzymatic analysis.

Part 2: Automation of enzymatic analysis on a small scale – Case study

Ronald Niemeijer

Marketing Director, R-Biopharm AG, Germany

Does automation only make sense for large laboratories with a high sample throughput? The answer is: no. We will present a portable, automated enzymatic analyzer that will fit in any laboratory, even with a low sample throughput. The RIDA®CUBE SCAN automated enzymatic analyzer processes individual samples. So even if you have only a few samples per day automation might be an option. We will present some examples of automated ethanol testing in alcohol free products like alcohol free beer, wine and kombucha.

September 7, 2022 (14:45–15:30)

Agilent

Trusted Answers

VENDOR SEMINAR:

Developments in Food Safety and Trace of Origin Testing

PART 1: FOOD SAFETY TESTING

From targeted to In Silico, the current state and future advancements of PFAS testing

Stephan Baumann

Academic Applied Segment Manager, Agilent Technologies, USA

Greaseproof packaging often contains per and polyfluoroalkyl substances, (PFAS) and there are more are a little over a hundred commercially available standards, and those tend to be expensive.

Tentatively identifying PFAS in food contact material (FCM) requires a screening instrument and annotation software. We introduced FluoroMatch software, which automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on retention time, precursor masses and fragment masses, annotation ranking, and confidence assignment.

To aid interpretation by making homologous series more identifiable, we have added a Visualizer tool to the FluoroMatch suite of software utilizing Microsoft PowerBI.

This is the first application of FluoroMatch automated PFAS annotation using in-silico PFAS fragmentation to food packaging.

PART 2: TRACING ORIGIN

The metabolomics-based chemical fingerprint of foods: tracing origin and post-harvest operations of nuts and wine

Luigi Lucini,

Università Cattolica del Sacro Cuore, Piacenza, Italy

The phytochemical profile of foods determines specific and peculiar quality traits, a condition determined by the combination of cultivar and terroir effects. Under the latter, we include pedo-climatic conditions, as well as agronomic and post-harvest operations that arise from tradition and local practices.

The comprehensive nature of untargeted metabolomics through UHPLC-ESI/QTOF mass spectrometry, followed by appropriate multivariate statistics (both unsupervised for naïve patterns and supervised modelling) allows elucidating the effect of different pre- and post-harvest factors.

A representative case referring to hazelnuts from different geographic origins and different cultivars, following optimal and non-optimal post-harvest conditions, and following different shelf-life conditions, will be discussed. The work will elucidate specific effects and highlight as the outcome of shelf-life primarily depends on post-harvest storage.

A second case-study, dealing with the wine Amarone, will discuss the effect of berries drying conditions and yeast genotype on the distinct chemical signatures of the resulting wine.

September 7, 2022 (14:45–15:30)

VENDOR SEMINAR:

New Developments for the Analysis of MOSH/MOAH and 3MCPD in Food

Development & Advances for MOSH MOAH Analytics

Govert Schröder

Axel Semrau GmbH & Co KG, Germany

Saturated and aromatic hydrocarbons, the so-called "MOSH MOAH contaminants", have been the focus of sustained public interest for some time. These contaminants are now considered undesirable in food, consumer goods and cosmetics. As a result, a relevant analytical test point has been established and manifested. The basic method for this was published in 2017 in the form of EN method 16995. With this method, it is possible to analyse MOSH/MOAH in vegetable oils and foods based on vegetable oils with the LOQ of 10 mg/kg. In the last two years new methods have been established (DGF C-VI 22 (20)), EN 16995 Version2022). With these methods, an LOQ of 1 mg/kg can be achieved, but some manual sample prep is needed to achieve this LOQs. In the session an optimized, automated workflow is presented, which allows to reach this LOQs in an automated way. Using a new version of epoxidation this workflow gives especially for samples with high interferences (Palmoil, Coconutoil) better results.

New developments for the Analysis of 3MCPD in Food

Govert Schröder

Axel Semrau GmbH & Co KG, Germany

For the analysis of 3MCPD in edible oil and fat are different methods available. Some of this methods are easy to automate. To automate the method developed by SGS, Hamburg,(3in1) is more challenging, because it requires storing the sample for a long time at -22°C which requires special hardware. The lecture shows the experimental setup, some real date achieved with an automated system for this method and a comparison of the different methods. It also shows some date for a method which analysis free MCPD instead of MCPD esters and gives an overview of new methods which have been established in China.

September 7, 2022 (14:45–15:30)

VENDOR SEMINAR:

Improved Characterization of Fatty Acids in Food for Reliable Nutritional Labelling

High throughput, reliable characterization of fatty acids in food using a rapid single-step microwave-assisted extraction and derivatization method followed by flow-modulated GC×GC-FID

Giorgia Purcaro

Gembloux Agro-Bio-Tech, University of Liège, Belgium

The optimization and validation of a highly throughput and sustainable method for accurate FAME determination is herein presented and discussed.

A single-step microwave-assisted extraction and derivatization (MAED) method for characterizing FAME in a wide variety of food commodities was optimized and compared with the results from two different official FAMEs preparations (AOCS Official Method Ce 2b-11; Cruz-Hernandez et al. J AOAC Int 87(2) 2004 545). Moreover, a higher level of information along with a faster characterization was obtained using reversed fill/flush flow modulation comprehensive two-dimensional gas chromatography (GC×GC) coupled to FID for the analytical determination. Such a configuration is not only more sensitive than a mono-dimensional GC separation, but also allow for a higher separation speed, in terms of peak separated per unit of time, and a more unambiguous identification of FAMEs in complex mixture.

September 8, 2022 (7:45–8:30)

VENDOR SEMINAR:

Improved Confidence with Superior Qualitative and Quantitative Data Using LECO´s GC×GC-TOFMS

Can LECO Pegasus BT 4D GC×GC-TOFMS help improve the analysis of pesticide residues in cannabis?

Michal Stupak, Petr Mraz, Jana Hajslova

University of Chemistry and Technology Prague, Faculty of Food and Biochemical Technology, Department of Food Analysis and Nutrition, Prague, Czech Republic

The unique properties of cannabis (especially its great potential in medicine) are due to its complexity, in particular the content of multiple constituents contains a wide range of different compounds belonging to groups of phytocannabinoids, terpenes, flavonoids, stilbenoinds, fatty acids, alkaloids, carbohydrates, and polyphenols. Therefore, a comprehensive implementation of analytical tools is necessary to obtain detailed information about its composition, medicinal properties, and bioactivity, as well as risk assessment including the analysis of solvent residues, heavy metals, and pesticides. The purpose of this study was to develop a sensitive and reliable analytical strategy to analyze pesticide residues in cannabis, including optimization of sample preparation and the GC×GC-TOFMS method. During the method development, emphasis was placed on the clean-up step; thus, five different sorbents were tested. Using an advantage of the separation of the target analytes in the second dimension, we achieved very good separation between the matrix and analytes. With regard to these facts, the LECO Pegasus BT4D GC×GC-TOFMS technique allows detection of significantly more pesticide residues at very low detection limits compared to the GC-MS/MS system routinely used for this purpose.

The characterization of recycled materials

Andrea Hochegger, Erich Leitner

Institute of Analytical Chemistry and Food Chemistry, Graz University of Technology, Graz, Austria

The average European citizen is producing approximately 33.5 kg plastic waste from packaging material, or 25 million tons in total annually [1]. So, the EU started several actions to reduce the produced waste. Recently the European Commission announced a strategy for reducing packaging waste [2]. This includes the following actions:

- improve packaging design to promote reuse and recycling
- increase recycled content in packaging
- tackle excessive packaging

- reduce packaging waste

So, a special focus must be put on point 2 (increase recycled content in packaging). It is clearly mentioned in the EU regulation 1935/2004 on materials and articles intended to come into contact with food, that materials and articles may not in foreseeable conditions release to food their constituents in amounts which could endanger human health or change the composition of the food in an unexpected way, or cause changes in the organoleptic characteristics of food, such as odour, taste or appearance. Due to the recycling process and due to various materials (pesticide formulations, mineral oils, hygienic products, medication…) there is the risk, that undesired compounds can be present in the recycled material and can be transferred to the packed goods. Unfortunately, there is a lack of official methods to verify the safety of the recycled materials. Several examples and strategies for the characterization and safety evaluation of the recycled materials by one- and two-dimensional separation will be discussed.

[1] https://de.statista.com/statistik/daten/studie/786353/umfrage/plastikverpackungsabfall-in-ausgewaehlten-eu-laendern-je-einwohner/

[2] https://ec.europa.eu/info/law/better-regulation/have-your-say/initiatives/12263-Reducing-packaging-waste-review-of-rules_en

September 8, 2022 (7:45–8:30)

VENDOR SEMINAR:

Introducing Novel Mass Spectrometry Techniques and Their Applications in Food Testing

Introducing novel mass spectrometry techniques and their applications in food testing

Jianru Stahl-Zeng, PhD, Daniel McMillan

SCIEX

Over the past two years, SCIEX have introduced several pioneering new technologies in the field of liquid-based mass spectrometry, including solutions for very high throughput routine as well as cutting edge research analyses.

In this seminar, we will briefly describe the new ZenoTOF 7600 accurate mass system, with its unique ZenoSWATH DIA and EAD techniques, and the EchoMS system, capable of confidently analysing thousands of samples in a matter of hours.

Using examples of real-world applications, created with researchers from leading regulated food testing laboratories, we aim to demonstrate how these new technologies have the potential to change the landscape of food testing and bring sensitive, accurate and fast analyses into routine use.

September 8, 2022 (13:30–14:15)

eurofins | Technologies

VENDOR SEMINAR:

Mycotoxins Risk Management: Industrial Solutions for On-site Testing

Mycotoxins risk management: industrial solutions for on-site testing

Giulia Rosar

Mycotoxins Product Manager, Eurofins Tecna s.r.l. a socio unico

Climate changes, drought and raw materials shortage have led to agricultural practice modifications, changes in foods and feed formulas, and sometimes a deep reconsideration of mycotoxins risk assessment. As mycotoxins may have their way into areas not previously seen, with unexpected patterns, or eventually at unpredicted levels indeed, their management revision must rely on affordable, smart, easy-to-use yet robust on-site screening methods.

Eurofins Technologies offers a wide port-folio of plate-based screening solutions designed for low and high analytical throughputs, both for manual execution and automatic implementation, to quantitatively analyse aflatoxins B and G, aflatoxin M1, patulin, deoxynivalenol, fumonisins, ochratoxin, zearalenone, T-2/HT-2 toxins in susceptible matrices. Kits have been designed to be reliable tools to food and feed industries; the goa of the seminar is to present their features and unravel doubts on the technologies and its fitness for the purpose.

How much do you know about immunochemical-based assays? How much do you trust them? Is that true that screening methods do always overestimate? How can methods and technologies be compared? Do you know how to correctly use control materials and reference materials to monitor routine quality?

An interactive workshop will be hosted by Eurofins Technologies to answers a few questions and debate over adequateness of immunoassays for mycotoxins risk management.

September 8, 2022 (13:30–14:15)

VENDOR SEMINAR:

Elemental and Isotopic Analysis: Solutions for Food Authenticity, Quality and Safety

Elemental and isotopic analysis: solutions for food authenticity, quality and safety

Dr. Niel Williams[1], Dr. Sukanya Sengupta[2]

[1] Sales Representative Inorganic Mass Spectrometry, Thermo Fisher Scientific
[2] Application Specialist Trace Elemental Analysis, Thermo Fisher Scientific

With the Thermo Scientific portfolio for elemental and isotope analysis, you can address QC, authenticity, origin and safety of your samples through scalable, software-driven workflows. Dr. Niel Williams will present how elemental and isotope analysis deliver QC and integrity answers to variety of feed and food samples such as crops, sugar, meat, rice, juice, tea or dairy. Dr. Sukanya Sengupta will provide insights into the characterization and monitoring of both nutrient elements and toxic trace metals in different kinds of food samples using ICP-MS techniques that allow high sensitivity analysis for quantifying even the smallest amounts of toxic metals like lead, mercury etc. in the foods. It will be demonstrated how laboratories can achieve this easily in an efficient manner using the developed method for reliable and robust analysis of hundreds of samples daily.

Learn more about integrating elemental and isotopic solutions in your analysis.

September 8, 2022 (13:30–14:15)

VENDOR SEMINAR:

Application of Trapped Ion Mobility Mass Spectrometry for Food Research

Migration screening of raw and food contact materials using Intuvo GC MS

Dr. Carsten Baessmann[(1)], Sofia K. Drakopoulou, M.Sc.[(2)]

[(1)] *Bruker Daltonics GmbH & Co. KG, Bremen, Germany*
[(2)] *Ph.D. Candidate, University of Athens, Greece*

Analysis of complex samples such as food is becoming more challenging as the trend develops from targeted to untargeted workflows. Untargeted workflows are the best way to rapidly identify multiple compounds from a specific group e.g., dioxins or PFAS molecules, plus they are a very useful starting point for food authentication studies. Untargeted workflows are more challenging because the more 'features' (compound spectra) that can be determined, the greater the number of spectral points exist for comparison purposes (authenticity studies) or ensuring all compounds of a specific group can be identified.

Ion mobility in combination with high resolution mass spectrometry is a powerful tool in aiding these studies. A fourth analytical dimension, Trapped Ion Mobility Spectrometry (TIMS) enables the above to be readily achieved. Several studies have been performed on a timsTOF Pro (Bruker Daltonics) linked to an UHPLC or GC. TIMS data rapidly provides an additional and important I.D. criterion namely cross collisional section values and has been found to be highly valuable in compound identification, separation and quantitation of isomers plus clean-up of chromatograms and spectra.

The benefits of using TIMS will be described in detail and illustrated with examples of pesticide analysis from onion QuChERS extracts, PFAS analysis, dioxin and POPs analysis by GC-APCI TIMS MS and food authenticity studies.

September 8, 2022 (14:45–15:30)

Agilent

Trusted Answers

VENDOR SEMINAR:

Analysis of Alternative and Conventional Proteins

I don't taste the difference: Analytical approaches to compare nutrition, safety & functional properties of alternative & conventional proteins

Stephan Baumann

Academic Applied Segment Manager, Agilent Technologies, USA

Plant-based and cell-based proteins including meat, dairy and other variants are gaining in popularity among consumers but also one of the largest focus areas to increase food sustainability and reduce green-house gas emissions associated with animal rearing going forward to feed the world's burgeoning population. While regulations are at an early stage to deal with many of these novel foods and ingredients, analytical testing is a key component to ensure safety, nutrition, and quality during product development and post commercialization. This seminar will discuss analytical tools critical for food companies to consider while doing product development to ensure that taste & flavor agents mimic traditional animal-based foods while also studying the profile, yield and chemical pathways of proteins and lipids during development to optimize them in the final food stuff. We will also discuss critical analytical techniques and parameters to test for regulatory and food safety testing while touching upon testing needs for vitamins, minerals, fats, sugars, amino acids, and other food nutrition parameters that alternative food manufacturers have to test their product for.

September 8, 2022 (14:45–15:30)

VENDOR SEMINAR:

Ways to Master Your Pooling Testing Approach for Food Pathogen Testing

Ways to master your pooling testing approach for food pathogen testing

Sandra Fréville

Marketing Tactical Manager, Thermo Fisher Scientific

Regulation transfers the responsibility for the food safety and quality to the food producers. They must establish and develop HACCP (Hazard Analysis and Critical Control Point System) and define the appropriate sampling plan. Pathogen monitoring programs play a crucial role in this control plan and efficiency of pathogen screening can be significantly improved with sample pooling approach. This enables to optimize the number of tests, get the right balance between a large-scale sampling plan and the required testing resources, and of course better manage the risk. The ISO 16140-4:2020 standard was recently amended to enable in-house validation of sample pooling together with the selected analytical methods. In this seminar, Sandra Fréville, Tactical Marketing manager in Thermo Scientific Microbiology, will explain the key point of the amendment of the ISO 16140-4:2020 and will present case studies and practical examples of implemented pooling methods with the SureTect Salmonella Assay.

September 8, 2022 (14:45–15:30)

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

VENDOR SEMINAR:

Make It Your Analysis with Waters' Solutions for Food Safety!

Application Solutions for Natural Toxins, Anionic Polar Pesticide, and PFAS

From field to fork - Solutions for mycotoxins and alkaloids detection

Nicola Dreolin

Waters Corporation

Waters and VICAM together offer complete field to lab solutions to address the needs for early detection and finished products verification. Our rapid, antibody-based strip tests provide a fully streamlined approach to preventive monitoring that allows quantitative results for up to 6 mycotoxins in less than 10 minutes. Immunoaffinity columns offer multiple functions: they can be tested with a field-based fluorometer or coupled with HPLC and UPLC for confirmatory methods of single or multiple mycotoxins. The high selectivity of Immunoaffinity columns makes them also an ideal clean-up in use with LC-MS. In a broader approach the well-known sample prep tools of the Oasis family provide superior performance in sample clean-up that can be applicable to multi-toxin and multi-residue methods. And if you have the need for high through-put a dilute and shoot method using a high-end mass spec is the answer.

In our workshop we will take you from field to lab and showcase how to achieve results fast using our strip tests or get highest sensitivity by HPLC and LC-MS/MS confirmatory methods for mycotoxins and alkaloids.

Anionic polar pesticides – A direct injection approach you can rely on

Jenny Davies

Waters Corporation

Routine analysis of anionic polar pesticides has become a requirement for many laboratories. These challenging analytes and their metabolites are not "amenable" to common multi-residue approaches, such as QuEChERs and mini-Luke, nor to reversed-phase chromatography. The area of anionic polar pesticide analysis has been evolving over the past ten years where the adoption of generic extraction methods, such as the QuPPe method, have enabled laboratories to take a multi-residue approach for the analysis of these challenging analytes.

Waters constantly improved it's work in the area of anionic polar pesticide analysis. The combination of the dedicated Waters' Anionic Polar Pesticide Column, ACQUITY™ UPLC and Xevo™ TQ Absolute mass spectrometer solves several of the critical challenges with this approach as well as expected extraction method performance. The workshop will focus on the demand for lower limits of quantification for the anionic polar pesticides. Those can be addressed with the enhanced

negative ion sensitivity of the Xevo™ TQ Absolute system, which now allows limits of detection in the low and even sub µg/kg region.

PFAS Analysis – Overcoming challenges to meet regulatory limits with a total solution

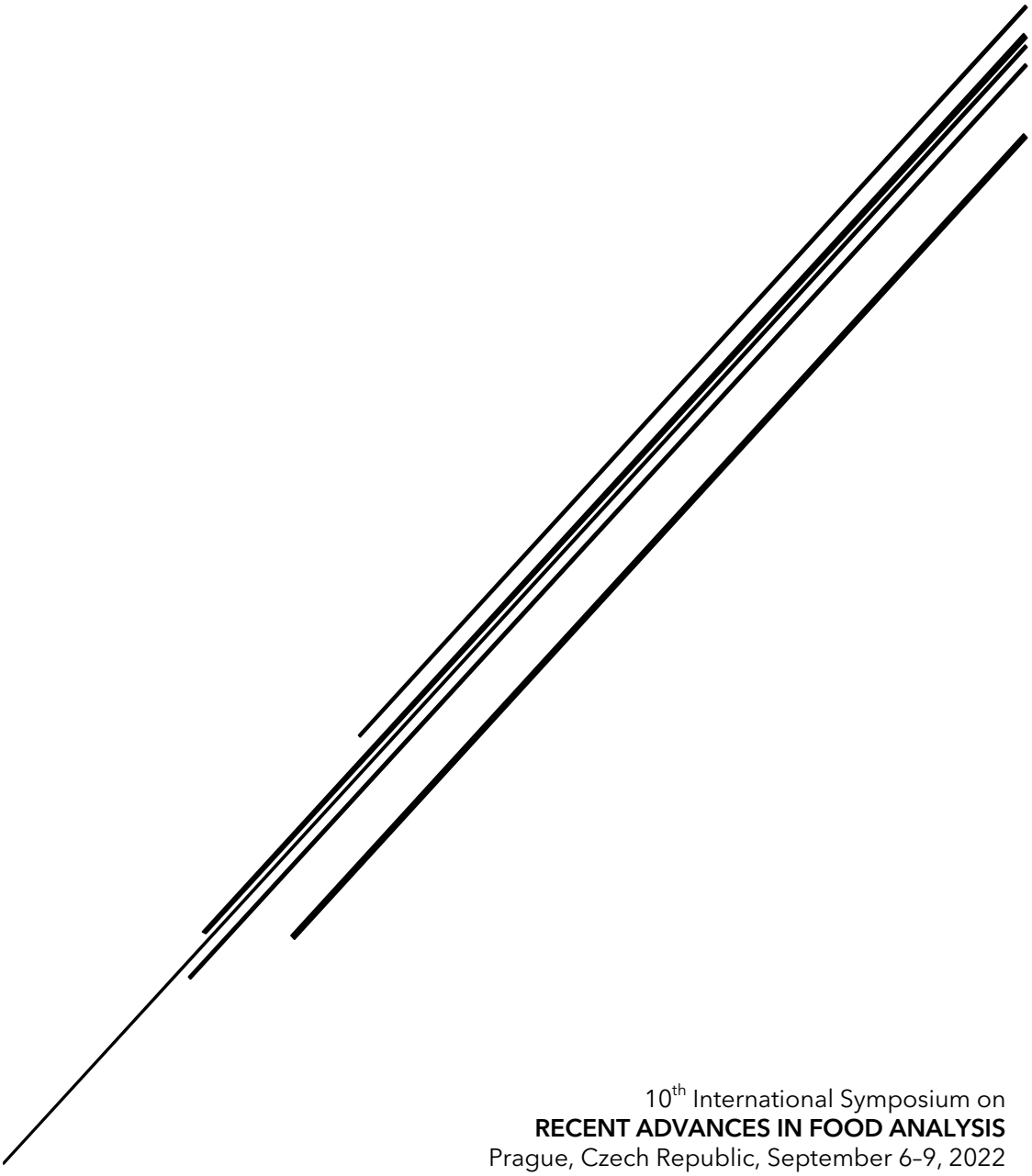
Hannah Willmer

Waters Corporation

Detection requirements for per- and polyfluorinated alkyl substances (PFAS) have been getting more challenging as regulations to protect consumers and environment continue to be created and updated.

In this workshop we discuss a minimal and rapid sample method by direct injection for PFAS analysis on a highly sensitive mass spectrometer to reach necessary performance criteria regulated by the EU. The enhanced negative ion sensitivity of the Xevo™ TQ Absolute Tandem Quadrupole Mass Spectrometer allows for utilization of the direct injection method for PFAS analysis with a reduced sample injection not compromising the method performance. Next generation Premier™ technology with MaxPeak High Performance Surfaces™ and novel ionisation techniques such as UniSpray™ can be further utilised to improve efficiency and robustness by reducing conditioning times and lowering detection limits or injection volumes. Waters is passionate in its continuing and evolving effort to offer total solutions for these notoriously tricky compounds to meet your laboratories specific needs and requirements.

LECTURES



10th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, September 6–9, 2022

LECTURES

L1

SEARCHING FOR THE UNKNOWN - ANALYTICAL APPROACHES TO UNCOVER FOOD ADULTERATIONS

Carsten Fauhl-Hassek*[1)]

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Official control laboratories have large product knowledge and a wide range of analyitcal methods available for food and feed authentication. However, the history of prominent food and feed adulteration incidences in global trade such as melamine, tells us that revealing unexpected additions (or contaminations) remains a major challenge in food control.
In the past, fraud has successfully been detected by whistle bowing, traceability approaches, audits or, then often "coincidentally", in the analytical laboratory. Once a "new" adulterant has been discovered, analytical methods are developed and established in control, which is often accompanied with the quick "disappearance" of the fraudulent practice.
Implementing non-targeted approaches has led to new perspectives in analytical food and feed authentication. Instead of measuring target analytes for specific adulterations, the product itself is comprehensively characterized by spectral "fingerprints", which is especially advantageous for the proactive detection of unknown and unforeseen adulterants.
The presentation will illustrate examples of food and feed adulteration incidents and their analytical detection. It will also focus on the scientific challenges of how non-targeted analysis can improve particular the detection of unexpected adulterations (e.g. appropriate statistical modelling, validation) and the associated difficulties of introducing these methods into routine analysis and surveillance.

Keywords: *authentication, adulteration, non-targeted, additions*

L2

CLIMATE CHANGE AND AGRICULTURE: HOW PLANTS COPE WITH RECLAIMED WATERS FOR IRRIGATION

Christian Klampfl*[1], **Franz Mlynek**[1]

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Water policy is a big topic worldwide and of course also within the European Union (EU), and several directives (e.g., 2008/105/EC, 2013/39/EU) focusing on the aim of ensuring a good water quality exist. Furthermore, the EU campaign "Water is too precious to waste", points out the increasing problem of water shortages and droughts across the EU in the recent years. Water scarcity already affects more than 11 % of the population in Europe and 17 % of the EU territory respectively. Consequently, by the year 2030, 50% of Europe's river basins might be affected by this issue [1,2]. In this regard, a promising approach for fighting water scarcity is to re-use treated wastewater (TWW) wherever possible. One field of application of TWWs is agriculture, as the extent of cultivable surface where irrigation is indispensable for successful farming is continuously growing. Countries like Malta and Cyprus where 90 % and 60 % respectively of the TWW is re-employed are in the forefront of water re-use. Other countries of the European south, recycle between 5 and 12 % of their TWW only, leaving a huge potential for improvement [2]. Despite the fact that more and more pollutants may be removed from wastewater by a continuous improvement of treatment plants and the processes employed therein, TWW still may contain a variety of micropullutants including pharmaceuticals and personal care products (PPCPs). When such TTWs are employed for irrigation in agriculture, these substances may be taken up by the roots, translocated to various plant parts (including those consumed by humans and animals) and eventually metabolized within the plant [3]. In our recent research projects, we investigated these factors on the example of a range of plants used for the production of food and feed (e.g., lettuce, tomato, maize, pea, amaranth, rice). Thereby we focused on a range of contaminants spanning from typical personal care products (such as sunscreens) to widely prescribed pharmaceuticals such as nonsteroidal anti inflammatory drugs, b-blockers, anti depressants and statins – nowadays almost ubiquitous in the aquatic environment. Besides studies on the uptake of these compounds by plants, cooperation partners from Brno also investigated the influence on plant growth. In our „in-lab" growing experiments actual TTWs from local treatment plants were applied as well as in-lab mixed artifial waste water samples for studying particularly the biotransfromation of these substances by a range of plants, showing that in many cases the presence of metabolites predominates and the parent drug is only found in lesser concentrations.

[1] European Commission, Water - reuse. http://ec.europa.eu/environment/water/reuse.htm.
[2] European Commission, Water – reuse factsheet. http://ec.europa.eu/environment/water/pdf/water_reuse_factsheet_en.pdf
[3] Klampfl CW (2019) Metabolization of pharmaceuticals by plants after uptake from water and soil: A review. Trends Anal. Chem. 111:13–26.

Keywords: *treated wastewater, water re-use, emerging contaminants, plant uptake, metabolization*

Acknowledgement: *This work was in part funded by Austrian Science Fund (FWF) project I-3046 (Pharmaceuticals in the Environment and their Interaction with Plants).*

LECTURES

L3

PANDEMIC IMPACTS ON FOOD ANALYSTS - AND VICE VERSA?

Michael Rychlik*[1]

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The presentation will outline some effects of the COVID-19 pandemic on researchers working in the field on food analysis. However, food analysts also contributed to perspectives how food and food components can help to strengthen immunity and alleviate a severe symptomatology of the infection. A particular focus of the talk will be laid on certain types of phenolic food components.

Keywords: *Covid-19, pandemic, food components, immune system*

L4

THE ROLE OF FOOD ALLERGEN ANALYSIS IN PROTECTING ALLERGIC CONSUMERS

Clare Mills*[1]

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Mandatory food allergen labelling is helping help food-allergic consumers practice food avoidance, but the presence of unintended food allergens and the precautionary allergen labels (PAL) used to warn consumers of such allergens, is problematic. Surveys of foods with and without PAL indicate confusion and a lack of a coherent approach, with some foods having been found to contain significant levels of allergens and yet not carrying a PAL. The use of risk-based approaches to managing allergens in foods is addressing this confusion but requires access to good quality data from clinical studies to allow. The FAO-WHO Expert consultation on food allergens is providing recommendations regarding global priority allergenic foods, and identified health based guidance values that allow identification of action levels for allergens in foods that are considered generally safe for most food-allergic consumers. The repertoire of allergen detection methods and their capacity to provide the required specificity and sensitivity for allergen analysis will allow many food allergens to be determined effectively in important food categories, although for some this remains a challenge. The importance of allergen reference materials in allowing harmonisation of test method reporting units and test method comparison will be highlighted. Finally, how new allergenic foods may emerge in future, especially in the context of the transformation of the food system towards plant-based diets and the role analysis will play in helping to identify manage emerging food allergens and novel foods will be discussed.

L5

EMERGING FOOD SAFETY CHALLENGES AND THE IMPORTANCE OF VALIDATED MULTI-CLASS METHODS

Rudolf Krska[1], **Michael Sulyok***[1], **David Steiner**[1]

[1] *University of Natural Resources and Life Sciences, Vienna, Austria*
**Corresponding author - E-mail: michael.sulyok@boku.ac.at*

Weaknesses of our food safety systems triggered by isolated events such as a zoonotic agent or a carcinogenic mycotoxin will be heavily compounded in the years to come by climate change, a shift in our food system towards a more plant-based diet and the need for a recircular economy. These developments will also impact on the occurrence of and exposure to chemical contaminants in food which continue to be an important food-borne public health concern in Europe [1]. Particularly, unintentionally present chemical contaminants in food, such as environmental and food process contaminants and natural toxins (esp. mycotoxins and plant toxins), can pose public health concerns if their concentrations are not kept at appropriately low levels as dictated by legislation.
Monitoring of food contaminants and residues has undergone a significant improvement in recent years and is now performed in an intensive manner [2]. Achievements in the area of chromatography-mass spectrometry coupling techniques enabled the development of quantitative multi-target approaches covering several hundred analytes. This paper provides an overview of relevant multi-class concepts based on LC-MS/MS instruments. Merits and shortcomings will be critically discussed based on current performance characteristics of the EU legislation system. In addition, a recently developed approach covering >1.000 agrochemicals including relevant biotoxins and other secondary microbial metabolites will be discussed as a case study to illustrate the current developments in food analysis and the importance of fully validated multi-class methods. The applicability and practicability of current guidelines for multi-analyte method validation will also be critically assessed. A major conclusion of our studies is clearly that more emphasis should be put on the investigation of relative matrix effects in the validation procedure.

[1] Eskola M., Elliott C., Hajšlová, J., Steiner D. & Krska R. (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.
[2] Steiner, D; Malachova, A; Sulyok, M; Krska, R. Challenges and future directions in LC-MS-based multiclass method development for the quantification of food contaminants. ANAL BIOANAL CHEM. 2021; 413(1): 25- 34.

Keywords: *mycotoxin, validation, LC-MS/MS, food safety, emerging contaminants*

Acknowledgement: *This work was created within a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence centre FFoQSI is funded by the Austrian ministries BMVIT, BMDW and the Austrian provinces Niederoesterreich, Upper Austria and Vienna within the scope of COMET - Competence Centers for Excellent Technologies. The programme COMET is handled by the Austrian Research Promotion Agency FFG.*

L6

INVESTIGATING THE POTENTIAL OF BERRY PLANT EXTRACTS TO INHIBIT PANCREATIC LIPASE: COMBINING IN VITRO ASSAYS TO SUSPECT METABOLOMIC SCREENING

Aristeidis Tsagkaris*[(1)], **Anna Louckova**[(1)], **Tereza Jaegerova**[(1)], **Jana Hajslova**[(1)]

[1)] *Department of Food Analysis and Nutrition, University of Chemistry and Technology, Prague, Czech Republic*
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Pancreatic lipase (PNLIP, EC 3.1.1.3) plays a pivotal role in the digestion of dietary lipids, a metabolic pathway strictly related to obesity. An effective strategy in obesity treatment is the inhibition of PNLIP, which is possible to be achieved by phytochemicals found in high abundance in plants [1]. In this study, a multidisciplinary approach is presented investigating the in vitro PNLIP inhibitory effect of 8 berry plant extracts belonging to the Ericaceae, Grossulariaceae and Rosaceae families, plants with proven anti-obesity potential [2]. Initially, a rapid and cost-efficient assay PNLIP 96-microwell plate assay was developed and important parameters were optimized, e.g., the enzyme substrate. The optimized assay was used to monitor the inhibitory effect of both aqueous and dichloromethane (DCM) extracts. Importantly, half of the tested aqueous extracts induced an inhibition rate higher than 60% (n=4), with blackcurrant (*Ribes nigrum*) and bearberry (*Arctostaphylos uva ursi*) extracts (10 mg mL^{-1}) almost completely inhibiting PNLIP activity. In the case of the DCM fractions, the monitored in vitro inhibitory effect was much lower, with blackberry leaves achieving the highest inhibition rate (44% ± 5%, n=4). Considering the significantly higher inhibitory effect of the aqueous fractions, it can be assumed that polar compounds were more potent to inhibit the PNLIP activity. In the next stage of the experiment, to tentatively identify the composition of the tested berry fruits a metabolomic suspect screening workflow was followed using a quadrupole time-of-flight mass spectrometry (UHPLC-q-TOF-MS) analyzer. Briefly, an in-house database containing 166 natural bioactive compounds was screened and work is under-way to complete the chemical characterisation of the analysed extracts. All in all, the presented approach combines in vitro bioactivity measurements to high-end metabolomics to identify molecules with potential medicinal and/or dietary applications.

[1] A. Kumar and S. Chauhan, Life Sciences, 2021, 271, 119115.
[2]. H. Jiang et al., Food Research International, 2021, 47, 110539.

Keywords: *pancreatic lipase, in vitro, bioprospecting, suspect screening, high resolution mass spectrometry*

Acknowledgement: *This project was supported from the UCT Prague Rector´s Junior Grant for the year 2022 from the funds of the Institutional Plan. Also, this work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

LECTURES

L8

WHEN MORE IS MORE IN PESTICIDE RESIDUE ANALYSIS

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A common paradox in many technical and scientific areas is that of "Less is more" referring to the idea that a smaller quantity might be of higher quality. However, considering the need to increase the scope for both the residues and commodities that are necessary in food control nowadays, this paradox cannot be considered relevant, the challenge being to look for "more is more". Accordingly, a clear target for most laboratories are scopes of more than 500 compounds, and dozens of commodities, in a single, fast multiresidue method, with the lowest LOQs down to 5 ppb - all complying with adequate quality control under internally accepted accreditation guidelines.
Covering these issues is a real challenge for routine laboratories. Nevertheless, they can be achieved when taking into account the rapid evolution of the high-end mass spectrometers coupled to chromatography as well as the help provided by automation and analytical workflow digitalization. These challenges are widely promoted by the EURLs through the support and training they give to the EU official laboratories. It is important to note that it is not only necessary to have effective instrumentation and analytical tools to carry out these objectives, but also the expert skills of the laboratory analysts - this latter focus forms the main effort of the EURLs in their work with the OFLs network. Various examples of these achievements, such as e-learning training activities and the latest proposed and optimized analytical methods, are presented in this talk.

Keywords: *pesticide residues analysis, food control, EURL*

L9

IMPROVEMENT IN ANALYTICAL PERFORMANCE FROM PARTICIPATION IN EU PROFICIENCY TEST ON CEREALS AND FEED

Mette Erecius Poulsen*[1], **Ederina Ninga**[1], **Elena Hakme**[1]

[1] *DTU, National Food Institute, Denmark*
**Corresponding author - E-mail: mpou@food.dtu.dk*

The EURL for pesticide residues in Cereals and Feedingstuff (EURL-CF) has organized 16 proficiency tests on cereals and feeding stuff (EUPT-CF) since 2007. The EUPTs were offered to EU and EFTA National Reference Laboratories (NRLs) and Official Laboratories (OfLs) both in EU and from Non-EU Countries. This has resulted in a high number of participants in each EUPT (up to 178). The cereals; barley, maize, oat, rice, rye, and wheat and the feeds; hay, compound fodder, and rapeseed cake have been used as test material. Data from the first 12 EUPT-CF showed that the Alg A standard deviations was generally decreased to less than 25%. The scope coverage for the NRLs was in general good, while a significant part of the OfLs had poor scope coverage and no clear improvement was seen. The performance concerning precision was positive for the NRLs for both the average z scores and the Average of squared Z scores (AZ^2). Only few NRL were underperforming. Also, the average z scores for the OfLs showed a positive development, even some improvement in AZ2 scores was seen. But a significant number of OfLs were underperforming. This presentation will include data from all 16 EUPT-CF and focus on the individual performance of the NRLs. Around 1/3 of the 38 NRLs has performed well in all the EUPTs. Another third has improved during the 16 years and the rest has not really made any stable improvement according the AZ^2 scores. In 2021 the test item for the EUPT was rapeseed cake, a challenging commodity to analyse, mainly due to the relative high amount of fat (in this material up to 20%). This PT revealed that a substantial part of the participants did not have an analytical method fit for purpose and the Alg A standard deviations were 25-38 %, thus higher than the typical standard deviations obtained in other EUPT-CF. Statistical ANOVA analysis have shown that the calibration approach, milling and water addition before extraction had significant influence the on the results, depending on the chemical property of the compounds.

Keywords: *proficiency test, pesticide residue, ANOVA, analysis of PT results*

L10

EXPERIENCES FROM THE EURL PROFICIENCY TESTS FROM THE EUROPEAN UNION REFERENCE LABORATORY FOR PROCESSING CONTAMINANTS

Arvid Fromberg*[1)]

[1)] *European Union Reference Laboratory for Processing Contaminants, National Food Institute, Technical University of Denmark, Denmark*
**Corresponding author - E-mail: arfr@food.dtu.dk*

The European Union Reference Laboratory for Processing Contaminants (EURL-PC) provides analytical methods for processing contaminants in foods, supporting the analytical quality for the National Reference Laboratories (NRLs) and disseminating relevant knowledge to the NRLs and provides scientific advice to the Commission.
One of the key tasks of development and documentation of the analytical performance by the NRLs and official control laboratories are proficiency tests in accordance with ISO/IEC 17043 as described in Commission Regulation 2017/625.
This presentation will outline the most recent approaches and results of the proficiency tests offered by the EURL-PC, hosted by the National Food Institute at Technical University of Denmark (DTU Food) within the food area. This include the selection and characterisation of the PT materials representing relevant samples for the complex marketed products to be analysed by the NRLs and the establishment of reference values as main benchmark for the assessment of PT results.
Laying down the methods of sampling and analysis for the control of the levels of processing contaminants in foodstuffs are described in Commission Regulation 2007/333 and the maximum levels for contaminants in foodstuffs set in Commission Regulation 2006/1881. With the inclusion of maximum levels for 3-MCPD fatty acid esters (and 3-MCPD) in this regulation the required scope of the analytical methods has been expanded. For acrylamide with the current Benchmark levels set in Commission Regulation 2017/2158, the focus has been on improving the limit of quantification for acrylamide to be able to meet the low levels set for some of the foodstuffs. Furan and alkylfurans, with the new Commission Recommendation 2022/495 on monitoring the presence of furan and alkylfurans in foods, on top of analysing furan, the recommendation ask for the inclusion of 2-methylfuran and 3-methylfuran and if possible 2,5-dimethylfuran, 2-pentylfuran and 2-ethylfuran. Thereby requiring methods of analysis reliable for this purpose.
The EURL-PC provides PT for acrylamide, furan and alkylated furans, 3-MCPD, 3-MCPD esters and glycidyl esters, and PAH for the EU National Reference Laboratories (NRLs).

Keywords: *EURL-PC, furan, acrylamide, PAH, 3-MCPD fatty acid esters*

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

L11

THE EU REFERENCE LABORATORY FOR MYCOTOXINS AND PLANT TOXINS: ACHIEVEMENTS AND CHALLENGES WITH IMPLEMENTATION OF NEW AND UPCOMING REGULATIONS

Patrick Mulder*[1], **Hans Mol**[1], **Monique de Nijs**[1]

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The past two years have seen the introduction of a number of new EU regulations, particularly in the area of plant toxins. Four regulations were published that have come into effect in the course of 2022. Commission regulation (EU) 2020/2040 describes maximum levels of 35 pyrrolizidine alkaloids in a variety of foodstuffs including (herbal) infusions, food supplements, pollen supplements, culinary herbs and spices. Regulation (EU) 2021/1408 describes MLs for tropane alkaloids in (herbal) infusions, certain unprocessed (pseudo)cereals, maize, as well processed cereal based foods. Regulation (EU) 2021/2142 defines MLs for opium alkaloids in poppy seeds as well as in bakery products. In the mycotoxin field, ergot alkaloids are now regulated in (EU) 2021/2001 for cereal milling products including rye, wheat gluten and processed cereal based foods for infants and young children.
More legislation is underway in 2022. A regulation on delta-9-tetrahydrocannabinol in hemp seeds and hemp seed oil and an updated and expanded regulation on ochratoxin in a wide variety of food matrices are likely to be published in 2022. Recently a commission recommendation (EU) 2022/561 has been published on the monitoring of glycoalkaloids in potatoes and potato derived products. Last, but not least, the current regulation on sampling of mycotoxins (EC) 401/2006 is under revision and a similar regulation is being developed for the sampling related to plant toxins.
The establishment of MLs for this wide variety of substances and matrices presents a considerable challenge for the EURL, NRLs and OLs. Methods need to developed and/or validated, accredited and implemented in the laboratories for Official Control. To facilitate this process continuous efforts are made by the EURL-MP to develop for the various toxin groups methods that are robust and easy to apply. These methods are made available via the EURL-MP website and when needed trainings are organised. The capabilities of NRLs and their progress made in implementing methods for Official Control are monitored via the organisation of proficiency tests. The results of these PTs will be discussed. Work in progress on toxin groups that may become relevant in the near future will be discussed as well.

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

L12

SMART INTERPRETATION OF RESULTS FROM FOOD ANALYSIS: HOW TO USE ALL INFORMATION AVAILABLE

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When validating screening methods, typical performance characteristics addressed in the study are sensitivity, specificity, false positive and false negative rates. If the obtained performance characteristics meet the criteria, the method is considered fit for the intended purpose. However, under real world condition the use of such methods may lead to results that do not corresponds well to the outcome of the method validation study. For instance, a significant fraction of suspect positive samples may turn out to be negative after analysis with confirmatory methods. An elevated number of false negative results will then trigger a couple of questions, such as (1) whether something went wrong during the validation study, (2) the screening method is actually not fit for purpose or (3) what is the overall impact on the selected measurement strategy. Likewise, low values for the specificity obtained in the validation study of the screening method do not necessarily mean that the method is not suitable for a measurement exercise. The purpose of the presentation is to demonstrate that by taking into account additional information during the evaluation phase, a more comprehensive conclusion of the expected benefit of the screening method can be reached. Such an evaluation will also solve the apparent contradiction of results obtained during the validation compared to the results obtained under real world conditions. This additional evaluation involves the application of Bayesian statistics, thus making use of the prior knowledge of the expected portion of non-compliant samples in all samples that are analysed within the screening exercise. The approach is demonstrated on real world examples [1] applying the validation scheme established by European legislation [2].

[1] Lattanzio V.M. T, von Holst C., Visconti A.: Experimental design for in-house validation of a screening immunoassay kit. The case of a multiplex dipstick for Fusarium mycotoxins in cereals. Anal Bioanal Chem (2013) 405:7773–7782
[2] Commission Regulation (EU) No 519/2014 as regards methods of sampling of large lots, spices, and food supplements, performance criteria for T-2 and HT-2 toxin and citrinin and screening methods of analysis. L147/29

L13

ANALYSIS OF METALS AND NITROGENOUS COMPOUNDS - EXPERIENCES AND ANALYTICAL CHALLENGES FROM THE PERSPECTIVE OF THE EURL-MN

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The National Food Institute, Technical University of Denmark, hosts the European Union reference laboratory for metals and nitrogenous compounds in feed and food (the EURL-MN). The EURL was formed in 2018[1] and succeeds the former EURLs EURL-HM (Heavy metals in feed and food) and EURL-CEFAO (Chemical elements in food of animal origin). The scope of the new EURL was extended to include all metals, and nitrogenous compounds was added as a new group of compounds. Hence, the EURL-MNs area of competence include all metals, other elements, and nitrogenous compounds in feed and food. The EURL leads a network of national reference laboratories in EU, EFTA and EU candidate countries and the network currently includes more than 60 laboratories.
As a EURL, we contribute to the improvement and harmonisation of analytical methods used for official feed and food control. Our activities covers the regulated[2, 3] compounds (e.g. arsenic, inorganic arsenic, cadmium, lead, mercury, nitrite and nitrite) and compounds of monitoring and/or possible regulatory interest (e.g. aluminium, nickel and N-nitrosamines. In this talk, we will present some of the experiences made and present ongoing work on method development, including some of the analytical challenges encountered. [1] Commission Regulation (EU) 2018/192 [2] Directive 2002/32/EC, and later amendments [3] Commission Regulation (EC) 1881/2006, and later amendments

Keywords: *EURL, regulation, MLs, feed, food*

L14

IMPORTANCE OF REGULATORY KNOWLEDGE WHEN TRANSFORMING THE SCIENCE TO THE COMMERCIAL FOOD AND FEED PRODUCTS

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A large amount of innovation, science and an array of scientific studies are needed throughout the Research and Development (R&D) process to create new food or feed products. New regulated food and feed products, such as novel foods, food and feed additives and food contact materials, require a pre-market authorisation prior to they can be lawfully sold on the markets, for example in the European Union (EU), United Kingdom (UK), United States (US), Canada, Australia, Brazil, China or Singapore. In the EU, the European Commission (EC) grants the authorisation following the application, while in the UK, Food Standards Agency (FSA) and in the US, Food and Drug Administration (FDA) are the respective authorising bodies.
When applying for authorisation of a new regulated product, an application dossier has to be supplied with administrative, technical and safety information to the evaluating body. In the EU, the dossier is evaluated by European Food Safety Authority (EFSA), and FSA in the UK and FDA in the US. Technical information comprises, e.g. data on manufacturing process, chemical composition of the new food or feed product and its chemical and microbiological contaminants. Safety information ranges from toxicological data on humans, animals and farm animals to the data on toxicological impacts on the environment. Also, data on nutrition, allergenicity and efficacy of the new product may be needed.
The above implies that the developer of the new food or feed product not previously authorised needs to know which data are required by the authorities and which quality these data need to have. As the R&D is very costly and time consuming, it is better to know the data requirements at an early stage of the entire R&D project in order to be on the right foot from the very beginning. The regulatory authorities provide detailed and extensive guidance documents on the technical and scientific data requirements. While these guides are very helpful, they also require a good scientific, regulatory and risk assessment knowledge to interpret them right. Sometimes the EC, FDA or FSA needs to be contacted directly to get the right legal interpretations.
It is of utmost importance for a successful dossier that its scientific data are generated with high quality methods; be them then toxicological or analytical methods. A laboratory accreditation and proper method validation following the internationally accepted protocols are the key elements for this success. Well-established targeted analytical techniques are very important in most of the analyses, while non-targeted analytical methods, e.g. different omics approaches, can provide highly useful tools both for compositional and toxicological studies.
Among other things, these aspects highlighted above will be further elaborated and discussed together with the examples how the regulatory knowledge assists in the R&D while converting the science to the new commercial regulated food and feed products.

Keywords: *novel food additives, regulatory-authorisation, analytics*

L15

HALOGENATED POPS IN FEED AND FOOD – RECENT DEVELOPMENTS IN THE EURL/NRL NETWORK

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With the extension of the scope of the European Union Reference Laboratory (EURL) for Dioxins and PCBs in Feed and Food to all halogenated persistent organic pollutants (POPs) in 2018, the focus of the network of EURL and National Reference Laboratories (NRLs) has been expanded to cover a broad spectrum of different halogenated POPs. This includes brominated contaminants such as polybrominated diphenylethers (PBDEs), hexabromocyclododecanes (HBCDDs) and emerging brominated flame retardants (eBFRs), fluorinated contaminants such as per- and polyfluoroalkyl substances (PFAS) and other chlorinated contaminants such as chlorinated paraffins (CPs) and polychlorinated naphthalenes (PCNs).
As a consequence, analytical methods to determine the above listed additional analyte groups have been developed and validated by the EURL POPs. Furthermore, core working groups (CWGs) have been formed within the EURL/NRL network covering different analyte groups. In these CWGs independent experts as well as experts from NRLs are involved to develop analytical criteria and guidance for the non-regulated analytes. The outcome of this work was published as articles and analytical guidance documents for the analysis of CPs, PBDEs and HBCDDs, and PFAS in feed and food (eurl-pops.eu).
The extension of the scope is also reflected in the organization of EURL proficiency tests and interlaboratory studies. In addition to proficiency tests for a broad spectrum of analytes (i.e. covering PCDD/Fs, PCBs, PBDEs, HBCDDs, PFAS, CPs and other brominated contaminants), specific proficiency tests and interlaboratory studies were organized for certain matrix/analyte combinations, in particular for CPs, PFAS, PCNs and brominated contaminants.
Another activity is the EURL POPs collaboration within the network of the four EURLs for contaminants, including 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).Guidance documents or recommendations on analytical and regulatory issues of interest to all four EURL/NRL networks are jointly discussed and published, aiming for a broader harmonization within the field of contaminants.

Keywords: *halogenated POPs, analysis, EURL, guidance document*

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.*

L16

MID-INFRARED PHOTONIC SOLUTIONS FOR SOURCE TRACKING OF FUNGAL CONTAMINAITON IN AQUAPONIC PRODUCTIONS

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Filamentous fungi are one of the most resilient food spoilage microorganisms which can survive under different processing conditions used in a modern farm-to-fork production. Under certain conditions some food spoilage fungi produce secondary metabolites – mycotoxins - which are often characterized by high cell toxicity and considered to be carcinogenic. The pre- and postharvest fungal spoilage and mycotoxins contamination is the cause of approximately 25% of the global food supply loss. Novel food productions such as aquaponic and hydroponic are closed systems characterized by a high temperature and humidity. Such production conditions can trigger undesired fungal growth and wide spread of fungi in the system. Fungi-associated spoilage outbreaks in aquaponic and hydroponic productions pose a clear challenge to the production and require early detection and identification of fungal contaminations. One of the reasons to the challenges is the very limited knowledge on mycobiota from aquaponic/hydroponic productions and lack of established approaches for fungal source tracking for this type of productions. Fungal detection and identification are critical steps in source tracking. These are usually performed by applying traditional phenotypic and genotypic methods which are expensive, time consuming and require involvement of specialized personnel. Therefore, there is an urgent need to for robust rapid and cheap technologies to perform fungal source tracking. Among the optical/spectroscopic methods, mid-infrared (MIR) spectroscopy has proven to be one of the most reliable analytical techniques for probing molecular fingerprints of microbial and chemical contaminations in food via molecular spectral signatures. The on-going EU project PHOTONFOOD aims to provide a portable solution for flexible farm-to-fork sensing of fungal and contamination in food products and along the food production chain. The EU-funded project is developing an integrated solution that combines innovations in MIR sensing with smart paper-based sample treatment, and advanced data analysis. The solution will be validated and demonstrated in real scenarios along the food value chain including aquaponic productions. As a part of a first study, we applied MIR spectroscopy to characterize the mycobiota present in the aquaponic production of herbs and established a source tracking approach to identify the sources of fungal contamination along the production. A set of samples of water, air, soil, plants, and fish feed materials were obtained in an aquaponic company. Fungi were isolated by standard agar plating method using selective media. Further, isolated fungi were typed by high-throughput screening (HTS) Fourier Transform Infrared (FTIR) spectroscopy and phylogenetic discrimination was done by multivariate data modelling using in-house FTIR database of food spoilage fungi. In addition, we evaluated a presence of chemical contaminants for samples from aquaponic production.

L17

RAPID AUTHENTICITY VERIFICATION AND FRAUD DETECTION USING A PORTABLE HANDHELD LASER-INDUCED BREAKDOWN SPECTROSCOPY (LIBS) SYSTEM

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The increasing incidence of food fraud cases necessitates the development of novel technologies for identifying fraudulent actions, which often entail substituting superior quality ingredients or commodities with cheaper, but lower-quality ones, mislabeling products, or using unapproved taste and appearance enhancements. Site visits involving inspections and sampling require quick, reagent-free, and portable detection systems for authenticity verification. Laser-induced breakdown spectroscopy (LIBS) is an emerging, highly promising technology initially developed for material science applications. It has recently gained recognition as a potential tool for the characterization of agricultural products and biomaterials due to its efficiency, minimal sample preparation, and speed. In our research, we demonstrated the applicability of a commercial handheld LIBS instrument initially marketed for material science applications, such as alloys testing, to food analysis and compared its performance to that of a custom-designed benchtop lab system for elemental analysis. In experiments, we prioritized high-value regional agricultural products. Both LIBS systems assessed solid samples consisting of seven coffee varieties, sixteen Alpine-style cheeses, and eight distinct spices. These specimens required no sample preparation. The liquid samples, including seven balsamic vinegars and six vanilla extract examples, were deposited onto a nitrocellulose membrane for measurements. Our analytical process utilized one hundred rapid LIBS laser shots, producing an averaged spectrum for a sample region. Multiple regions were interrogated to account for product inhomogeneity. The low signal-to-noise spectra resulting from errors in sample positioning were automatically discarded. The remaining standardized LIBS spectra were processed for dimensionality reduction using a univariate feature selection procedure. Subsequently, the most predictive spectral features were utilized for training and evaluating several state-of-the-art supervised classifiers. The ante-hoc explainability and embedded multivariate feature selection capability favored the elastic net-regularized multinomial classifier over competing techniques such as neural networks and support vector machines. In contrast to many "black box" machine learning systems, the proposed approach not only reached remarkably high accuracy but was able to identify the key combination of predictive chemical elements, characterizing the analyzed samples. The results indicate that field-deployable, portable LIBS devices that require minimal sample preparation provide an easy-to-use platform for agricultural product verification. Our findings also suggest that portable analytical methods, such as LIBS, should play a more significant role in the detection of food fraud, supplementing, or, in many cases, replacing the more complicated and expensive laboratory benchtop devices.

Keywords: *LIBS, food fraud, spectroscopy, authenticity, machine learning*

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L18

PORTABLE LAMP DIAGNOSTICS FOR FOOD SAFETY

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Safe and healthy food is essential for life. With the current shift towards a more sustainable economy and society, and the increasing effects of climate change, new challenges and risks are emerging in the food production and distribution chains. In parallel, a shift is observed in consumer awareness and participation with respect to food and its safety, production and security. Consumers nowadays expect food on the market to be safe, authentic and traceable, but are simultaneously aware of the potential fallibility of the food production system.
The combination of these emerging challenges, risks and increased consumer awareness has greatly contributed to the rise of rapid diagnostic tools in food safety control. Portable and consumer-operable tools that can be used on-site by producers, consumers or inspection authorities show applicability for food safety analysis, so that they can act promptly in case of a non-conformity.
Loop-mediated amplification (LAMP) is an isothermal amplification technique that is ideal to be used on-site due to the fact that no expensive, power consuming equipment is needed. LAMP can be used to a broad range of applications, utilizing various detection options, such as (real-time) fluorescent detection, colorimetric dyes, and lateral flows. However, although sensitivity and specificity of LAMP is similar to qPCR, in some cases, additional specificity and/or robustness is required, e.g. when discrimination between pathogenic and non-pathogenic species is only possible at single nucleotide level or when very crude DNA extracts are used. To achieve increased specificity and robustness, we have studied a one-pot LAMP/CRISPR-Cas system without adding any additional operational complexity.
Here, we report the applicability of *in house* designed easy-operable LAMP and LAMP/CRISPR-Cas assays for a variety of targets in *real-life* food samples.
A duplex poultry LAMP assay shows sensitive and specific detection down to 0.01% chicken or turkey in processed sausage, even when a simple, on-site DNA extraction method is applied – achieving similar sensitivity to singleplex qPCR in combination with lab-based DNA extraction. For food pathogens*Vibrio parahaemolyticus, Salmonella spp.* and *S. enterica enterica* LAMP/CRISPR-Cas assays have been developed for detection in complex shell fish matrices. Down to 180 copies of *V. parahaemolyticus* per reaction results in amplification in a clean matrix, and in swabs taken from spiked mussel intestine 1000 copies are detected in less than 45 minutes from sampling to result. In addition, general animal, plant and shell fish LAMP assays have been developed as internal controls to show correct performance of the procedure.
These results show that the combination of LAMP with on-site DNA extraction and specific, sensitive and robust detection has great potential to help combat the emerging risks and challenges in food safety and authenticity control.

Keywords: *LAMP, LAMP/CRISPR-Cas on-site, Salmonella*

L19

MONITORING OF FERMENTATION PROCESSES BY GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY (GC-IMS) AND MACHINE LEARNING

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Gas chromatography hyphenated to ion mobility spectrometry (GC-IMS) is a powerful two-dimensional separation and detection technique for volatile organic compounds (VOC). Low detection limits (low ppb to ppt by volume), high selectivity and robust operation characterize it as an ideal tool for non-target screening (NTS) of complex sample materials. Due to the high sensitivity, headspace sampling without enrichment is commonly used. Exhaust gas analysis from fermentations allows to generate profiles of volatile, extracellular metabolites without interference or contamination of the process, as it is easily available. The obtained 3-dimensional GC-IMS data can then be used to characterize the fermentation and predict target variables that are difficult to be measured directly e.g., the formation of a product or potential deviations in a process. This talk presents results from an offline proof-of-concept study which demonstrates that *E. coli*, *S. cerevisiae*, *L. brevis* and *P. fluorescens* can be categorized simply by VOC profiling as a first step towards detecting contaminations. Further, the transition to online measurement and data analysis with a new GC-IMS prototype are explained. Data analysis was carried out using the new inhouse-developed Python package "gc-ims-tools" for data specific I/O, preprocessing, and visualizations which is available under the BSD 3-clause license at https://github.com/Charisma-Mannheim/gc-ims-tools.

Keywords: *non-target screening, contamination detection, python, chemometrics, exhaust air VOC profiling*

L20

TOWARDS PORTABLE ON-SITE MYCOTOXIN DETECTION: PAPER MICROFLUIDICS WITH MID-INFRARED SPECTROSCOPIC DETECTION OF DEOXYNIVALENOL IN WHEAT

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The increasing demand for food and feed products is stretching the capacity of the food value chain to the extent that in the forthcoming years it will reach a limit. Moreover, the food industry needs to comply to ever more stringent standards for food and feed safety, and other sharpened guidelines. Disturbingly, over the past decade mycotoxin occurrence increased as a result of climate change, which endangers both food safety and security [1,2]. Achieving food safety along the entire value chain requires fast, easy, and accessible monitoring of contaminants. However, to date samples are often taken at the production location and then sent to an accredited laboratory for analysis. Mycotoxin analysis is typically performed with high-end instrumentation, after extensive sample preparation, which can take days. Therefore, there is a pressing challenge to translate laboratory-based procedures into rapid and truly on-site approaches suited for non-experts. These challenges include (i) sample acquisition & extraction, (ii) selective capture & enrichment, (iii) detection and (iv) interpretation.
Here, a proof-of-concept is presented to tackle this challenge in the context of the analysis of a potent mycotoxin, deoxynivalenol (DON) in wheat samples. In this concept, we established the first the combination of paper microfluidics with mid-infrared spectroscopy detection. Such analysis of an analyte on a paper substrate requires careful design of the methodology. Thus, we established criteria for the (Attenuated Total Reflection – Fourier Transform Infrared) ATR-FTIR detection of DON, as a framework for a standardized on-paper mid-infrared spectroscopy approach. For this framework, we developed a data analysis methodology with which we demonstrate concentration dependency of deoxynivalenol on paper. For this, we have selected a spectral region in which we systematically discriminate DON from paper in the ppm range. Furthermore, to lower the limit of detection and comply to the current regulation limits for DON an immuno-enrichment approach is exploited [3]. Here, paper microfluidics was used to selectively capture and enrich DON from wheat samples and analyze by ATR-FTIR. In this proof of concept, An immuno-enrichment zone was created on the paper substrate that is specific for DON and to obtain chemical information from the enrichment zone, we exploited the developed ATR-FTIR framework proposed above.
This proof-of-concept for DON detection, in a real-life wheat sample is the first demonstration of paper-based mid-infrared detection for mycotoxins. Furthermore, the established framework addresses challenge (ii) of selective capture and enrichment, advancing towards on-site approaches for non-experts. In the future, this framework can be implemented with miniaturized mid-infrared techniques that are developed within the EU project PhotonFood, as a novel approach for portable food contaminant detection.

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Keywords: *paper-based microfluidics, mid infrared, mycotoxins, on-site analysis, spectroscopy*

L21

RAPID ON-SITE TOOL FOR SEMI-QUANTITATIVE SCREENING OF THC ANALOGUES IN CANNABIS BY "DIGITAL" CHROMATOGRAPHIC SEPARATION FROM INTERFERING CBD ANALOGUES, FOLLOWED BY CHROMOGENIC SMARTPHONE DETECTION

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The expansion of the cannabis industry and related products urgently requires high-throughput, low-cost methods for cannabinoid analysis in a variety of matrixes, to assess the presence and amount of psychoactive compounds. The main psychoactive cannabinoid Δ9-tetrahydrocannabinol (THC), its acidic precursor tetrahydrocannabinolic acid (THCA, potentially psychoactive), and degradation product cannabinol (CBN, ~10% THC potency) are of most interest in this regard. However, these cannabinoids (THC analogues, characterized by a pyran ring and a single alkene C=C bond) usually coexist with cannabidiol (CBD) and its analogues (characterized by two olefinic C=C bonds and a second phenolic group, e.g., cannabidiolic acid (CBDA) and cannabigerol (CBG)) in cannabis varieties and products. Although THC analogues and CBD analogues have completely different physiological activities, most colorimetric tests used for quick screening produce color in the presence of members from either group, hampering the selectivity towards only the psychoactive species.[1] Therefore, it is crucial to develop on-site screening tools that allow full separation of THC and CBD analogues prior to a fast and convenient readout.
In a previous work, we have separated THC from CBD by argentation HPLC, and induced discriminable fragmentation of silver adducts in mass spectrometry. This was based on the different affinity of Ag (I) towards a single double bond (weak; e.g. in THC analogues) and 1,5-dienes (strong; e.g. in CBD analogues).[2] Here, we hypothesized that modifying a silica gel TLC plate with an Ag(I) retention zone and non-coated detection zone would enable a "digital" separation of strongly retained CBD analogues and poorly retained THC analogues. After extensive optimization, "digital" chromatographic separation was achieved within minutes, with CBD analogues kept in the Ag(I) retention zone and THC analogues moved up to the detection zone. This separation was assessed using colorimetric reagents (Fast Blue BB) and further confirmed by HPLC-MS/MS. The resolution (Rs) between the closest two spots from the two groups was 4.7, almost 8 times higher than the resolution on an unmodified silica TLC plate. Next, smartphone-based color analysis enabled semi-quantification of the total percentage of THC analogues. The method was validated by analyzing artificial samples and real samples and showed a good correlation with HPLC-UV (R^2 = 0.97). In a desiccator, Ag(I) TLC plates can be stored for at least three months without performance loss. The developed method requires no special equipment. Due to its simplicity and low operating cost, it has great promise for on-site applicability, e.g., for conducting large-scale surveillance programs for the quick detection and content determination of cannabinoids, cannabis freshness identification or THC screening in CBD products.

(1) Bruni et al., *Braz. J. Anal. Chem.* 2021, 9(34), 52-78.
(2) Huang et al., *Anal. Chem.* 2021, *93*, 3794-3802.

Keywords: *THC analogues, CBD analogues, "digital" chromatographic separation, smartphone detection, rapid on-site tool*

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L22

WHICH TECHNIQUE TO ASSESS THE PRESENCE AND ABSENCE IN FEED OF AUTHORISED AND NON-AUTHORISED INSECT SPECIES?

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Since the emergence of the bovine spongiform encephalopathy (BSE) in 2001, the European Commission takes a series of measures in order to protect the consumer. With the intention to keep control over the presence of unauthorised processed animal proteins in feed, two methods have been developed and validated at European level and were included in the Commission Regulation (EC) No 152/2009. These official methods are light microscopy (LM) and polymerase chain reaction (PCR). These methods are applied according a Standard Operating Procedure (SOP) established by the European Reference Laboratory for Animal Proteins (EURL-AP) to assure a harmonised implementation across the different European national laboratories.
Based on EFSA's recommendation, the European authorities agreed to introduce the use of insects for feeding aquaculture animals (farmed fish) as of July 2017. A closed list of seven insect species was firstly authorized to be reared and used in aquaculture was established. More recently, in August 2021, the European Commission has decided to revise the feed ban by authorising the use of processed animal proteins derived from farmed insects to be used in pig and poultry in feeds. This introduction raises questions about the methods to be used for quality control as well as contamination and fraud detection. The purpose of this presentation will be to give an overview of official/existing methods, the limitations and the need of developments of new analytical methods and strategies.

Keywords: *processed aniamal protein, feed, insect meal, control, analysis*

L23

EXPLORING FLAVOR DEVELOPMENT IN FERMENTED FOODS BY REAL-TIME HIGH-THROUGHPUT PTR-MS ANALYSIS

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Humans have used fermentation for millennia as a strategy to preserve perishable food and improve nutritional value. Currently, fermented products represent around one-third of our food consumption. The result is an extraordinary variety of products that provide the basis of the world's enogastronomic heritage and hold considerable economic, social, and environmental importance. Now fermentation is being repurposed as a sustainable strategy to improve the quality and safety of agri-food production.
During fermention processes, microorganisms produce volatile organic compounds (VOCs), which are present in the final products and these VOCs may influence sensory perception during product consumption. Therefore, following the generation of VOCs during fermentation enables an improved understanding and control of *i)* the bioprocess dynamics, *ii)* the quality/safety of the products, and *iii)* the flavour of final products. In addition, it will support the development of improved fermented meat and dairy analogue products.
Direct injection mass spectrometry (DIMS) allows direct and green analyses, providing on-line and real-time measurement of a given sample's VOCs without treatment, extraction or chromatographic separation. In recent years, we used DIMS to investigate VOC generation across diverse fermented matrices (e.g., dairy-based, cereal-based, plant-based, meat-based fermented products) and the impact on VOCs of different microorganisms (i.e. bacteria, yeasts, filamentous fungi) and process parameters (e.g., fermentation time and temperature).
Here we outline applications of DIMS, with a focus on how technical advancements associated with Proton Transfer Reaction Time-Of-Flight Mass Spectrometry (PTR-TOF-MS) have led to an improved understanding of VOCs generated in fermented foods and beverages. We report how integration of an autosampler, and tailored data analysis have allowed an effective high throughput approach to simultaneously follow fermentations from start to finish across several strains, substrates and fermentation temperatures with robust replication. We also show how additional tools (switching reagent ion system, fast GC, tailored sampling) have improved the depth of the analytical information collected.
The case studies (wine, beer and bread) discussed demonstrate how the analytical strategies outlined above, allow an improved understanding of fermentation processes and VOC generation pathways. More specifically they show the ability to characterize *i)* different starter cultures, *ii)* diverse microorganism/food matrix combinations, *iii)* how flavor generation is affected by interactions between microbial strains, and *iv)* indroduction of specific flavor notes by microbial strains.
Microbial-based processes are key technologies for a sustainable future food production, with DIMS offering a time-saving, and low-impact analytical approach, which together can support a sustainable agrifood system.

Keywords: *volatilome, fermentation, direct injection mass spectrometry, proton transfer reaction mass spectrometry, microbial strains*

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L24

ALKYL PYRAZINES DETERMINATION BY GAS CHROMATOGRAPHY – ION MOBILITY SPECTROMETRY. THE ROASTED HAZELNUT CASE STUDY

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Gas chromatography coupled with ion mobility spectrometry (GC-IMS) is an emerging analytical technique which is rapidly gaining popularity in food flavour analysis due to its robustness, high sensitivity and the second-dimension separation provided by IMS.
Most of the current studies are based on untargeted fingerprinting and qualitative approaches. However little research is focused on quantitative studies targeting specific classes of aroma compounds. This is due to two peculiarities of the IMS working principle that makes the quantification challenging: (i) the formation of multiple ionized species (monomer and dimer) from a single analyte, and (ii) the non-linear detector response.
In this study, we focused on alkyl pyrazines and their content determination in roasted hazelnuts. Pyrazines, which are Maillard reaction products generated during thermal treatment, are responsible for roasty and earthy notes characteristic of roasted food matrices. Several alkyl pyrazines have been reported as roasted hazelnut key-odorants. Due to their low odour-thresholds, they are crucial to determine the aromatic profile even though their concentrations in kernels is low (ng/kg), thus providing an interesting case study for a GC-IMS targeted and quantitative approach.
The concentration-response curves of 8 alkyl pyrazines over several orders of magnitude of concentrations (0.1-100 µg/g) have been studied in two different model matrices (a mix of medium chain triglycerides – MCT – and a hazelnut paste physically treated to remove the majority of volatiles components). The results showed a non-negligible matrix effect, explained by the different fat percentage in MCT and hazelnut paste, and a relevant impact of the pyrazine ring substitution pattern on the concentration-response curve trends, highlighting the need of an external standardization approach to perform a reliable quantification. Five alkyl pyrazines have then been identified in hazelnut paste samples obtained by roasting kernels from different geographical regions (Italy and Turkey).
The implementation of a quantitative approach extends the GC-IMS applicability for targeting specific aroma compound classes. This methodology could be successfully applied for food flavour characterization in the agro-industrial field.

Keywords: *GC-IMS, quantitative targeted, aroma compounds*

Acknowledgement: *Maria Mazzucotelli is grateful to Soremartec Italia and Giract (Giract's European PhD in Flavor Research Awards Programme) for the financial support.*

L25

ISOALLERGENE SPECIFIC QUANTIFICATION OF THE APPLE ALLERGEN MAL D 1 IN DIFFERENT APPLE SAMPLES

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Due to their bioactive compounds apples contribute to a healthy diet, but in Northern and Central Europe up to 70% of patients suffering from birch pollinosis develop an adverse reaction to fresh apples.[1] This is due to a cross reactivity between the allergen Mal d 1 in apples and Bet v 1 in in birch pollen. Clinical studies indicate a marked difference in the allergenic potential of different varieties.[1-3] However, these discrepancies in allergenicity cannot sufficiently be explained by the Mal d 1 content. Therefore, one hypothesis is that the isoallergens of Mal d 1 exhibit different allergenic potentials. So far, immunochemical and gene expression studies are applied for allergen quantification, but these methods are not suitable to quantify Mal d 1 isoallergen specific. To elucidate the impact of different isoallergenes on the allergenicity requires a reliable isoallergene specific quantification method.
Therefore, a mass spectrometric bottom-up proteomics approach for the quantification of Mal d 1 isoallergenes by isotope dilution analysis was developed. Isoallergen specific markers for Mal d 1.01-1.06 were identified as well as combination markers representing several isoallergens. Based on two global markers the total Mal d 1 (1.01-1.09) was quantified. The application of individual, combination and global markers enables the verification of results. A modified r-Mal d 1 used as an extraction standard allows to monitor and to correct differences in extraction efficiencies between various varieties.
Variety specific differences in the isoallergen profiles were obvious. A trend to lower Mal d 1 contents in traditional varieties was found, but some commercial varieties, described as highly allergenic, also showed lower Mal d 1 contents in the range of traditional apples. Marginal effect of the cultivation method was observable in the samples we investigated. However, a marked increase in the Mal d 1 content and changes in the isoallergen profiles, highly dependent on time and conditions, were evident during storage.
Our new approach using bottom-up proteomics allows the isoallergene specific quantification of Mal d 1 and additionally compensating differences in extraction efficiencies among varieties. So far, we demonstrated for the apples investigated that in addition to the variety, different parameters also influence the Mal d 1 content in apples. In future research this method will be applied to further clarify the impact of the Mal d 1 content and profile on the allergenicity. Moreover, it allows the identification of cultivar and exogenic factors, e.g., storage conditions, influencing the Mal d 1 content and profile in apples.

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Keywords: *bottom-up proteomics, apple allergy, storage effects, inter-replicate variation, traditional vs. commercial apple varieties*

Acknowledgement: *Andreas Siegele is thanked for the apple samples provided. Rachel Wood and Sven Richter are acknoledged for their help freeze drying the samples and Jens Brockmeyer and Alexandra Klußmann are thanked for discussions during method development.*

L26

FAST CENTRIFUGAL PARTITION CHROMATOGRAPHY: DEVELOPMENT OF THE METHOD FOR ISOLATION OF PHYTOCANNABINOIDS

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Fast centrifugal partition chromatography (FCPC) is a preparative chromatography method based on the interaction of two immiscible solvent mixtures. The FCPC is primarily used for the separation and isolation of biologically active compounds. In this work, a new method was developed for the separation and isolation of phytocannabinoids, biologically active compounds of the plant *Cannabis sativa* L. The primary goal was to separate the positive-acting cannabidiol (CBD) and its precursor, cannabidiol acid (CBDA), from psychotropic compounds of the tetrahydrocannabinol group (Δ^9-THC, Δ^9-THCA-A) in hemp extract. Based on literature, 38 model mixtures of solvents were selected. Partition coefficients (K_D) and separation factors (α) were calculated for each mixture based on the phytocannabinoids presence, which was determined by UHPLC-HRMS. The fallowing system was selected as the most suitable; heptane:ethyl-acetate:ethanol:water (1.5:0.5:1.5:0.5; *v:v:v:v*), which meet the goal of the work – allow the separation of CBD/CBDA from substances of the tetrahydrocannabinol group. Additionally, this solvent system allows to separate CBD from CBDA, which could be used for the creation of analytical standards or for the further research of biological activity. The purity of CBD and CBDA fractions was finally evaluated by UHPLC-DAD and UHPLC-HRMS and the results were discussed with literature.

Keywords: *cannabis sativa L., counter-current chromatography, isolation and separation method, phytocannabinoids*

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L27

COMPARISON OF CHROMATOGRAPHIC CONDITIONS FOR THE TARGETED TANDEM MASS SPECTROMETRIC DETERMINATION OF 344 MAMMALIAN METABOLITES

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Metabolomics, the process of measuring a wide range of small molecules in any biological system, has become an increasingly popular "omics" approach in biomedical research and nutritional analysis [1]. Comparatively, the use of modern metabolomics technology in livestock research is still lagging behind [2]. To develop a set of targeted multi-analyte LC-MS/MS methods for metabolite quantification and biomarker discovery, we constructed a metabolite library with 344 mammalian metabolites from 19 compound classes, including sugars, amino acids, carboxylic acids, nucleotides, and various lipid classes. We then optimized multiple selected reaction monitoring transitions for each compound on a triple quadrupole mass spectrometer. Subsequently, we compared the retention profiles of our metabolite library across different chromatographic conditions: three reversed-phase (RP) methods (C18, F5, C18 under lipidomics conditions), three hydrophilic interaction liquid chromatography (HILIC) methods (bare silica-, zwitterionic-based HILIC, zwitterionic-based HILIC at pH 9) as well as anion exchange chromatography (IC). In line with previous findings [3], our results show that RP and HILIC are complementary to each other. Both IC and RP using apolar solvents increase analyte coverage. Compared to RP, HILIC methods improve the coverage of polar metabolites such as amino acid related compounds or biogenic amines, while carboxylic acids, nucleotides, and sugar phosphates are predominantly targeted by IC or HILIC under basic conditions. This extensive survey of both chromatographic and MS properties provides a diverse and comprehensive dataset, which will facilitate the development of quantitative targeted LC-MS/MS methods for livestock metabolomics.

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Keywords: *metabolomics, mammalian metabolites, targeted LC-MS/MS methods, high-performance liquid chromatography, retention profile*

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L28

QUALITY ASSURANCE SAMPLES IN NON-TARGETED ANALYSIS – MAKING USE OF THE WHOLE SPECTRA INFORMATION THROUGH MULTIVARIATE ANALYSIS IN A USER-FRIENDLY ROUTINE

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The authentication of food and feed materials is of high relevance to prevent consumer deception and reveal possible health risks due to fraudulent practices. For this purpose, spectroscopic techniques like nuclear magnetic resonance spectroscopy (NMR) or infrared spectroscopy (IR) are more and more applied in a non-targeted manner. From the perspective of the German Federal Institute for Risk Assessment it is essential that similar to classical targeted methods an adequate quality assurance is implemented to get reliable results and observe systematic errors. For this purpose, quality control samples are typically measured along with the routine samples, with increasing frequencies also in non-targeted studies. However, in literature these quality control samples are mostly only evaluated in a quantitative way, neglecting the multivariate data evaluation applied in non-targeted analysis.
With regard to the use of non-targeted methods in routine analysis and official control there is a demand for reliable and widely accepted analysis of the quality assurance samples. Recently multivariate analysis of quality assurance samples based on different distance and density measures was reported by Lörchner et al. [1]. This is a promising approach to evaluate quality assurance samples in a multivariate way, but still using established evaluation charts. In the current work, we adapted the approach to allow the evaluation of NMR measurements with related preprocessing of raw data. Furthermore, partly the detection of outliers reported by Brownfield et al. [2] was implemented in the workflow to extend the range of different outlier detection methods. The entire process of data analysis has been developed as user-friendly KNIME workflow for routine analysis. This includes aspects like reading the raw data, preprocessing, statistical analysis, and the summary of the results in an interactive way. Taken together, we propose the combination of different distance-based outlier detection methods with the visualization as multivariate quality control charts in a KNIME workflow and we like to demonstrate that the assessment of quality assurance samples in non-targeted analysis can be performed quick, easy, reproducible and objective on a daily basis.

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Keywords: *quality assurance, non-targeted spectroscopic analysis, multivariate statistics, authenticity*

LECTURES

L29

PARC PROJECT REAL-LIFE MIXTURES

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The European Commission and the 27 Member States are funding the Partnership for the Assessment of the Risk from Chemicals (PARC) to address future regulatory needs as described in the European Commission's Chemical Strategy for Sustainability. With over 200 partners from all over Europe and a total budget of €400 million, PARC is one of the largest projects of its kind in the world and is thought to be capable to address innovation in regulatory risk assessment. It has a duration of 7 years and is coordinated by the French agency for food, environmental and occupational health, and safety (ANSES). PARC will collect new human biomonitoring data, will improve hazard data collection of untested chemicals, and will deliver new methods for regulatory risk assessment.
There is growing societal concern about combined exposure to multiple chemicals e.g. mixtures of PFAS, mycotoxins, heavy metals or pesticides. Exposure might occur via several exposure routes e.g. oral, inhalation or dermal contact. National institutes responsible for risk assessment of combined exposure to multiple chemicals are frequently asked to respond to societal concerns and consequently scientific methods needs to be developed and harmonised throughout Europe. The PARC project real-life mixture will study the exposure to mixtures on the basis of human biomonitoring (HBM) data. The HBM4EU project, which data collection will be continued in PARC, collected HBM data all over Europe. This HBM data will be studied for mixture patterns in several countries. The combined effect of the chemicals to which an individual is exposed, needs to be summed according to scientific criteria set by the European Food Safety Authority (EFSA). Furthermore, the kinetic conversion of chemicals within the human body needs to be understood. Chemicals might be metabolised before reaching a targeted organ, some chemicals will be excreted within hours while other persistent chemicals such as PFAS might remain for longer years in the human body. Mixtures of chemicals to which individual are most frequently exposed will be further tested to understand the real-life mixture effect.
PARC will also provide innovative methods and capacities to monitor chemicals in appropriate human. Analytical developments will also be performed to identify non-targeted and suspect screening methods to detect emerging contaminants and support the monitoring of real-world mixtures. However, data from such methods might not be accepted for the purpose of regulatory risk assessment. The observation from these analytical techniques might set priorities for developing biomarkers of exposures.

Keywords: *human biomonitoring, risk assessment, mixture risk assessment, mixtures*

Acknowledgement: *PARC – Partnership for the Assessment of Risks from Chemicals. Co-funded by the European Union*

L30

EDIBLE OIL QUALITY: RAPID ASSESSMENT OF PROCESSING CONTAMINANTS AND OTHER QUALITY INDICATORS USING CHROMATOGRAPHY AND MASS SPECTROMETRY

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Edible oils and fats are key ingredients of the human diet. They are not only important for the nutritional value of foods, but also for the flavour, taste and mouthfeel. Edible oil quality and safety are primarily determined by the nature/origin of the oil, as well as by the processing applied. Numerous chemical compounds from a large number of chemical classes are relevant. These include waxes and alkanes, MOSH/MOAH contaminants, PAHs, pesticides, dialkylketones, tocopherols, glycidylesters, 3-MCPD esters, oxidized lipids, residual solvents and other volatiles, cyanogenic glycosides, polyphenols, etc. Some of these species are naturally present in the oils, others are formed during processing of the oil or during storage of a finished food product. Some compounds are typical 'baddies' that should not be there, like PAHs and pesticides or the naturally occurring cyanogenic glycosides, others are 'goodies', like tocopherols and polyphenols. Processing can change the levels of both the goodies and the baddies, where the ideal processing would remove the baddies that are present with neither generating new baddies nor affecting the levels of the goodies. Driven by the desire of the consumers for more natural, less processed foods, food industry is looking into new methods for food preparation. The question how this affects the levels of goodies and baddies is essential. Advanced analytical methods are needed to support this research.
In the presentation new, broad-scope chromatography-MS methods will be discussed that allow rapid monitoring of the levels of multiple classes of edible oil and fat ingredients in one run. Given the large variety of compounds groups that are relevant, no single chromatographic system will be able to cover the huge variety of species ranging from non-polar alkanes to highly polar oxidized lipids, or from the smallest residual solvents to the very large, polymerised lipids. Comprehensive LC×LC will be used for a broad coverage of oxidized compounds, multi-compound LC-MS/MS is shown to be able to detect numerous compounds if levels are not too low, and fast GC-MS will be employed for studying volatiles in fresh and aging foods. GC×GC finally is employed to identify the cause of off-flavour development in plant-based foods over time.

Keywords: *edible oils and fats, comprehensive chromatography, edible oil quality, processing contaminants*

L31

BENCHMARKING OF SOLID-PHASE MICROEXTRACTION, STATIC HEADSPACE AND DYNAMIC HEADSPACE COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY FOR FURAN QUANTIFICATION IN INFANT FOOD

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Furan is a possible carcinogen for humans. The First Infant Total Diet Study conducted by ANSES concluded that furan is a priority contaminant for infants and toddlers. Intended to better assess risks raised for infants, it is necessary to dispose of reliable and robust analytical techniques leading to a more precise quantification of furan. As furan is a volatile compound, analytical methods are based on gas chromatography-mass spectrometry (GC-MS) coupled with an extraction technique. Considering the reactivity and high volatility of furan, the critical aspect is the extraction technique. Most often, Solid-Phase MicroExtraction (SPME) is used for furan quantification. This technique offers a good recovery rate as the fiber has a good adsorption capacity, which is interesting to extract small quantities. However, the adsorption layer is still weak and competition phenomena can lead to variability in measurements from one series of analyses to another and over time. As possible alternatives to reduce these variabilities, two techniques were studied in this work. The first is Dynamic Headspace (DHS) which uses TENAX, a fiber with greater adsorption that could reduce competition phenomena. Automate systems are available, contributing to more reproducible analyses than manual systems. The second approach is Static Headspace (SHS) which lets get rid of competition phenomena between different components on the adsorbent phase, allowing a better reproducibility. However, in the SHS case, the furan recovery rate is weaker due to the absence of concentration step link to the absence of a fiber and the mass detector sensitivity becomes critical: thus Q-Exactive Orbitrap (MS detector) was used in this work. Benchmarking between SPME, SHS and DHS was performed with emblematic infant foods, revealed by ANSES and EFSA as major sources of risks regarding the furan levels present. This concerns apple puree, mixed vegetables, fish meal, powdered infant formula, and infant cereals, highlighting different sources of furan generation (carbohydrates, amino acids, polyunsaturated fatty acids, and carotenoids). To validate the most convenient method for each matrix, a statistical comparison was made by comparing the accuracy profiles of the three methods.

Keywords: *infant food, furan, quantitative method, headspace extraction techniques, gas chromatography-mass spectrometry*

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L32

TARGET AND NON-TARGET FOODOMICS INVESTIGATION OF CHEMICAL CHANGES IN MEAT SAUSAGES INDUCED BY VARIOUS PROCESSING CONDITIONS

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The intake of processed red meat has been associated with several adverse health outcomes, most importantly risk of colorectal cancer. Colorectal cancer is one of the most common cancers in the western world. The mechanism behind carcinogenesis and processed meat consumption is not fully understood. However, this association may involve the presence of processing contaminants classified as potent carcinogens, for example, N-nitrosamines formed during nitrite curing among other factors. Processed meat is a broad term referring to any meat that has been transformed through one or several processes such as salting, curing, cooking, or fermenting. The multitude of potential processes applied, the introduction of additives, as well as the complexity of the chemical composition of meat, could generate a wide range of potentially harmful compounds. In order to get more insight into chemical changes occurring during red meat processing, a new exploratory study is needed involving both target and non-target foodomics approaches.
The present study focuses on the detection and identification of compounds formed in beef and pork sausages during manufacturing. For this purpose, model meat products were prepared under controlled conditions. Chemical changes occurring in those meat products after nitrite curing, liquid smoke flavouring, and barbequing were examined. The target analysis revealed the presence of six volatile and two non-volatile N-nitrosamines in most of the analysed samples. Subsequently, LC-HRMS deep MS/MS profiling coupled with metabolomic and chemometric data processing workflows, were developed and successfully applied revealing chemical differences in profiles and chemical changes occurring as a result of applied manufacturing conditions. The prioritization of detected compounds for further identification was made based on principal component analysis and t-test. Various mass lists, experimental MS1&MS2 database, and ChemSpider were used for supporting the identification of prioritized features. Moreover, if reference spectra were not available in the databases, suggestions for fragmentation of suspect candidates were computed using either MetFrag or CMF-ID. Metabolic pathways were also investigated to provide potential metabolism pathways of detected compounds. For example, 36 metabolites listed in the degradation pathway of naphthalene were detected in samples containing liquid smoke. This study may help in understanding the mechanisms linking processed meat intake and adverse health outcomes by delivering the chemical explanation of changes occurring during red meat processing and manufacturing.

Keywords: *chemical profiling, foodomics, non-target screening, processed meat, LC-HRMS*

L33

SAMPLE POOLING STRATEGY: A REALISTIC OPTION TO STRENGTHEN THE SURVEILLANCE OF FOOD CHEMICAL SAFETY

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Current food chemical safety surveillance relies on high-sensitivity but costly and low-throughput analytical methods, which limits the scope and frequency of monitoring by health authorities and virtually prohibits their use for industrial self-monitoring. To overcome these limitations and improve the surveillance system, a promising option is to implement an approach based on sample pooling (Mallapaty, 2020). Instead of analyzing the samples one by one, this approach consists of using high sensitivity methods to evaluate mixtures of *n* samples in order to identify contaminated samples at higher throughput and lower cost.
This proof-of-concept study undertaken in the frame of the SENTINEL collaborative research project aims to evaluate the potential of the sample pooling strategy for food chemical safety monitoring starting with the analytical monitoring of non-dioxin-like polychlorinated biphenyls (nDL-PCB) in meat. After validating the practical feasibility of producing pools of meat made up of a large number of individual samples (*n* between 10 and 200), evaluating the linearity and analytical uncertainty of the measurement carried out on pools of samples, mathematical simulations based on the pool testing strategy proposed by Dorfman (Dorfman, 1943) have been implemented on a database of meat contamination with PCBs (Dervilly-Pinel et al., 2017) in order to determine the optimal number of individual samples to be grouped together in order to obtain the best cost-benefit compromise. The first results of the different simulations carried out on the meat/nDL-PCBs pair show an expected gain especially in terms of cost by a factor of between 5 and 10.

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Keywords: *chemical food safety, sample pooling, PCBs, mea, mathematical simulation*

Acknowledgement: *This study was supported by the French National Research Agency, project SENTINEL, Contract No. ANR-19-CE21-0011. SENTINEL: High-throughput screening tools for a reinforced chemical safety surveillance of food. Available at https://anr.fr/Project-ANR-19-CE21-0011.*

L34

A MULTI-PLATFORM METABOLOMICS APPROACH TO CHARACTERIZE THE EFFECTS ON THE METABOLISM IN PIGS DUE TO CHRONIC EXPOSURE TO LOW DOSES OF NON-DIOXIN-LIKE POLYCHLORINATED BIPHENYLS

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Recent epidemiological studies show that current levels of exposure to polychlorinated biphenyls (PCBs) remain of great concern, as there is still a link between such exposures and the development of chronic environmental diseases. Adverse effects of PCBs have been reported in humans after accidental and massive exposure, but little is known about the effects of chronic exposure to low-dose PCB mixtures. In this sense, food represents the main source of exposure to PCBs. Furthermore, most studies have focused on the health effects caused by exposure to dioxin-like PCBs (DL-PCBs), although chemical exposure to non-dioxin-like PCB (NDL-PCB) congeners is more significant.

Dietary exposure to Aroclor 1260 (i.e. a commercially available mixture consisting of 98% NDL-PCB congeners) in pigs is investigated as a new contribution to the risk assessment of NDL-PCBs. This animal model has been selected for its similarities with human physiology and to complement previous toxicological studies carried out with more standard animal models. Dietary exposure doses of 20 ng/kg body weight (b.w.) per day during 21 days were applied based on reported population NDL-PCB exposure levels. The metabolomics and lipidomics studies carried out in the framework of this work involve the use of liquid chromatography and gas chromatography coupled to high resolution mass spectrometry (LC-HRMS and GC-HRMS) methods, showing the importance of multiplatform approaches to discover as many biomarkers-of-effect as possible. In this context, the study of metabolic perturbations induced by exposure to a given chemical hazard has recently emerged as an interesting alternative approach to apply in chemical risk analysis [1].

The combination of data from all three platforms provided a significant picture of the effects of NDL-PCBs on metabolism in pigs, mirroring evidence from the literature. In particular, the effect of NDL-PCBs on central energy metabolism, such as the citric acid cycle, the glycolysis, and the pentose-phosphate pathway, could be highlighted. This was also reflected in an enhancement of lipogenesis and linoleic acid metabolism. The kynurenine-serotonin balance and the bile acid production also seemed to be affected. Such metabolic alterations are related to chronic oxidative stress and inflammation that are important factors in metabolic syndrome, obesity, and ultimately cardiovascular disease. Notably, none of the analytical platforms provided a robust overview of changes in metabolism in pigs, highlighting the importance of approaching the metabolome from different points-of-view in analytical chemistry to decipher its complexity. These observations are consistent with other toxicological and epidemiological studies and suggest that chronic exposure to current low levels of NDL-PCBs may still cause adverse health effects, but our study does not show whether these effects are reversible.

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Acknowledgement: *Grant IJC2019-040989-I funded by MCIN/AEI/ 10.13039/501100011033. Project PID2020-120020RA-I00 funded by MCIN/AEI/ 10.13039/501100011033 'A way to make Europe'.*

L35

ADVANCES IN THE ANALYSIS OF TRACE ELEMENTS IN FOOD - RECENT DEVELOPMENTS FROM RESEARCH - REFERENCE LABORATORY - AND STANDARDIZATION ACTIVITIES

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Confidence in the quality and safety of food is a high priority worldwide. The presence of undesired chemicals as well as the lack of essential chemical substances to fulfill the dietary requirement can potentially lead to serious consequences for human health. The trace elements have their own place in this context and comprise both essential and toxic elements. When assessing the quality and/or safety of foods there is a demand for reliable experimental information, which in turn is based on the availability of fit-for-purpose analytical methodologies.
Trace element speciation analysis has been among the most important research topics within the field of trace element analysis over the last decades. Food samples are comprised of high variety of chemical compounds from which many can interact with metals and metalloids forming complex elemental species with various influence on the human body. In order to achieve the full picture, it is important not only to determine the total amount of a certain trace element present in the food sample but also to identify the chemical form in which given element occurs in given sample (i.e. its speciation). Selected examples on trace element speciation will be presented.
The increasing World population has led to an increased demand for food and research initiatives in exploitation of novel food ressources. In the western part of the world several projects have been initiated on exploration of increased use of e.g., insects and seaweed as ingredients in food production. The use of novel bioressources demands that these matrices are characterised for their content of essential and harmful chemicals, incl trace elements. Examples from the determination of trace elements in seaweed and how data is used to evaluate food safety will be provided.
Recently, DTU FOOD were appointed as hosts of the European Reference Laboratory for metals and nitrogenous compounds in feed and food (EURL-MN). The EURL-MN collaborates closely with the network of NRLs (National Reference Laboratories) in the EU members states and organises proficiency tests, workshops and training for the NRL with the aim of harmonising and increasing the analytical competences of the laboratories involved in official food control of trace elements. An important player here is also the European Standardisation Committee (CEN) and the Working group 10 on Elements and their chemical species in Food, which develops standardised methods and procedures for analysis of trace elements in food. Recent activities and future plans within the EURL-MN and CEN standardisation work will be adressed.

Keywords: *trace elements, speciation, EURL, standardization*

L36

INTERACTIVE SEMINAR: STEP BY STEP STRATEGIES FOR FAST DEVELOPMENT OF SMART ANALYTICAL METHODS

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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 for 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.

L37

SAFETY PROFILE AND RISK ASSESSMENT OF FOOD SUPPLEMENTS

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Global sales of food supplements are growing steadily, sustained by consumers perceiving them as a safe and healthy option. Quality control of these products is a multifaceted process, whose complexity is often overlooked by the narrative surrounding them.
Likely therefore, a variety of contamination and standardization concerns emerged during the last decade. Many supplements are in fact reportedly suffering from unreliable content of active principles, adulterations with foreign (and sometimes illicit or synthetic) substances, substitution with cheaper ingredients and pesticide or mycotoxin contamination. While these issues may emerge separately, they may also occur at the same time, altering trustworthiness of research data, safety of use and efficacy.
Such scenario has prompted researchers to advocate for a closer attention to quality, for more widespread controls and for a stricter legal framework, to close the gap between reality and consumers perception in terms of quality and safety.
Within this context, we will present two paradigmatic case-studies, regarding red yeast rice (RYR) and algae-based (i.e. spirulina, klamath, etc.) supplements. In both cases, a comprehensive analytical protocol based on LC-HRIMS was developed, to allow retrospective analysis and identification of unexpected contaminants.
In the case of red yeast rice, the occurrence of mycotoxins at significant levels, mainly citrinin and ochratoxin A, was determined in a range of products from the market (1). A preliminary risk assessment was also performed under a Margin of Exposure approach, showing that exposure to citrinin from RYR supplements may pose a risk for the consumers.
Regarding blue algae supplements, the presence of cyanotoxins was carefully monitored in samples from the e-commerce, confirming the results already pointed out by EFSA in 2016 (2). Taken altogether, our results point out that a more rigorous standardization and monitoring plan must be encouraged to extrapolate data for risk assessment with respect to the prevalence of natural toxins in food supplements.

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(2) Testai et al., 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. EFSA supporting publication 2016:EN-998. 309 pp.

Keywords: *mycotoxins, risk assessment, ion mobility, marine toxins*

Acknowledgement: *The authors kindly acknowledge Maria del Mar Aparicio-Muriana and Prof. Ana Maria García-Campana from the Department of Analytical Chemistry, University of Granada, for the fruitful collaboration in the method development and analysis of cyanotoxins.*

L38

CIGUATERA FISH POISONING OUTBREAK IN EUROPE LEADS TO A NOVEL CIGUATOXIN-3C GROUP CHARACTERIZATION FROM THE INDIAN OCEAN

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Ciguatera poisoning (CP) is a serious seafood-related illness in humans, resulting from the consumption of seafood containing ciguatoxins (CTXs). CTXs are produced by microalgae and are found in marine animals from various food webs and habitats in tropical, subtropical, and (some) temperate zones globally. Outbreaks of CP reported from the southwestern coast of India were first described in 2016, however, the causative CTXs associated with the reported illness and environmental sampling efforts have yet to be fully elucidated. Herein, we report for the first time a description of the CTX3C-group compounds present in a snapper species (*Lutjanus bohar*) originating from the coast of India and traded in a large (7,000 kg) international shipment among nine EU countries and the United Kingdom and ultimately causing an outbreak of CP in the Netherlands in 2020. Two consumer packages containing seven pieces of fish from the related product lot were collected from commerce in Germany and investigated for CTXs. All samples were positive for 'CTX-like toxicity' containing a range of 0.79-5.39 ng CTX3C equivalents per gram wet tissue equivalent, as determined by an *in vitro* cell assay (N2a-MTT). Liquid chromatography-tandem mass spectrometry revealed the (potential) presence of several marine biotoxins of the CTX3C-group in all batch samples. The CP outbreak and subsequent testing of related material described in this study indicate that multiple bags of fish portions from this lot exceeded the official controls for CTXs in fishery products sold in the European Union. The description of CTX3C-group toxins in products originating from India suggests a re-evaluation of the CTXs associated with the Indian Ocean region.

Keywords: *ciguatera, fish poisoning, outbreak, human health, ciguatoxin*

L39

QUANTIFICATION OF CONJUGATED TYPE A TRICHOTHECENES IN CEREALS USING IMMUNO-AFFINITY CLEAN-UP AND ENZYMATIC HYDROLYSIS

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Currently, increasing attention has been paid to conjugated mycotoxins in plant-based foods because of their assumed bioavailability and health risks. However, analytical standards for most mycotoxin glycosides are not available and risk assessment processes have not yet been completed because of the lack of occurrence data. Our study presents a method for efficient pre-concentration of type A trichothecene glycosides using immuno-affinity columns (IAC), followed by indirect determination after enzymatic hydrolysis. Cross-reactivity of antibodies of four types of commercially available IACs dedicated to HT-2/T-2 was tested for HT-2/T-2 glycosides. For enzymatic hydrolysis of β-glycosidic bond between HT-2/T-2 and releasing the saccharidic moiety, two different β-glycosidases were tested. Separation of free and glycosylated mycotoxins before and after hydrolysis was performed on Acquity UPLC® HSS T3 reversed phase column (100 mm x 2.1 mm, 1.8 mm; Waters), with subsequent detection assured by Q-orbitrap mass spectrometer in full-spectral acquisition mode with systematic fragmentation of precursor ions in ESI+mode. This method was applied to 52 various oat-based products. Free T-2 toxin and T-2-monoglucosides were present in 92% and 69% of samples, respectively. Free HT-2 toxin and HT-2-monoglucosides were present in 92% of samples and HT-2-diglucosides were detected in 38% of samples. The percentage of HT-2 present in the form of glycosides was up to 56% of the total HT-2 content.

Keywords: *HT-2 glycosides, T-2 glycosides, immunoaffinity clean-up, enzymatic hydrolysis, high-resolution mass spectrometry*

Acknowledgement: *This work was financially supported by the Czech Science Foundation (project No 20-14649S), METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, and a grant for specific university research – A1_ FPBT_2022_005 and grant No A2_FPBT_2021_043.*

L40

TOWARDS NOVEL GREEN SAMPLE PREPARATIONS FOR MULTI-MYCOTOXIN DETERMINATION IN FOODS

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Sample preparations usually employed in multi-mycotoxins analysis present some disadvantages such as involving high volume of organic solvents, sample dilution and time-consuming procedures. This is in disagreement with the new trends in Green Analytical Chemistry (GAC) that are linked with the 10 principles of Green Sample Preparation (GSP) recently presented [1]. In order to avoid these drawbacks, miniaturized sample pretreatment techniques such as Dispersive Liquid-Liquid Microextraction (DLLME), or its variant with solidification of a floating drop (DLLME-SFOD) are a great choice to minimize matrix interferences, and especially, for the enrichment of mycotoxins. This allows to determine mycotoxins at trace levels in complex matrices such as food samples in order to fulfill current legislation requirements.
Furthermore, Natural Deep Eutectic Solvents (NADESs) have emerged as a novel generation of green solvents which can be employed in sample treatments as an alternative to the toxic organic solvents commonly used so far. Their use offers numerous advantages including biodegradability, low toxicity, solute stabilization, sustainability, low costs, and simple preparation [2].
Taking the above mentioned into consideration, in this work it is proposed, for the first time, a sample treatment based on DLLME using a NADES as dispersive solvent and 1-octanol as extraction solvent for the isolation and pre-concentration of mycotoxins. The mycotoxins included are patulin (PAT), *alternariol alternata* mycotoxins such as alternariol (AOH), alternariol monoethyl eter (AME) and tentoxin (TEN), as well as aflatoxins (AFB1, AFB2, AFG1, AFG2). They are mostly found in fruits and their processed products, therefore, apple puree samples destined to infant consumption has been chosen as a high interest matrix which has been scarcely investigated until now.
In addition, in order to evaluate the benefits of this methodology it has been also compared with a traditional DLLME method developed in parallel but involving organic solvents. For this purpose, a new metric tool recently developed known as "AGREEprep" was used. AGREEprep is an analytical greenness metric for the sample preparation which take into consideration the 10 principles of GSP showing a final assessment score from 0 to 1 that allows us to evaluate the greenness of different sample treatments [3].
To conclude, the main goal of this work has been to evaluate and demonstrate the applicability of a new and environmentally friendly sample preparation in the fields of mycotoxins and food safety.

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Keywords: *mycotoxins, apple puree, food safety*

L41

INTEGRATED BIORECOGNITION-MASS SPECTROMETRY APPROACHES FOR IMPROVED FOOD SAFETY TESTING

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The presence of contaminants in food may lead to loss of quality or potential risk for consumers. The current strategy in the EU for monitoring food contaminants consists of a two-tiered approach. First, screening is performed employing, among others, bioassays. If the screening is suspect, the second step is confirmation with liquid or gas chromatography (LC- or GC-) coupled with mass spectrometry (MS), which aims at the unequivocal identification and, when possible, quantitation of the suspect contaminant. However, changes in socioeconomic, environmental, and ethical issues shape the future food safety monitoring methods, so alternative approaches will need to be developed to cope with the increased demand for contaminant monitoring. In this line, different integrated biorecognition-MS approaches are presented, consisting of modified monoclonal antibody-based (mAb) assays focusing on the biorecognition-based isolation to replace the screening, and direct ionization MS is used for subsequent confirmation. Different bioassay surfaces were employed in combination with various ionization and mass detection methods. Incorporating biorecognition elements with direct MS analysis eliminates the time-consuming LC- and GC- separation analysis and leads to high throughput, improved specificity, and selectivity in contaminant identification. The novel integrated biorecognition - mass spectrometry approaches have demonstrated application in detecting food contaminants, i.e., the mycotoxin deoxynivalenol (DON) and the marine toxin domoic acid. Those toxins are strictly regulated in the EU, with a specified maximum permitted level; however, their structural analogs, i.e., masked forms of DON and kainic acid, cross-react with the biorecognition elements, while their presence in food commodities is not regulated. This cross-reactivity makes a screening assay insufficient to differentiate between the regulated and the non-regulated forms; thus, confirmation is essential. The developed integrated biorecognition-mass spectrometry approaches are of high scientific interest and undoubtful social significance because they will cause a paradigm shift in how food safety testing and toxin monitoring could be conducted soon.

Keywords: *deoxynivalenol, domoic acid, biorecogntion, immunoassay, direct/ambient mass spectrometry*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie grant agreement No 720325 and from the Dutch Ministry of Agriculture, Nature, and Food Quality under projects KB-37-002-005 and KB-37-002-016.*

L42

ANALYTICAL STRATEGY FOR IDENTIFICATION OF UNKNOWN TRANSFORMATION PRODUCTS OF MYCOTOXINS AFTER THEIR DECONTAMINATION BY PULSED ELECTRIC FIELD TECHNOLOGY

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Mycotoxin contamination of cereals is a problem of a global scope. Different decontamination technologies are in use today, including the pulsed electric field (PEF). Our recent research on this topic revealed significant influence of PEF on decontamination of mycotoxins present in cereals. The overall reduction of trichothecenes, zearalenone, enniatins, beauvericin, and tentoxin in barley was up to 31, 48, 84, 36 and 46%, respectively. As we found out, besides the increased extractability into the electrolyte (water), the major reason of this reduction was mycotoxins' degradation and/or transformation into the different reaction products. As the identification and characterization of these unknown reaction products is of a major importance in terms of follow up toxicity and risk assessment, we developed an analytical strategy enabling characterization and confirmation of these products by using the tools of advanced analytical chemistry, including the *in silico* modelling and high resolution mass spectrometry (HRMS). For creating the database of predicted reaction products, Zeneth software was used, and these compounds were further pre-screened by ultra-high performance liquid chromatography coupled with Q-orbitrap HRMS. Positive hints of this pre-screening were further verified by evaluation of their retention times, and compliance of their HRMS/MS spectra with the *in silico* predictions. The determined degradation / transformation products of mycotoxins were mostly products of hydrolysis, elimination and/or oxidation.

Keywords: *mycotoxins, pulsed electric field, degradation, transformation, high-resolution mass spectrometry*

Acknowledgement: *This work was primarily supported by GACR 20-14649S Pulsed electric field as an innovative tool for decreasing Fusarium micromycetes and mycotoxins in the barley-malt-beer production chain. Further it was supported by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) and from the grant of Specific university research – grants (A1_FPBT_2022_005).*

L43

SAFETY AND AUTHENTICITY OF DIETARY SUPPLEMENTS: ANALYTICAL CHALLENGES AND STRATEGIES

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Dietary supplements are intended to correct or maintain adequate intake of certain nutrients, such as vitamins, minerals, amino acids or fiber, or to support specific physiological functions. They have become very popular among people who want to improve their health, especially those who are seeking natural alternatives to pharmaceutical products. Unfortunately, dietary supplements can be subject to adulteration and also contamination. Intentional adulteration of dietary supplements is economically driven and may lead to safety concerns in certain cases, such as adulteration with active pharmaceutical ingredients, which is a dangerous practice affecting especially sexual enhancement, weight loss and sports supplement categories. Contamination can occur at various stages of the manufacturing process, with the ingredients typically being the most important sources of potential chemical residues or contaminants, including mainly pesticide residues, heavy metals, mycotoxins or pyrrolizidine alkaloids and other plant toxins. The analysis of chemical residues, contaminants and adulterants in dietary supplements is difficult and requires fit-for-purpose analytical methods and strategies to adequately address challenges stemming from the nature of the analyzed compounds and also from the complexity, diversity and variability of dietary supplement product and ingredient matrices. For instance, authenticity testing of botanical ingredients often employs orthogonal analysis approaches to verify their identity. Potential adulteration with active pharmaceuticals can be assessed using methods targeting well-known suspects or by non-targeted analysis that can detect and identify unexpected drugs or their analogs. And analysis of various chemical residues and contaminants typically requires increased selectivity and/or sensitivity do deal with the myriad of matrix-related challenges and effects that are far more complex when compared to the analysis of conventional foods.

Keywords: *dietary supplements; authenticity; adulteration; residues; contaminants*

L44

APPLICATION OF ARTIFICIAL INTELLIGENCE IN THE DEVELOPMENT OF HONEY DIFFERENTIATION MODELS. A COMPARISON AMONG THE EFFICIENCY OF SEVERAL MACHINE LEARNING ALGORITHMS

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The present study aims to illustrate the potential given by the application of Artificial Intelligence in food authentication. In this regard, a comparison among the efficiency of several Machine Learning (ML) algorithms (i.e. Artificial Neural Networks - ANN, Support Vector Machines - SVM and K-Nearest Neighbors - KNN) for honey differentiation with respect to the botanical and geographical origin and harvesting year was performed. The choice of honey as working matrix for the development of the ML recognition models is strongly related to the fact that honey is one of the top falsified food commodities in the world and, therefore, the need for the development of reliable honey authentication approaches is undisputable. The constructed prediction models had as input data an association between the isotope composition and elemental fingerprint of the investigated honey samples. The isotope composition of the extracted water (δ^2H, $\delta^{18}O$) from the honey samples was measured using a liquid-water isotope analyzer (DLT–100, Los Gatos Research), while for the $\delta^{13}C_{honey}$ and $\delta^{13}C_{protein}$ determinations, an IRMS - isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific) connected with a dual inlet system was employed. The elemental composition (i.e. macro-, micro- and potentially toxic elements) of the investigated honey samples was measured through inductively coupled plasma mass spectrometry (ELAN DRC (e), Perkin Elmer SCIEX). Distinct pre-processing approaches have been applied prior to the construction of the ML-based models in order to increase the classification performance. As feature selection, both unsupervised (i.e. Analysis of Variance – ANOVA) and supervised (i.e. Partial Least Squares – PLS) techniques were tested and compared. It was proven that selecting a subset of the original attributes by maintaining only the most relevant features associated with each differentiation criteria conducts to higher accuracy measures and, therefore, represents an important step in the development of reliable discrimination models. Nonetheless, the obtained results highlight that Artificial Intelligence can successfully be applied in food authenticity control, though special attention should be paid to selecting proper hyperparameters for the development of the ML models and to the pre-processing phase, as it can drastically influence the performance outcome.

Keywords: *machine learning, honey, food authentication, pre-processing, recognition models*

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L45

HAZELNUT PRODUCTS TRACEABILITY THROUGH ISOTOPE RATIO MASS SPECTROMETRY APPROACH

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The geographical origin of hazelnut (*Corylus avellana* L.) products is nowadays a relevant aspect for high-quality food characterization. Isotope Ratio Mass Spectrometry (IRMS) could play a key role in origin discrimination. The present study aims to assess the geographical provenience of Italian raw, roasted hazelnuts, and paste of hazelnuts, by analysing relative isotopic ratios of carbon and oxygen, through Elemental Analyzer (EA) – and Thermal Conversion (TC) – IRMS. Method development is performed by evaluating test samples' repeatability, reproducibility, and robustness, varying mass parameters. Outcomes highlight reproducible and robust results, having acceptable standard deviation values (from 0.15 to 0.7). One-way ANOVA test demonstrates the significant statistical difference between Italian and not Italian hazelnut samples (from 1 to 4-5 δ of difference). A Design of Experiment (DoE) is created to sample correctly, taking into account factors such as variety, processing, and percentage of peel. A total of n=48 hazelnut lots, from Italy, Turkey, Georgia, and Azerbaijan, are analysed for the geographical assessment: this strategy demonstrates highly promising potentialities, as food isotopic abundances reflect ground and climate-related features, typical of precise locations. Moreover, this approach consists of limited, or even inexistent sample preparation and provides for high sensitivity.

Keywords: *EA-IRMS, TC-IRMS, food authenticity, geographical origin claim, corylus avellana L.*

L46

VOC-BASED PROFILING OF OILS AND JUICES WITH GC-MS/MS-IMS AND MACHINE LEARNING

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In recent years, gas chromatograph-ion mobility spectrometry (GC-IMS) has become a more and more popular method in different fields of analytics. GC-IMS allows for a rapid analysis of volatile organic compounds (VOC). These VOCs are monitored in food and fermentation processes or they can serve as an indicator for quality and shelf life of different food products. For an increase in selectivity, our group has previously described the use of GC IMS-MS systems [1]. This prototype proved to be valuable for the simultaneous generation of electron ionization mass spectrometry (EI-MS) spectra and IMS spectra in particular for differentiation of isomers in complex matrices. Safety and authenticity of foods and flavors are highly relevant and challenging topics. Due to the multitude of different production types in worldwide trade, generally confirmatory analytics are complex. As examplary products, citrus oils such as orange peel oil, lemon peel oil, lime oil and grapefruit peel oil were selected. Due to their substantial price differences, they are frequently adulterated products. Apart from the citrus oils, also the respective juices are commonly affected by food fraud. The quality of citrus juices decreases with storage duration or may be subject to adulteration with water, sugar or other juices of inferior quality. While it could be demonstrated that the parallel detection of IMS and MS delivers a clear benefit for the classification of citrus juices, most of the volatile compounds were only detected in the IMS, not in the EI-MS system [2]. One difficulty to face is the strong fragmentation of analytes in EI. Notably terpenes show very similar fragments, which leads to interfering data in case of coelution.To address this issue, a prototype GC-(ion-trap)MS-IMS setup with ammonia-based chemical ionization (CI) is a central part of the project. The softer chemical ionization technique results in less extensive fragmentation and allows for the detection of the intact quasi molecular ions. The ion trap detector allows MS/MS experiments based on the intact precursors, which is expected to deliver higher selectivity particularly for chemometric data analysis, which is the main focus of our study. The combination of GC-CI-MS/MS and GC-IMS based on a single-injection system is expected to deliver more comprehensive data than the individual methods alone and will be evaluated for its suitability for data fusion approaches in conjunction with powerful machine learning and deep learning algorithms.

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Keywords: *GC-MS-IMS for simultaneous detection, VOC profiling of citrus juices and oils, chemometrics, machine & deep learning*

L47

ARTIFICIAL INTELLIGENCE SMELLING MACHINES BASED ON TWO-DIMENSIONAL GAS CHROMATOGRAPHY: A HIGH-INFORMATIVE TOOL FOR FOOD AUTHENTICATION AND QUALITY ASSESSMENT

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The European hazelnut (*Corylus avellana* L.) is a tree nut mainly used by the confectionery industry. The industrial quality assessment of the raw materials is mainly based on human inspection to exclude damaged kernels and on sensory analysis to detect sensory defects and rancidity. A modern concept of food quality should implement molecular resolution methodologies capable to support traditional and well-established procedures while adding extra information about product authenticity, storage stability, and technological quality.
The volatile fraction of raw hazelnuts, also referred to as *volatilome*, encrypts most of the quality-related information on cultivar/geographical origin, post-harvest treatments, bacteria/moulds contamination, oxidative stability, and sensory quality. This latter relates to the peculiar quali-quantitative distribution of potent odorants that are capable to elicit distinctive yet unique sensory features resembling the identity of a specific food. A workflow capable to extract, isolate and quantify the key-aroma compounds of a product (*i.e.*, the aroma blueprint) has been recently defined as a Sensomics-Based Expert System (SEBES) acting as an Artificial Intelligence (AI) smelling machine.
This contribution realizes the AI smelling machine conceptualized by sensomics with some improvements related to analytical efficiency and information capacity. By comprehensive two-dimensional gas chromatography coupled with mass spectrometry and flame ionization detection (GC×GC-MS/FID) a single-step measurement is possible. Multiple headspace solid-phase microextraction (MHS-SPME) allows the accurate quantification of about 40 analytes including key-aromas, spoilage markers, and geographical tracers.
Results, visualized as odor activity values (OAVs) maps, resemble identity sensory features of the samples while facilitating the comparative process through their aroma blueprint. Moreover robust yet reliable quantitative data can be used for the development of authentication/discrimination models.
The proposed methodological approach, transferable on a routine basis, offers a great increase in resolution compared to traditional quality control protocols. From a single analytical run, multi-level molecular information is readily and reliably extracted.

Keywords: *European hazelnut, volatilome, sensory maps, chromatographic fingerprinting, artificial intelligence smelling machine*

L48

COMPARISON OF THREE METHODS (DNA METABARCODING, REAL-TIME PCR, DNA ARRAY) FOR SPECIES IDENTIFICATION IN FOOD AND PET FOOD SAMPLES

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Species identification and differentiation in meat and their products, which are prone to adulteration, have become a major issue in food authentication. One of the most common frauds worldwide is the replacement of high priced meat species by low-cost ones. Numerous analytical methods can be used for the authentication of food, also to ensure correct information for customers who prefer certain foods over others for religious, ethical or health reasons. Next generation sequencing (NGS) technologies, in particular massively parallel sequencing of PCR products based on the analysis of species-specific differences in DNA sequences (DNA barcoding), is being considered as promising alternative in food science. Currently, multiplex real-time PCR assays and DNA arrays are mainly used for detecting food adulteration in official food laboratories.
Our working group has developed a DNA metabarcoding method allowing the identification of mammalian and poultry species based on a DNA library preparation protocol from Illumina [Dobrovolny et al., 2019, *Food Chemistry*]. In-house validation experiments were performed and an interlaboratory comparison confirmed the feasibility of this previously published screening assay [Dobrovolny et al., 2022, *Foods*]. The aim of this work was to compare qualitative and quantitative results obtained by the DNA metabarcoding method with those obtained by real-time PCR assays and/or a DNA array. Individual DNA extracts from 25 reference samples of known composition (2-14 meat species in a ratio from 1.0 to 99.9% (w/w), 56 food products, such as sausages, vertical rotating meat spits, pâtés, convenience foods and milk products, together with 23 pet food products were analyzed. A subset of seven reference samples was analyzed in two independent laboratories. DNA extraction was performed using three commercially available kits. All samples were sequenced on the MiSeq® instrument and the resulting FastQ files were processed with an analysis pipeline using Galaxy.
The qualitative results indicated that the DNA metabarcoding method, real-time PCR assays and the DNA array allowed correct identification of the meat species. After analyzing quantitative data and calculating a relative quantification error, the results obtained by DNA metabarcoding were in line with those obtained by real-time PCR. For example, the 25 reference samples, including 13 sausages with major components (>85%) and minor components (between 20% and 5%) showed error rates of less than 10% and 30%, respectively. The two laboratories got comparable results for the subset of the seven reference samples. In a number of food products and pet food products, undeclared species were detected at a level of more than 5%.
In conclusion, DNA metabarcoding is a suitable screening method for meat species authentication and could be an attractive alternative to real-time PCR assays. A break-even analysis shows that the use of NGS is cost-effective from the tenth sample onwards.

L49

INFORMING EFSA ON CIRCULAR ECONOMY FOOD AND FEED PRACTICES. WHAT IS THE EVIDENCE FOR EMERGING RISKS?

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Circular economy (CE) is an approach that decouples economic activity from the consumption of finite resources, designs out waste, and instead promotes an economic model based on sharing, leasing, reuse, repair, refurbishment and recycling, in an (almost) closed loop. In the framework of enabling regulatory and policy drivers (e.g. Circular Economy Action Plan, Integrated Nutrient Management Plan, Farm to Fork strategy, new Water Reuse Regulation, the European Bioeconomy Strategy, Single Use Plastics Directive etc), the European Food Safety Authority (EFSA) has been undertaking a 2-year investigation into "Food and feed safety vulnerabilities in circular economy" (2021-2022). As part of this we carried out an extensive literature review to gather and evaluate the evidence for vulnerabilities in the CE approach for food and feed safety, plant, animal and human health and the environment. As a new driver, implementation of CE approaches might bring about a set of emerging risks.
This review identified and categorised CE practices within all stages of the food and feed production chain in Europe. Four broad macro areas were identified within which CE practices are envisaged or currently used in Europe: primary production of food and feed; reducing industrial/manufacturing/processing waste; reducing food and feed waste in wholesale, food retail, catering and households; and reducing food and feed packaging waste. In each macro area, there were a variety of practices of interest regarding emerging risk to plant, animal, human health and the environment. A focus on evidence relating to emerging risks to plant, animal, human health and the environment from 'novel foods and feeds within the framework of CE' found a bias towards research investigating the suitability of novel feeds in terms of animal productivity parameters rather than on emerging risks of novel food/feed for animal, human, plant health and the environment. Those studies that investigated risk were almost entirely focused on the biological and chemical hazards, risks to health, and environmental impacts of insects as food or feed and the substrates that they are reared on. Emerging risks are characterised and recommendations made for future research. We recommend that future primary research in novel food and feed in the CE focuses on areas other than insect farming, and that there are further investigations into the potential risks associated with importation into the EU of livestock/goods that may have been subject to different restrictions/legislation.

Keywords: *novel food, novel feed, emerging risk, food waste, circular economy*

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L50

RECYCLING OF FORMER FOODSTUFFS IN ANIMAL FEED: HOW TO DISTINGUISH AUTHORISED COLLAGEN FROM PROHIBITED ONE?

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Circular economy is one of the major issues promoted by the European Union. In this context, the use of former foodstuffs (FFS) containing ruminant collagen and/or gelatine was recently authorised in non-ruminant feed (Commission Regulation (EU) 2021/1372). FFS retain a significant nutritional value and thus constitute a very promising alternative feed that could contribute to the sustainability of the food chain. However, their use remains limited. One of the reasons for this limited use is probably that, from a safety point of view, FFS containing animal products follow the same rule that other products of animal origin for their use in animal feed. Their high content in ruminant DNA coming from authorised by-products such as dairy products and gelatine make them challenging to be distinguished from the use of prohibited proteins. Mass spectrometry-based proteomics has proofed to be a power method perfectly adapted to animal-by products regulation requirements by providing information about the tissue and species of origin based on the detection of specific peptide sequences. However, some proteins, i.e. collagen, are present in both authorised and unauthorised by-products.
The objective of this work was to optimise the sample preparation protocol to increase the sensitivity and the specificity of bovine processed animal proteins (PAPs) detection. The analysis on the sediment fraction, as used in the official light microscopy method was evaluated.
Pig feed adulterated with 0.1 % and 0.5 % (w/w) of Bovine PAPs or Porcine PAPs were used to evaluate the sensitivity of the protocol. Specificity was verified by analysing pig feed with 10 % former foodstuffs containing or not gelatine. These compound feed were adulterated or not with 0.1 % (w/w) of Bovine PAPs. Feed samples were prepared following the two sample preparation procedures. First procedure was based on sample extraction with in a buffer containing 200 mM TRIS-HCl pH 9.2, 2 M urea followed by trypsin digestion and purification with tC18 SPE (Waters). The second includes preliminary sedimentation of the feed using high density solvent (tetrachloroethylene). Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQ-XS, Waters). Peptide markers identified in previous studies were simultaneously monitored.
Results obtained with the two approaches (standard v/s optimized method) were compared. The optimised method was able to detect Bovine PAPs at the 0.1 % (w/w) level based on haemoglobin and collagen peptides. This level corresponds to the LOD of the official methods for animal proteins detection. In addition, sedimentation step has cleaned the sample regarding the gelatine provided by the FFS, avoiding a false suspicion of the presence of prohibited PAPs.

Keywords: *former foodstuffs, processed animal proteins, mass spectrometry, proteomics, gelatine*

L51

CHARACTERIZATION AND VALORIZATION OF FRUIT SIDE STREAMS AS UNCONVENTIONAL SOURCES OF FUNCTIONAL INGREDIENTS

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In order to valorize an underexploited side stream of the fruit processing industry and to potentially fulfil the growing global demand for alternative functional ingredients for the food/feed/cosmetic sectors, this research addresses the characterization of the chemical/nutritional composition of different fruit residues (mainly seeds and kernels) and a defined processing scheme based on the cascade biorefinery concept, to obtain high yields and high purity valuable compounds (oils, proteins and fibers).
After providing an overview of the proximate composition of residuals from different fruit categories (stone, tropical, citrus fruits), the recovery of nutrients was performed by a bio-refining approach based on mild enzymatic-assisted extraction (EAE) with *Bacillus licheniformis* protease. Then, further detailed analysis was performed on the obtained fractions: the oil was characterized in terms of fatty acid profile and unsaponifiable compounds by GC-MS; proteins by amino acid profiling by HPLC-MS and hydrolysis degree; fibers by molecular weight, monosaccharides profile and esterification degree.
The overall enzymatic process obtained variable ranges of protein and lipid extraction yields based on the fruit residuals (20-90%). In some cases, the oil extraction required an acid pre-treatment, whereas the mild EAE allowed the recovery of a specific soluble fibers fraction (arabinogalactans).
After proteolysis, the protein fractions resulted rich in oligopeptides with high digestibility and low allergenicity (MW < 3kDa).
Fruit seed oils resulted rich in total MUFA for high oleic acid (C18:1, >25%) and PUFA for high linoleic acid (C18:2 n-6, till 65% in blackberry seeds) and α-linolenic acid (C18:3 n-3, 15% in lemon seeds), except mango seed oil, which showed a cocoa butter-like fatty acid profile with 45% of stearic acid (C18:0). About 5% of alpha-eleostearic (9-cis,11-trans,13-trans, C18:3 n-5), previously suggested to modulate adipogenesis, was found in cherry seeds. The unsaponifiable matter composition (about 1% fat) depended on fruit oils but it was generally characterized by β-sitosterol as predominant sterol compound (>2000 mg/kg), squalene, policosanols and tocopherols at different isomer proportions.
Soluble fibers of citrus peel/seeds and stone fruits presented the same composition of arabinogalactans from Arabic gum, with lots of potential industrial applications.
The residual pellet, contained up to 90% of fibers to be further extracted and enzymatically processed to obtain other oligosaccharides (as xylooligosaccharides and pectooligosaccharides) and applied for functional foods (prebiotics).
Next steps will be the functionalization processing of fractions for bioactivity optimization and final safety assessment and the advancement from pilot to demonstration scale.

L52

PFAS IN FOOD CONTACT MATERIALS – ANALYZING THE HIDDEN TREAT

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Poly- and perfluorinated alkyl substances (PFAS) are a large heterogenous group of chemicals consisting of several hundred different structures. Due to their stability and water/lipid repellent properties they are used in various applications, like production of non-stick cookware, stain resistant products or fast food packaging. However, because of their persistent and bio accumulative nature, these substances are omnipresent in the environment. Unfortunately, many of them present concern for human health.
PFAS could end up in human body through various sources. However, one of their major pathways is through food consumptions. [1] Consequently, in recent years a lot of focus was also put at materials containing these substances, which are in direct contact with food.
Recently some PFAS have begun being regulated or phased out. Only few of the substances have been risk assessed by the European Food Safety Authority (EFSA) and Environmental Protection Agency (EPA) [2, 3]. Currently, there are various regulatory initiatives that specify acceptable limits of some PFAS, however they have been imposed mainly for environmental matrices.
Until today, more than 5000 different PFAS are present on the market, with different physical and chemical properties. Furthermore, there are numerous precursors, many of which with unknown structures, making their analytical determination quite challenging.
In this work, the targeted approach for detecting and quantifying 24 PFAS commonly found in paper and board matrices is shown. PFAS are extracted using accelerated solvent extraction, followed by analysis via high-performance liquid chromatography coupled with triple quadrupole mass spectrometry. The method that we report exhibits chromatographic separation of all the 24 substances. Average linearity and reproducibility are 0.99338 and 1.6, respectively. Recovery values are in the 85 – 94% range.
The model that we present here could lead to an establishment of simple and efficient method for quantification of PFAS that could be used for quick and easy monitoring of PFAS in paper based FCM that are currently available in the market.

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Keywords: *PFAS, food contact materials*

L53

MULTIMODAL CHARACTERIZATION OF MICROPLASTICS IN DRINKING WATER

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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. Yet, limited and incoherent information is available about the occurrence of MPs and NPs in food, water, and beverages. Several parameters have to be assessed in order to fully characterize MP contamination: number of particles, particle size, particle size distribution, particle shape, chemical composition, and particle mass. Currently, no single analytical or physical method is able to provide all this information. Optical techniques, used to measure and count the particles, can be combined with vibrational spectroscopic techniques for their chemical characterization. However, those techniques are subject to interference related to aging, weathering, surface contamination, and chemical damage of the micro and nano particles' surface.
In this presentation, we propose the combined detection and quantification of MPs by Nile Red staining and fluorescence microscopy plus chemical characterization of individual particles by ambient ionization mass spectrometry (MS) using an Atmospheric Solids Analysis Probe (ASAP). A sample preparation procedure for the analysis of MPs in bottled water, including the Nile Red staining of MPs, was optimized and a macro was written in ImageJ for the automated MP detection and quantification. Repeatability, in-laboratory reproducibility, linearity and linearity range, method limit of detection, and limit of quantification were assessed. For the chemical characterization, a rapid MS method was developed and spectra of MPs from 19 polymers were acquired as both molecular ions and in-source fragmentation pattern.
This multimodal characterization method, the first in MP analysis to report an exhaustive assessment of quality parameters, excels in the discrimination of MP polymers belonging to the same chemical class and in the identification of polymer mixtures. The method overcomes the interference from MP surface contamination and, as a bonus, enables the MS characterization of the adsorbed contaminants. This method represents a step forward in MPs analysis toward both the reliable quantification of MPs in food and the applicability of MS to the characterization of MPs.

Keywords: *microplastic, nanoplastic, mass spectrometry, drinking water, nile red*

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L54

HUMAN BIOMONITORING AS TOOL FOR EXPOSURE ASSESSMENT TO PESTICIDE MIXTURES

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Exposure- and risk-assessment of pesticides is typically based on residue data from food monitoring and food consumption data. The enormous variety in diets and residues therein make it challenging to estimate to what (mixtures of) pesticides the (individual) consumer is exposed. Another point is that in most cases residue data are only available for the raw agricultural commodities, and effects of processing (industrial or home-cooking) are difficult to address due to availability and reliability issues with processing factors. Finally, besides dietary intake there might be other routes of exposure which are not covered when restricting exposure assessment to food monitoring only.
In food safety matters human biomonitoring (HBM) is an alternative option for exposure assessment and can bring added value for chemical risk assessment because it can reduce the assumptions needed regarding consumption rates, residue occurrence and processing effects, and it integrates exposures from the diet and potential additional sources (house-hold use, environmental). In HBM, biomarkers of exposure (parent pesticides or their metabolites) are measured in human matrices such as urine (most commonly used), blood, feces and hair. There is an increasing interest in the use of HBM over past 10 years from both academia and stakeholders (including EFSA, EEA) and significant improvements in analytical methods, applications and interpretations have been made. In this presentation an overview is given of the current status of HBM as exposure assessment strategy for pesticides. Emphasis will be on the analytical approaches for determination of pesticides/metabolites in urine, blood, feces and hair. Findings from recent studies (national and in the frame of EU projects https://www.hbm4eu.eu/ and https://sprint-h2020.eu/) will be presented. Besides pesticides/metabolites that were expected from dietary intake, also pesticides no longer approved for crop protection but still used as biocide or for treatment of farm and companion animals (e.g. certain pyrethroids, fipronil) were found. The results show that HBM provides insight in aggregate exposure and exposure to mixtures, both at individual and at population level. HBM is developing into a useful tool to complement existing procedures for pesticide risk assessment.

Keywords: *human biomonitoring, pesticides, suspect screening, metabolites, exposure assessment*

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L55

RAPID APPROACH FOR THE DETERMINATION OF ETHYLENE AND PROPYLENE OXIDE IN DIFFERENT FOODSTUFFS

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The occurrence of the banned insecticide, ethylene oxide (EtO), and its transformation product, 2-chloroethanol (2CE), has recently been reported in the Rapid Alert System for Food and Feed (RASFF) in various food commodities (more than 800 notifications since September 2020). In this study, two alternative approaches based on gas chromatography coupled to tandem mass spectrometry (GC–MS/MS) were developed to control the maximum residue limit of EtO (defined as the sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide), propylene oxide (PPO) and its transformation product 1-chlor-2-propanol (1C2P). The first approach offered a rapid screening of 2CE and 1C2P (the contamination markers) in an aqueous acetonitrile extract purified by dispersive solid-phase extraction (dSPE). Total EtO and PPO were determined using the second approach, which involved conversion of EtO to 2CE and PPO to 1C2P by acid hydrolysis in the presence of chloride; the ethyl acetate extract was purified prior to instrumental analysis by dSPE. The quantification limit achieved for EtO (the sum of EtO and 2CE expressed as EtO) was low enough to ensure compliance with regulatory requirements. The precision of the results was successfully verified by analysis of the EUPT test material (EUPT-SRM16 - 2021).

Keywords: *ethylene oxide, propylene oxide, 2-chloroethanol, GC-MS/MS*

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L56

TOWARDS AUTOMATION OF HIGH THROUGHPUT ANALYSIS OF PESTICIDES IN FEED

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Feed matrices are diverse and rather difficult matrices in terms of residues analyses. They cover of wide range of commodities with large differences in their chemical composition. Feed can consist of animal or plant origin materials, which makes the analyses much more complicated. Additional, there is always a high demand for increasing the scope of matrices and of pesticide residues in the routine control analyses. To satisfy such requirements the best overcome is often to develop new and improved analytical methodologies. In this study we developed and validated a high throughput micro-SPE (µ-SPE) clean-up method for feed and fatty matrices. A new automated clean-up workflow was custom designed according to our laboratory requirement and was implemented on a stand-alone Thermo Scientific™ TriPlus™ RSH autosampler. Commercially available µ-SPE cartridges containing magnesium sulphate, primary-secondary amine, C_{18}, and graphitized carbon X were used in the study. Rapeseeds, rapeseeds cake, rapeseeds meal and rapeseed oil matrices were validated according to the SANTE/11312/2021 guideline using three different spiking levels and extraction using citrate buffer QuEChERS method. Instead of dispersive SPE, the automated µ-SPE system was used to clean-up the extracts. The analysis of the cleaned extracts were done by GC-MS/MS and LC-MS/MS. For data quality purposes, the EURL-CF rapeseed cake EUPT-CF15 Test Item was included in the study as reference material. The results showed a successful implementation of the custom made workflow, with low standard deviation. For the majority of the compounds in this study, satisfactory validation data for the use of µ-SPE cartridges as clean-up was obtain for all matrices.

Keywords: *micro-SPE (µ-SPE), pesticides residue analysis, liquid chromatography (UHPLC), gas chromatography (GC), tandem mass spectrometry (MS/MS)*

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DETERMINATION OF AMINOGLYCOSIDE ANTIBIOTICS IN FEED AT CROSS-CONTAMINATION LEVELS – UNEXPECTED CHALLENGES

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Antimicrobial resistance (AMR) is of growing concern in the European Union (EU). Infections by multidrug-resistant bacteria are responsible for about 33 000 annual deaths in the EU. Moreover, AMR causes an estimated annual cost of 1.5 billion EUR in the EU due to healthcare expenditures and productivity losses. In addition, the decreasing number of newly developed antibiotics entering the market draws to combatting AMR being a key priority in the EU in this health-threatening scenario.

Since 2006, a plethora of initiatives involving the European Commission (EC), the European Centre for Disease Prevention and Control (ECDC), the European Medicines Agency (EMA), the European Food Safety Authority (EFSA), feed industry and farmers have been launched to reduce, replace and re-think the use of antimicrobials. Such initiatives target many different sectors such as: human medicine, veterinary medicine, research, animal husbandry, agriculture, environment and trade.

Ensuring the absence of antibiotics in feed is certainly an effective measure to curtail the emergence of AMR. Four aminoglycosides (apramycin, paromomycin, neomycin and spectinomycin) are included in the list of priority antibiotics to be controlled in feed (Regulation EU 2019/4) at cross-contamination level. These antibiotics are challenging to analyse due to their high polarity and the ability to interact with labware and feed components. Four published standard operating procedures based on alternative principles of sample preparation (mixed mode cation exchange, weak cation exchange, reverse phase and molecularly imprinted polymer SPE associated with various extraction protocols) were investigated for the analysis of pig and poultry feed. None of these methods generated satisfactory results serving the needs for a method that can be applied within the frame of official control.

This presentation aims at clarifying the root causes for abandoning the above mentioned method protocols and proposing an amended one. The different stages of the sample preparation (extraction, pH adjustment and SPE) were carefully assessed and key parameters have been identified and optimised. The developed method involves an efficient clean-up and analyte concentration hence enabling the quantification of aminoglycoside antibiotics in feed by LC-MS/MS at the low levels of interest. Finally, the extension of the scope to other highly water soluble antibiotics (e.g. amprolium, colistin) is being investigated. This work is part of the overall JRC commitment and effort to provide the EC policy makers and the Member States with robust and validated methods as reliable analytical tools for monitoring the 24 antibiotics listed in Regulation EU 2019/4 at cross-contamination levels by 2023.

Keywords: *aminoglycoside antibiotics, feed, cross-contamination, antimicrobial resistance*

L58

FEASIBILITY OF COATED BLADE SPRAY AS A SMART SAMPLING APPROACH FOR TESTING OF RESIDUES IN FOOD

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In food control laboratories, the analytical workflow includes sampling, transportation, and sample preparation procedures before the subsequent determination of target compounds using chromatographic separation techniques with different detection systems. Therefore, the number of samples that can be analyzed is relatively low compared to the high increase in notified food safety emergency alerts. Therefore, the development of new approaches for sampling which are preferably directly compatible with analytical detection techniques is of great interest for the fast screening of food samples. Under this scenario, ambient ionization mass spectrometry (AIMS) techniques which allow a rapid, real-time, high-throughput, and in-situ mass spectrometric analysis of liquids, gases, and solids, have shown a great advantage in the food analysis field [1]. Besides, AIMS with miniaturized MS systems also permits on-site analysis, reducing transport costs, the analysis time and increasing the laboratories throughput [2].
Among the existing AIMS techniques, Coated Blade Spray (CBS) combines solid phase micro extraction (SPME) onto a conductive metal strip, thus providing a selective sample enrichment and direct ionization by applying a high voltage onto the metal strip. Depending on the analyte(s) of interest, this sorbent layer can be changed for optimal extraction and enrichment. CBS-MS contributes to smart sampling by simplifying the sampling, shipping, and increasing the number of samples tested in a similar timespan. Additionally, CBS has already been combined with a portable USB device to provide the high voltage needed for electrospray [3]. Therefore, CBS is considered an encouraging advancement to achieve future on-site analysis of food-related samples since it can be used without the need for gasses and external power supplies. This work will present the results of the CBS smart sampling approach for food safety related applications and its potential for future on-site testing. These examples highlight the possibility of using CBS for the quick, high-throughput analysis of food safety hazards in food-related samples.

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Keywords: *ambient ionization, coated blade spray, mass spectrometry, on-site testing*

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ANALYTICAL QUALITY ASSURANCE AND METROLOGICAL TRACEABILITY OF MEASUREMENT DATA IN PROCESS ANALYTICAL TECHNOLOGIES FOR FOOD CONTROL ASSESSMENT

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Among the approaches devised in response to emerging food safety and quality issues, as well as for food authenticity and traceability testing, traditional techniques are based primarily on usually expensive and time-consuming off-line laboratory methods that cannot guarantee neither in-situ analysis nor real-time snapshot of the production process [1]. New approaches are therefore required to improve the effectiveness of food safety and quality management systems. The future of food testing includes rapid, cost-effective, portable and simple methods for both qualitative screening and quantification of food contaminants and quality parameters on-site and off-site. Process automatization through the Process Analytical Technology (PAT), which was initiated by the Food and Drug Administration in 2004, is an increasing trend in food industry as a way to consistently achieve high-quality products, production speed and labor shortage [2,3]. In this context, the analytical laboratory must be moved into the process through the implementation of analyzers directly in the production plant. This requires the attention of the research community and devices' manufacturers to ensure the reliability of measurement results from novel methods through the demonstration and critical evaluation of performance characteristics. The fitness for purpose of methods in real-life conditions is a priority that should not to be overloooked in order to maintain an effective and harmonized food safety policy. Data from multiple analytics platforms need to be handled, and quality control and quality assurance systems have to ensure the robustness of analytical processes. The role of metrological traceability as a prerequisite for the reliability of measurement results in food analysis will be highlighted.

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Keywords: *metrological traceability, analytical quality assurance, food control, process analytical technology*

L60

STABLE ISOTOPE METROLOGY IN FOOD AUTHENTICITY AND TRACEABILITY

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In food control, the measurement of stable isotope ratios has become a powerful tool in adulteration and origin identification. Its power relies on having a unified database of authentic samples of sufficient numbers with which a 'suspect' test sample can be compared. Also, authentic samples must be statistically 'screened' to identify the key tracers that discriminate between different types of plants, regions or countries of interest and farming practices.Ensuring that all stable isotope measurements are metrologically sound, for example, improving the quality of datasets and model evaluation by incorporating measurement uncertainty and, more generally, having the necessary metrological support, is equally important. A part of this support is the availability of suitable RMs that allow the analyst to follow the "principle of identical treatment" and enable users to normalize measurements of samples to isotope−delta scales. This presentation will cover the use of official, standardized methods, reference material (RM), data handling, evaluation of measurement uncertainty and participation in interlaboratory comparisons, including Consultative Committee for Amount of Substance (CCQM) comparisons. It will also discuss new, versatile international food matrix stable isotope RMs (USGS82 to USGS91) of plant and animal origin. These topics will be discussed within the strategy of the METROFOOD Research Infrastructure (https://www.metrofood.eu/), designed to provide high-quality metrology services to the food sector.

Acknowledgement: *The presentation will be put in the context of relevant projects and infrastructure including METROFOOD-RI, ISO-FOOD, ITN FoodTraNet, FoodMetNet, AgroServ.*

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CHALLENGES IN FOOD ADDITIVES ANALYSIS FOR ENSURING TRANSPARENCY IN THE FOOD CHAIN

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The variety of food additives (FAs) approved for usage in many kinds of foods is wide and their use is regulated in the EU by regulation 1333/2008. Consumers demand full transparency on food labelling, inspection services should control their labelling and respect of the maximum limits, member states will monitor the use and assess the dietary intake, and there is a constant demand from EFSA for usage data for the (re-)evaluation of FAs. Most analytical methods are dedicated to specific FAs and are applicable to a restricted number of food matrixes. Only few multi-methods are available. The extraction of FAs from different food groups might be challenging due to the diverse nature of food and the complexity of composite foods. The concentration range of the usage levels in different food groups vary considerably. There is also a lack of guidance documents on the validation of analytical methods for FAs. And there is a complete lack of metrological tools such as certified reference materials, standardized methods and proficiency tests. Nanotechnology is increasingly applied in the food sector, and certain FAs that are not typically considered as nanomaterials, such as E172, E174 and E551, contain a fraction of nanoparticles. Therefore, EFSA defined criteria for assessing the presence of a fraction of small particles, and set out information requirements for FAs. This, and actions such as banning E171, necessitates development and validation of methods for the characterization of (nano)particles in FAs and requires new approaches for food analysis.
The food industry is pursuing clean and clear food labels, where (artificial) additives are excluded from labels and replaced by natural ingredients. However, molecules present in natural ingredients are also authorized as FAs (e.g. lycopene E160d). It is almost impossible with the current methods to distinguish between the use as a natural ingredient or whether the component was added as a FA. In a global food market, FAs not authorized or illegal in the EU might be imported from third countries via imported ingredients or foods or even by fraudulent addition. Some of these "illegal" FAs might be harmful to the consumer and pose a direct public health concern. FAs authorized in an ingredient might be present in the composite food via the carryover principle of FAs, even if the FA is not authorized for the composite food, under the condition that the additive has no technological purpose in the composite food. Under this condition, the FA must also not be labeled, complicating the interpretation of analytical findings. Recent studies in Belgium have revealed that up to 10% of the samples taken were suspected of infringing FA legislation or labelling, and even illegal FAs were found. There is an urgent need for guidance for (multi-)methods, validation and interpretation of results, in establishing a network of laboratories and EU-RL, and finally for reference materials to tackle the current challenges.

Keywords: *food additives, nanomaterials, metrology, E171*

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TOWARDS AI-DRIVEN FOOD SCIENCE AND SOCIETY: OPPORTUNITIES AND CHALLENGES

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In the last decades, a great amount of work has been done in predictive modeling of issues related to human and environmental health. Resolution of issues related to healthcare is made possible by the existence of several available biomedical vocabularies and standards, which play a crucial role in understanding health information, together with a large amount of health data. However, focusing solely on healthcare data limits the potential benefits that our lives and societies could have from the rapid development of artificial intelligence (AI) and its enormous capabilities. As such, Lancet Planetary Health in 2019 noted that the focus of future improvements in our wellbeing and societies will depend on investigating the links between food systems, human health, and the environment. However, despite a large number of available resources and work done in the health and environmental domains, there is a lack of resources that can be utilized in the food and nutrition domain, as well as their interconnections. In particular, this is important during the current pandemics of COVID-19, when food provision and security, as well as healthy nutrition and environment, are tremendously needed for quick recovery and long-term sustainable development of our societies.
For the purpose of attaining human and societal wellbeing through advances in the field of artificial intelligence (AI), the talk will focus on opportunities for utilizing big data from food and nutrition and their interrelations with biomedicine and the environment. Huge amounts of data containing valuable information are now available in various datasets, registries, and scientific and grey literature, which makes it possible to use advanced Artificial Intelligence (AI) methods. However, before applying AI methods to real-life data, that is heterogeneous (i.e., of different types and formats), unstructured (textual) data needs to be structured and normalized with other structured data. In this talk, we will explain AI methods and resources that can be used on different levels in the modeling process, starting from raw data to discovered knowledge. Finally, the existence of such methods and resources will be linked to several application scenarios of utilizing food and nutrition data in predicting emotional distress, COVID-10 mortality rate, and food chain traceability.

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ANALYTICAL EVALUATION OF SAFETY AND QUALITY OF FOOD BYPRODUCTS IN THE CONTEXT OF CIRCULAR FOOD SYSTEM

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At the food system level, the competition on resources – agrifood raw materials, land, water and energy - will increase in the near future. At the same time, the quantity of food waste is increasing a major challenge being the accumulation, handling and disposal of waste from food processing. Some category of food waste are by-products which still contain important quantities of valuable nutritive compounds and which can be re-introduced in the food system as new raw materials. The most by-products are part of agri-food materials that are removed from the food system and further, are usually used for feed or compost producing. For re-introducing of by-products into the food system, logistic procedures are needed in order to maintain and to assure the safety of them, as new food raw materials. Within this study, an evaluation of the safety and quality of different byproducts was performed, using different analytical methods such as: photo chemiluminescence assay (PCL) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,20-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), the ferric reducing antioxidant power (FRAP), and cupric ion reducing antioxidant capacity (CUPRAC) for the measurement of lipid-soluble antioxidant capacity (ACL) or microbiological tests, mycotoxin and heavy metal analysis, etc. The results showed a great antioxidant potential of these and more, a good food safety level, which recommend them to be re-introduce in food system. Valorization of such waste and obtaining of food ingredients with functional potential used for increasing the nutritional value of new foods means an important contribution to the circular food system.

Keywords: *byproducts, quality, safety, circular food system, analytical methods*

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DURUM WHEAT ORIGIN BY MEANS OF COMBINED NOT CONVENTIONAL ISOTOPES AND MULTI-ELEMENTAL ANALYSIS

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Geographical origin of durum wheat is an important and emerging challenge, due to the added value, perceived by the consumers to the final products (e.g. pasta). It is also an emerging requirement needed to comply with specific national legislations. The values of the 87Sr/86Sr were successfully exploited in the first step of a tiered approach; adding then a second step by a Support Vector Machine Classification modelling SVMC based on Al, Mn, Mo, P, S, Ti, Y and Zn percentage in each sample. This study attests therefore the potentialities and successful validation of an innovative approach for the geographic discrimination of durum wheat on a global scale. It relies on combined information from multi-elemental analyses & stable isotope ratio values collected in authentic samples from different Italian, European and non-European regions during different harvest years. The correspondently developed predictive model is already routinely employed for the control of industrial lots.

Keywords: *wheat, geographical origin, not conventional isotopes, multi-elemental analysis*

L65

THE GERMAN PURITY LAW – METABOLITES OF WHEAT, CORN AND RICE IN BEER

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As one of the most significant historical food legislations, the Purity Law (1516) limits the ingredients for beer brewing to barley, hops and water. It aimed to ensure the safety and durability of the cultural beverage. Many aspects of this law are still applied in Germany and other countries. Nowadays, when the shelf life and quality of beer can be ensured on an industrial scale, numerous beers using other raw materials can be found on the global market. In particular, the use of other starch sources is considered a decisive factor in minimizing costs.
A widespread sample set of 400 beers was analyzed by ultra-high resolution (DI-FT-ICR-MS) and chromatography coupled mass spectrometry (UPLC-ToF-MS). Against the background of the German Purity Law, the comprehensive non-targeted approach allowed us to elucidate molecular signatures of different cereal varieties in the finished beer.
The mass resolution and wide chemical range of metabolites accessible by DI-FT-ICR-MS were used to describe and compare the beers at their molecular level. Unrivaled mass accuracy of Fourier transform ICR-mass spectrometry allowed to assign molecular compositions to over 7,700 resolved mass signals. The resultant comprehensive picture of the beers' chemical complexity was searched for metabolites specific for the use of wheat, corn, and rice in brewing using multivariate data analysis (OPLS-DA). Molecular networking (mass difference networks) of statistically specific accurate masses was performed based on the compositional information available for each singal. Across hundred potential marker molecules, metabolite signals specific for wheat, corn and rice as a starch sources were put in (bio)chemical relation. Including and extending from a few known plant metabolites, it provides a comprehensive molecular imprint of Purity-Law-contradicting cereals in beer.
Complementary UPLC-ToF-MS analysis of the beer samples confirmed the putative identity of key metabolites. Tandem mass spectrometry added the level of structural information, was used to create MS^2-similarity-networks and revealed the chemical compound classes of statistically conspicuous molecules. Besides wheat phytoanticipines and corn lipids, the aspartic acid conjugate of N-β-D-glucopyranosyl-indole-3-acetic acid as a potential marker for brewing with rice was described and identified using an isotope-labeled standard.
The non-targeted metabolomic study on cereal imprints in beer provides a comprehensive overview of the beer metabolome, molecular networks as specific fingerprints and ultimately single-marker molecules that open up the possibility to transfer the findings to other (targeted) analytical systems. It demonstrates that beer authenticity control is feasible on a big data metabolomics scale and the extensive molecular information available can be harnessed to find specific molecular targets [1].

[1] S. A. Pieczonka et al., *Front. Chem.* 2021 (715372)

Keywords: *metabolomics, beer, purity law, FT-ICR-MS, authenticity*

Acknowledgement: *We thank Prof. Hisashi Miyagawa for kindly cooperating with regard to the deuterated Asp-IAA-N-Glc standard that enabled level-1-identification of a potentially novel grain maker.*

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HS-GC-IMS RAPID FINGERPRINTING OF FOODS: UNTARGETED AND TARGETED ROUTES APPLIED TO QUALITY, AUTHENTICITY, AND SAFETY ASSESSMENT

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Food ingredients and food products are characterised by distinct volatilome fingerprints, composed of many volatile organic compounds (VOCs), acting as indicators of quality, often useful to assess authenticity, integrity and safety. Genotype, ripening process, processing of raw ingredients, storage, and spoilage can affect volatile fingerprints of foods, due to the impact of technology, enzymes, and microorganisms, leading to distinct VOCs. Moreover, the food contact materials (FCM, e.g. recycled plastic materials) can release specific volatile compounds affecting the flavour of foods, sometimes representing a risk for human health. Gas chromatography coupled with mass detectors, declined in different analytical approaches, as well as the comprehensive GCxGC-MS technique are powerful tools that are able to solve analytical challenges correlated to quality and safety items. HS-GC-IMS (Headspace Gas Chromatography coupled with Ion Mobility Spectrometry) is a rapid and sensitive method useful to describe the complex volatilome of foods. The reduction of time-consuming sample preparation, minimising handling and pre-treatment steps, as well as the usefulness of this analytical facility for rapid and on-site screening, represent the main values of this technique.
This lecture will be directed at the demonstration that HS-GC-IMS technology is capable to solve authenticity problems (e.g., integrity of traditional vinegar from Modena, Italy), quality challenges (e.g. the overall quality of vanilla pods and spreadable creams, related to ageing and oxidation; flavour of tomato products) and safety issues (particularly regarding the detection of molecules released from recycled food contact materials), also fitting with the monitoring of the at-line industrial processing of foods (chocolate, ice creams). Some case studies for each specific target will be shown reporting the advantages of this rapid approach of fingerprinting. HS-GC-IMS confirmed sensitivity, reproducibility, and usefulness regarding the at-line analysis, reducing the time to obtain the outcomes and costs (particularly regarding the quick and "green" samples preparation, avoiding the use of solvents and extractions).
The implementation of the AI-based strategies in the post-analytical processing of data will be also elucidated, opening new perspectives in rapid and partially automated food analysis.

Keywords: *GC-IMS, untargeted, targeted, integrity, quality, safety*

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DETECTION OF MECHANICALLY SEPARATED MEAT IN SAUSAGE AND COLD MEAT BY "TARGETED" LC-MS/MS ANALYSIS

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Background: The use of mechanically separated meat (MSM) from poultry in meat and sausage products is subject to declaration. Previous methods such as microscopy or calcium analysis have proven to be insufficient to ensure specific detection of MSM in meat and sausage products.
Hypothesis: when mechanically separated meat is used in the production of sausage products, disc and cartilage specific proteins from chicken, turkey or pork inevitably enter the products.
Methods: A pseudo-MRM-LC-MS/MS-based assay was developed and validated using disc- and cartilage-specific peptides to detect MSM from chicken in meat and sausage products. The five final selected marker peptides for chicken-MSM were assigned to collagen II alpha 1, which constitutes a large part of the proteome of intervertebral discs and cartilage.
Validation: To assess the validity of the methodology, a total of 23 positive controls (MSM content 5-90%) and a total of 19 negative controls were examined in a blinded study. Collagen hydrolysates were also added to two negative controls so that their influence could also be evaluated. After unblinding, 22 of 23 positive controls were correctly classified. Only one self-prepared sample containing 5% MSM was declared as a negative case (overall sensitivity 96%). In contrast, all negative controls were correctly classified as negative (specificity 100%) [1].
Summary: In summary, the new test can detect much lower amounts of MSM (10%) in commercial meat samples compared to all currently established standard methods such as microscopy, calcium detection, liquid scintillation counting (20%) or TXRF (40%). In addition, the method has the advantage of eliminating the need for extensive biochemical and chemical characterization of the sample material (lipids, proteins, ash, Ca, carbohydrates, etc.) because the high specificity of the pMRM transitions allows selective detection of MSM-specific marker peptides.
Outlook: Currently, developments are underway for the replication of MSM from turkey and pork. First results of these investigations will also be presented

[1] C. Wilhelm, M. Hofsommer, S. Wittke, FAM, 2022

Keywords: *mechanically separated chicken meat (MSM), targeted-LC–MS/MS assay, food authenticity, food fraud, blinded validation*

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DISCRIMINATION OF ITALIAN GRAPE MUSTS USING NMR METABOLOMICS

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As product mislabeling in wine industry remains a significant problem in the recent years, leading to significant losses in sales, employment, and revenue for this important component of food industry. Therefore, effective control measures require the assistance of the more advanced and precise analytical techniques for wine authentication to root out this problem. Grape must or grape juice is the primary precursor of wine on the road from grape berries, and physicochemical investigative methods have long been applied to it. Among the spectroscopic approaches used in wine analysis, nuclear magnetic resonance (NMR) spectroscopy is promising, and it has indeed been used on a wide scale recently. However, with respect to grape musts it has been much less applied. The reason for this consists in the fact that must is a "living" object that undergoes rapid transformation during fermentation, both alcoholic and malolactic; and it is extremely difficult to stop this process completely by any available approaches, be it addition of strong preservatives or deep freezing. In the terms of spectra quality this process results in severe non-linear misalignment of many signal groups leading to unsatisfactory results of statistical data treatment.
We have proposed to apply the untargeted NMR profiling to the grape musts from several wine producers from 17 Italian regions. After recording of the NMR spectra, specific data post-processing approach was used consisting in dividing the spectrum into three regions (organic acids, carbohydrates and aromatics), carrying out the dynamic alignment (or warping) via several software solutions such as MatLab and R scripts. Finally, several techniques of multivariate statistics were used with such methods as PCA, random forest etc. considering different parameters of the sample groups, e.g., geographical locations, agricultural zones, periods of harvest, types of grapes. We also address challenges encountered during this investigation as well as strengths and weaknesses of each approach.

Keywords: *grape musts, NMR Spectroscopy, food authentication, metabolomics*

Acknowledgement: *The research was funded by the FESR 2014–2020 Program of the Autonomous Province of Trento (Italy) with EU co-financing (Fruitomics).*

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IMPLEMENTATION OF SPME–GC–HRMS METHOD FOR DETECTING ADULTERATION OF SAFFRON BY MIXING IT WITH OTHER PLANT SPECIES

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Saffron is by far the most valuable spice in the world and, therefore, it often becomes an object of adulteration. The saffron spice is obtained by drying the scars of saffron flowers (*Crocus sativus* L.). The addition of other plant spices to saffron is one of the most common ways of its adulteration. Fraud is facilitated by the fact that saffron is sold mainly as a ground powder. Saffron is known mostly for its unique aroma, which is why this spice has been used for decades to season food. The aroma profile of saffron has already been described in many studies. However, only a few of them used aroma profile analysis for fraud detection. In this study, solid-phase microextraction followed by gas chromatography coupled to high-resolution mass spectrometry (SPME–GC–HRMS) was applied to analyze volatile profiles of 38 saffron samples and 37 samples of potential adulterants (safflower, calendula, capsicum, turmeric, arnica montana, beetroot, bixa orellana and pomegranate). For processing the obtained data chemometric methods such as principal component analysis (PCA) and partial least squares discriminative analysis (PLS-DA) were used. The chemometric analysis of volatile profiles of the spices and plant materials mentioned above showed good separation of saffron samples from adulterant samples. Significant markers for each group of adulterants as well as for saffron were tentatively identified. Another part of the experiment was devoted to the analysis of adulterated saffron samples. These samples were prepared by mixing a sample of saffron with selected plant material (safflower, calendula, capsicum and turmeric) at 20%, 10%, 4% and 2% levels. The SPME–GC–HRMS method allowed the revealing the adulteration of saffron samples by mixing with other plant species. It was able to detect an addition of adulterants even at a 2% level.

Keywords: *authentication, saffron, GC-MS, volatiles, chemometrics*

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DNA METABARCODING FOR THE SIMULTANEOUS DETECTION OF INSECTS IN FOODS

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There are thousands of insect species that are already consumed by humans in different geographic zones. The overall idea towards the exploitation of insect farming in EU was historically started mainly as a feed alternative over the use of imported soya, i.e., to foster the development of alternative feed material and to decrease EU dependency on critical imported feed material (De Visser et al., 2014). EU Regulation 2017/893 gave the green light for the use of seven insect species for use as feed. The suitability of additional species to be included in the catalog of insects that are suitable for commercial applications is already under examination (Rumbos and Athanassiou, 2021). Because insects are easy to farm due to their high conversion rates and are prevalent in most regions of the world, they have become an important source of nutrients for developing countries and nations prone to hunger crises (Halloran et al. 2014, Tao, Li 2018). In the meantime, insects have also become an attractive alternative to animal proteins in the food industry and manufacturing in Europe. FAO also indicates that insects are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content.
Under Horizon Europe, which is a funding programme for research and innovation, insect-based proteins are considered one of key areas of research. However, according to national and international food regulations, food consisting of insects or containing parts of insects must also be safe and authentic. Across Europe, edible insects and insect-containing foods are considered novel foods and require prior health assessment and approval under the Novel Food Regulation (EU) 2015/2283. So far, three insects have been approved as novel foods in the European Union, *Tenebrio molitor* (Yellow Mealworm), *Locusta migratoria* (Migratory locust), *Acheta domesticus* (House cricket), and a further nine are currently being evaluated by the European Food Safety Authority (EFSA).
In order to identify the possible use of insects or parts of insects in processed foods, reliable methods are needed. Metabarcoding approaches using NGS (Next Generation Sequencing) techniques for the detection of insects in food can provide a remedy for this problem by detecting many insect species simultaneously and also differentiating between them. Metabarcoding approaches are already successfully used in official control for the differentiation of mammals and birds (Dobrovolny et al., 2022, Preckel et al., 2021). Other methods have recently been published or are under development, including methods for the differentiation of fish and other seafood (Gense et al., 2021), and for the identification of plant ingredients. We present here a metabarcoding method for the detection of insects in food, which is able to detect the most common insects also in processed food (Hillinger et al., in preparation).

Keywords: *metabarcoding, food fraud, authenticity, edible insects, species identification*

Acknowledgement: *We thank our master student (Sophie Hillinger) for her phenomenal contribution and all colleagues who provided insight and expertise that greatly assisted the research.*

L71

CURRENT APPROACHES TO DATA HANDLING IN METABOLOMICS: FROM BASIC TO ADVANCED CONCEPTS

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Metabolomics is a fast-evolving strategy not only in food control but also in many other scientific fields. Data processing and handling is a crucial step and has far consequences on the results of metabolomic experiment i.e. the efficiency of statistical models used for food authentication or discovery of novel marker compounds. This lecture aims to give overview of data processing and handling to newcomers as well as experienced users addressing typical and novel tools to achieve sound results.

Keywords: *metabolomics, lipidomics, data handling, data processing*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

L72

DATA FUSION – AN EFFECTIVE TOOL FOR THE DEVELOPMENT OF RECOGNITION MODELS FOR HONEY AUTHENTICATION

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Honey is one of the most falsified food commodities, especially some particular varieties, due to the limited available quantities corroborated with high market demand. This is because some of the most important honey features, either in terms of taste or medicinal effects, are directly related to the botanical and geographical origin. Therefore, the temptation of some unfair producers or sellers to commercialize honey either under a false declaration of botanical variety or geographical origin must be discouraged through the development of easy-to-use and reliable tools for honey authenticity control. During the last years, vibrational spectroscopy (IR and Raman) in conjunction with advanced statistical methods have proved to be a suitable candidate for this purpose. This is because the main advantages of these techniques consist in an affordable analytical cost, rapidity, and reliability of the provided results. Moreover, the very fast development of the portable equipment market enhanced the applicability of these techniques, especially due to their easy-to-use operation mode. The complementarity of the results achieved through these two vibrational techniques is related to the fact that some vibrations which are active in IR are inactive in Raman spectroscopy and vice versa or by differences in band intensities but also by other specific features of each technique. Therefore, the corroboration of the information provided by these two analytical techniques is expected to enhance the discrimination capability of the developed recognition models as compared to the case when only one method, either IR or Raman spectroscopy is used.
In this regard, this work presents the development of honey recognition models based on a data fusion approach, namely by considering as input data the association between IR and Raman spectra. The obtained models' accuracies were compared to the achieved differentiation potential of the developed models when only one vibrational technique was used for the models' construction. Moreover, the study highlights the importance of the preprocessing phase for increasing the performance of the developed models. Apart from this, to enhance the classification accuracy of the honey differentiation models, a careful feature selection step was performed (i.e. corresponding to each of the investigated measurement types) in order to identify the most important markers that allowed the discrimination of the samples according to some pre-established criteria.

Keywords: *data fusion, IR spectroscopy, Raman spectroscopy, food authentication, honey*

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L73

RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY BASED – NOVEL MACHINE LEARNING VERSUS ESTABLISHED CHEMOMETRIC ANALYSIS FOR FISH FRAUD DETECTION

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Rapid evaporative ionisation mass spectrometry (REIMS) has been repeatedly demonstrated as a capable, fast and flexible platform to rapidly analyse a wide variety of different sample types, as diverse as biofluids and cancerous tissues through to biopolymers and faecal material. Its use to detect food frauds and provide quality measurements for food analysis has been equally widely demonstrated.
Its use for food analysis is a result of the flexible sampling interface options and the high speed at which samples can be analysed. It is possible to analyse very large numbers of samples (>100/h) and to undertake many types of analysis with little or no sample preparation. The high throughout and minimal sample preparation allows new or novel ways if sampling and sample types previously difficult to analyse are now more accessible using REIMS.
The use of REIMS on a high-resolution time-of-flight (ToF) mass spectrometer, combined with the high throughput nature of sampling creates new problems, however, with the REIMS system generating extremely large quantities of data. The typically data processing pathways used for REIMS include LDA and OPLS-DA discriminant analysis models. These techniques may not make the most effective use of the data generated by REIMS, and in some cases show increasing difficulty in processing the very largest REIMS data sets.
An alternative approach, using machine learning algorithms, is increasingly being adopted to better handle and utilise REIMS data.
This talk will discuss the use of established chemometric modelling and novel machine learning approaches to analyse a large fish data set comprising more than 2000 samples from 14 different commercially valuable species. Fraud within the fishery sector is a widespread issue, made more acute by new sanctions placed by Western nations on the Russian fisheries sector in response to the Ukraine invasion, and the ongoing shortages of seafoods as a result of climatic change.
These issues make substitution and mislabelling frauds particularly common within the seafood sector, and as a result, make the use of a rapid and robust screening technology capable of identifying a wide range of similar species highly desirable.
For this project cod, haddock, coley, whiting, herring, mackerel, rainbow trout, wild and farmed salmon were analysed using iKnife-REIMS. The resulting datasets were analysed using established chemometric models (LDA, PLS-DA and OPLS-DA models) and then compared to Random Forest and Support Vector Machine modelling which showed accuracies of 0.895 and 0.980 respectively, additionally the machine learning strategies eliminated the need to pre-process data, substantially cutting data processing times.

Keywords: *REIMS, ambient ionisation, mass spectrometry, food fraud, fish fraud*

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L74

VALIDATION OF LONG-TERM STABILITY OF CHEMOMETRIC MODELS EMPLOYED FOR VARIETAL AUTHENTICATION OF WINES (FOLLOW-UP STUDY)

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On the occasion of RAFA 2021 (www.rafa2021.eu), we presented a metabolic fingerprinting-bases multiclass strategy for varietal authentication of wines, which was developed within a joint research project of University of Chemistry and Technology Prague (UCT Prague) and German Federal Institute for Risk Assessment (BfR Berlin). 201 samples of five red and five white wine varieties were analysed and used for the creation of classification models. These models were arranged into decision trees and used for wine varietal identification. With classification rates ≥ 94% for both red and white wines, this strategy proved to be highly reliable. In the follow-up study, an additional sample set of 138 white wine samples was analysed and used for the verification of long-term stability of the previously created classification models. With a classification rate of 87%, the tested white wine decision tree appeared to be robust. Hence, the developed authentication strategy is very promising.

Keywords: *wine authenticity, metabolomics, UHPLC-HRMS, chemometrics, long-term stability*

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L75

EXPLORATION OF HIDDEN AUTHENTICATION PATTERNS IN NATIONAL FOOD CONTROL DATA

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Consumer demand for more information about the geographic origin of food is increasing. To determine the geographical origin, multiple data and statistical analyses are often needed. Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), generally known as dioxins, and polychlorinated biphenyls (PCBs) are toxic environmental contaminants. These lipophilic groups of compounds accumulate in fish with a high-fat content (e.g. salmon). Maximum levels are set in the EU regulation No 1881/2006 and further regulations for official tests are specified. The PCB and dioxin profiles found in salmon are typical for certain geographical origins (Sørensen et al. 2016). The data can therefore not only be used to test the compliance with the EU regulation but also to further exploit the potential of their underlying patterns to reveal authenticity features. A supervised classification model (one-class soft independent modelling of class analogy) was built based on the combined PCB and dioxin data from Baltic Sea salmon collected between 2004 and 2020. This model was then tested on a Baltic Sea salmon dataset from the year 2021 and salmon samples coming from other parts of the world (China, Chile, Canada, Norway, USA and Vietnam). The test samples were correctly classified according to whether they came from the Baltic Sea or not. This illustrates the potential of the model to predict the geographical origin of salmon samples in the future. The presented study contains data collected by the Danish Veterinary and Food Administration and the National Food Institute in Denmark over 17 years, showing the strong potential of this smart data handling approach. It should be further explored how to promote its way from authentication research into routine applications by official food authentication testing laboratories.

Sørensen S, Lund KH, Cederberg TL, Ballin NZ. 2016. Identification of Baltic Sea salmon based on PCB and dioxin profiles. Food Control. 61:165–171. https://doi.org/10.1016/j.foodcont.2015.09.044

Keywords: *authentication, salmon, geographical origin, chemometrics*

L76

MONITORING FOOD ADDITIVES AND IMPACT OF "CLEAN" LABELLING: WHEN THE TRENDS MATTER!

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Analytical methods have a crucial role in food additives (FAs) monitoring. More than 330 food additives are authorised in Europe and listed in Annex II of Regulation (EC) No 1333/2008. These compounds are very different, resulting in a need for different extraction procedures and analytical techniques.
Furthermore, Member States are required to monitor the consumption and use of FAs using a risk-based approach. However, dietary habits and FAs usage evolve quickly, and potential changes in the intake by the population need to be considered. Additionally, food manufacturers are under considerable pressure to minimise the use of FAs, often replacing them with "natural" alternatives. These reformulations are often accompanied by advertising claiming "no colourings", "no preservatives", or "no artificial additives". This trend is most commonly called "clean" labelling.
The aim of this work is to present a practical approach to prioritisation in FAs' monitoring plans.
First, an extensive market survey was conducted, and the presence of food additives in the Belgian market was mapped in an in-house dataset covering 36.601 food and beverages. The data mining step allowed assessing the prevalence, distribution, and co-occurrence of FAs within the dataset, giving a snapshot of the FA use in Belgium. The match of these criteria with authorised Food Category, Health-Based Guidance Values, and possible toxicological effects, allowed the creation of a risk-based ranking of the FAs. Next, the new trends were investigated by performing a market study using the Mintel Global New Product Database. The results highlighted a general reduction in the use of FAs in the last five years in Belgium and swap with other ingredients. The use of fruit and vegetable extracts to replace colours, citrus concentrates and vinegar powder to substitute acidifiers, use of a combination of sweeteners or sweetener with sugars are just a few examples. These reformulation trends put stress on new analytical methods for FA's.
Finally, prioritization of FAs for Belgium was achieved and led to applying in-house validated methods for a selection of FAs.

Keywords: *food additives, monitoring strategy, market survey, reformulation practices*

L77

ANALYSIS OF PFAS IN FOOD ITEMS, FOOD PACKAGING MATERIALS, HUMAN MILK AND SERUM

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At several places in the world the production of per- and polyfluorinated alkyl substances (PFAS) has led to high levels of these compounds in the neighbourhoods of PFAS producing plants. We analysed blood samples of inhabitants of Antwerp living close to a PFAS plant, as well as tap water, and fish, sediment, and surface water from the river Western Scheldt. Also, we determined PFAS in human milk from women living close to a Teflon manufacturing plant in Dordrecht, the Netherlands, and living close to a PFAS plant in Lyon.
Blood samples were taken from 45 persons that lived and/or worked within a few kilometers from the 3M site in Zwijndrecht. All persons completed a questioinnaire in which they answered a series of questions about their background, age, gender, die, etc. Flounder, marine vegetables, and surface water were sampled from the Western Scheldt at the Dutch/Belgian border and Antwerp harbour. Drinking (tap) water was sampled in Antwerp. Sets of 17-30 PFAS were determined by LC-MS/MS analysis on an LC-Sciex Exion LC AD coupled to a Sciex 6500+ triple-quad MS, using an Xbridge BEH C18 XP 150 x 2.1 mm, 2.5 µm column and electrospray ionisation.
The recent opinion on PFAS of the European Food Safety Agency (EFSA) gives a safe level of PFAS in human blood that is based on the sum of four PFAS congeners: PFOA, PFOS, PFHxS and PFNA. This sum should not be higher than 6.9 mg/L. The maximum value in human blood from Antwerp was 1154 mg/L, which is 167-fold the norm. Most of the persons studied consumed vegetables or fruit from their own gardens and/or ate eggs from their own chickens. Based on these results a larger study on PFAS in human blood was initiated by the Flemish authorities in 800 persons living within a circle of 3 km around the 3M site. That study confirmed the here presented results. 93% of the PFAS concentrations found in the larger study exceeded the EFSA guidelines of 6.9 mg/L. Western Scheldt fish, sediment and water samples confirmed the PFOS pollution from Antwerp. The PFOS concentration in flounder was 24 mg/kg, lower than determined by Chu et al. [4] who found 322 mg/kg in 2006. The perfluoro-1-butane-sulfonamide (FBSA) detected by Chu et al. was also found by in our study. The results of this study urge much better inventories of environments around current PFAS production plants.
Within the European REVAMP project new native and labeled PFAS standards are being produced, which were also used in a study on PFAS compounds in food packaging materials.

Keywords: *PFAS, food items, food packaging materials, human milk and serum*

L78

PARTS PER TRILLION LEVELS OF PFAS IN FOOD

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Per- and polyfluorinated compounds (PFAS) are anthropogenic chemical compounds, non-degradable with an increasing concern due to their accumulation in the environment and humans with potential toxic effects. These substances, known to have water-, dirt- and grease-repellent properties, are largely used in household utilities, cosmetics, food-packaging materials and clothing, among other things. PFAS are persistent environmental contaminants that can be found not only in water compartments (surface, drinking, waste, and ground waters) but also in all food types.
Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) as well as their derivatives are considered as persistent organic pollutants (POPs) by the EU POP Regulation, which implies of restricting their use. PFAS alternatives, which are presumed to be less toxic, have been manufactured to replace the traditional ones in the market. However, a continuous accumulation of PFAS in the environment and human samples is still reported with unidentified effects in human health.
Challenges linked to the analysis of PFAS in foodstuffs are numerous and comprised their huge number (ca. 7500 as listed in the US-EPA CompTox Dashboard [1]) with new ones regularly discovered [2,3]), the low limits of quantification that have been recently drafted by the European Commission, e.g., from 0.001 to 0.004 µg/kg for some of them, and the subsequent difficulties to avoid the numerous sources of contamination when taking various samples for analysis (being raw commodities from the agricultural side, processed ingredients from suppliers, or packaged products from the factory line, etc.) to the laboratory.
This presentation will highlight these analytical challenges, also in regard to the recently published EURL POPs guidelines for PFAS that are applied for the analysis of other types of food contaminants.

1. USEPA, *PFAS Masterlist of PFAS Substances. https://comptox.epa.gov/dashboard/chemical_lists/pfasmaster.*
2. Barzen-Hanson, K.A., et al., *Discovery of 40 Classes of Per- and Polyfluoroalkyl Substances in Historical Aqueous Film-Forming Foams (AFFFs) and AFFF-Impacted Groundwater.* Environ Sci Technol, 2017. 51(4): p. 2047-2057.
3. Liu, Y., et al., *High-resolution mass spectrometry (HRMS) methods for nontarget discovery and characterization of poly- and per-fluoroalkyl substances (PFASs) in environmental and human samples.* TrAC Trends in Analytical Chemistry, 2019. 121: p. 115420.

Keywords: *PFAS, food contaminants, food analysis*

L79

ENHANCED FOOD SAFETY AND QUALITY ASSESSMENT THROUGH HYPHENATED AND AUTOMATED SAMPLE INTRODUCTION COUPLED TO GC×GC

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Food analysis is a rapidly evolving field involving more advanced analytical techniques to answer more complex questions respecting the requirements of green and white analytical chemistry. In this framework, along with the miniaturization and reduction of solvent consumption, it is fundamental to maximize the amount of information for every single analysis. These advanced chromatographic techniques coupled with efficient, automated, and miniaturized sample preparation techniques are extraordinary tools. The selection of the proper sample preparation technique, along with its proper tuning may add an important extra dimensionality to a comprehensive multidimensional system (GC×GC), especially coupled with a mass spectrometer (MS). In this scenario, the selection of the proper sample preparation system may become a hyphenated extra dimension that can be automated and tuned to maximize the level of information extractable from a single set of analyses. Both sorptive headspace techniques and liquid sample introduction techniques can contribute to maximize the information. Different sample introductions will be examined to evaluate their contribution to the overall analysis. In particular the use of a completely hyphenated LC- GC×GC-TOFMS/FID system for safety control in the field of mineral oil contamination in food, and the use of headspace sorptive techniques (i.e., SPME and HISorb) to answer to quality and authenticity food-related questions will be presented, showing the extraordinary potentials and flexibility that the systems can provide.

Keywords: *MOSH & MOAH, volatiles, food quality, high concentration capacity tool, LC-GCxGC-TOFMS/FID*

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L80

NON-VOLATILE SUBSTANCES EXTRACTIBLE FROM INNER COATINGS OF METALLIC CANS FROM THE FRENCH MARKET AND THEIR OCCURRENCE IN THE CANNED VEGETABLES

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Regulatory pressure against bisphenol A for food contact material (FCM) applications led can coating manufacturers to develop polyester-based alternatives. Besides intentionally added substances, the investigation of the presence of non-intentionally added substances (NIAS) has been raising the interest of the scientific community in response to manufacturers and consumers concerns. NIAS, generally of unknown structures, can be impurities from starting materials, degradation or decomposition products, contaminants from production process or oligomers principally formed during the synthesis of the coating. Such oligomers can be formed during resin synthesis or upon degradation over time. Although a wide range of reactions are possible (which poses an analytical challenge), these NIAS may be predicted since polyalcohols and carboxylic polyacids involved in the polycondensation reaction are generally known. In order to increase chemical food safety of packaging, it is now necessary to produce qualitative, quantitative and toxicological data.
This work aimed to contribute to the risk assessment of NIAS from polyester-based coatings by developing an innovative analytical strategy dedicated to their characterisation. A comprehensive database of predictable oligoesters was developed based on known monomers. Combined with the acquisition of a fingerprint from polyester resins extracts by LC/HRMS coupling, it allowed for the annotation of the signals of interest. Eventually, previously unavailable genuine analytical standards were synthetized, including linear and cyclic, native and deuterium-labelled combinations of neopentyl glycol and isophtalic acid (lengths of 4 and 8 monomers), allowing structure identification at the highest level of confidence and semi-quantification. The developed methodology allowed to pinpoint more than 125 compounds in a set of 40 coatings from 12 vegetable cans sampled in a supermarket. Among the 75 oligoester combinations (involving 6 diols and 4 diacids) identified in the coatings, 61 migrated to the can contents. Cumulative semi-quantification levels averaged 330 ng/g in the drained vegetables (43 to 1600 ng/g).

Keywords: *coating, non-intentionally added substance, oligoester, high-resolution mass spectrometry*

Acknowledgement: *Synthesis of oligoesters standards of this project was implemented by the IBISA core facility CHEM-Symbiose (CEISAM, UMR CNRS 6230) as part of the Biogenouest network devoted to the custom synthesis of molecules for academic consortium. The authors are grateful to Julie Hémez and Laurence Arzel (AMaCC platform, CEISAM, UMR CNRS 6230, University of Nantes) for their mass spectrometry analytical contributions to this work, as well as to Maxime Lelièvre and Charles Lenclume (LABERCA) for their help in sample preparation developments. Elsa Omer, who contributed to the characterisation of the synthesised standards is gratefully acknowledged.*

L81

BISPHENOL A BY LC-MS/MS: A CHALLENGING INCREASE IN SENSITIVITY

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Among other fields of application, bisphenol A (BPA) is used in the production of food contact materials made of polycarbonate plastics (e.g. tableware, reusable beverage bottles, storage containers), epoxy resin coatings of cans, thermal paper and toys. In the case of food, canned products (e.g. meat and fish) can be affected with bisphenol A migrating from can coatings into food. The widespread application of BPA results in the fact that BPA is now ubiquitous and therefore challenging the analyst regarding blank values and spot contaminations.
The European Food Safety Authority (EFSA) has revised its risk assessment for bisphenol A because of new data regarding the effects on the immune system and published a draft of a new scientific opinion on 15.12.2021, in which a TDI value of 0.04 ng/kg body weight per day was proposed. This TDI value was lowered by a factor of 100,000 than the previous provisional TDI value of 4 µg/kg body weight per day. Due to the new toxicological rating a significant lowering of the limit of quantification is necessary in order to achieve a reasonable evaluation of the safety of food.
The application of a modern LC-MS/MS system is mandatory, but the biggest challenge, however, is the ubiquitous occurrence of BPA.
To reduce the background signal from BPA contained in components of the HPLC system we established a trap column. Also many reagents, chemicals and other labware contain traces of BPA prior not affecting the result. To keep the method blank as low as possible several actions are taken: All glassware is baked out and single-use plastic labware as well as SPE materials are cleaned by a solvent extraction prior to use. Nonetheless, the sample preparation procedure has to be kept as simple as possible. Since the contamination of BPA is relevant for many different kinds of food materials, another challenge was the establishment of various sample preparation procedures for several different matrix groups, i.e. low/high fat, high carbohydrates etc.
Here we present the outcome of our studies of several extraction solvents and clean-up techniques on the reliability of a method for the quantification of BPA in the ppt range for various food matrices. Although some procedures result in cleaner extracts and lower matrix effects in LC-MS/MS many reagents cause unacceptable high blank values, e.g. the general QuEChERS clean-up and so-called molecular imprinted particles (MIPs).
As a result of the numerous blank control actions and pre-cleaning we achieve low limits of quantification in the range of 10-100 ng/kg depending on the matrix. Based on the new TDI and achievable LOQ, ingredients and intake that were not necessarily considered before can potentially be reexamined.

Keywords: *bisphenol, food contaminant, food contact material, ubiquitous contaminant, ultra trace analysis*

L82

ANALYTICAL STRATEGIES IN THE EDIBLE OIL INDUSTRY TO CONTROL MOSH-MOAH CONTAMINATION

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Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) are compounds of potential health concern that end up in food ingredients and products. In anticipation of upcoming legislation, food industry is taking a diversity of actions to control mineral oil contamination aiming to avoid risks and meet customers' demands for mitigating. Considering that MOSH-MOAH consist of hundreds of thousands of compounds derived from a wide variety of sources such as environment, farming, storing, transportation or processing, it is key to have a strong and reliable analytical strategy to face this challenge. For the edible oils and fats industry the challenge is even greater, since the supply chains are very complex and moreover, these ingredients contain a huge amount of natural compounds that interfere with the chemical analysis. In this communication, we will present the analytical toolkit developed in the edible oils and fats industry to characterize MOSH-MOAH, their analogs and natural interferences in a diversity of oils and fats. This analytical strategy includes advances in the purification steps, and qualitative and quantitative analytical methodologies (i.e., LC-GC-FID, on-line-LC-GCxGC-MS/FID, and GPC-HPLC-Fluorescence). In addition, new insights on the proper use of mineral oil markers, sources of analogs and natural interferences will be discussed.

Keywords: *MOSH-MOAH, mineral oil, contaminants, analytical toolkit, edible oils and fats*

L83

FOODOMICS & THE HOLOMETABOLOME: HIGH RESOLUTION TAILORED METABOLOMICS IN THE FOOD-NUTRITION-HEALTH CHEMICAL CONTINUUM

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Metabolomics, as the comprehensive study of metabolic reactions in complex dynamic living systems and thus in holobionts is growing very rapidly, and integrates analytical approaches (LC-MS, NMR and ICR-FT/MS) covering the possible description of only 10% of the experimental signals in databases. Important approaches thus are related to the description of the dark metabolome with adapted strategies. Especially direct injection FTICR/MS enables a long term high throughput description of highly complex mixtures and holometabolomes at the level of the elementary composition space. Foods are complex chemical mixtures/systems themself composed of original plant/animal metabolites and or transformed metabolites i.e. from fermentation (acidic, alcoholic) or thermal processes (Maillard-type). FTICR/MS will be presented is a strong tool to describe the known/unknown chemistry and chemical diversity in various study fields of food chemistry, microbiomes towards the discovery of new bioactives.

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L84

GUT MICROBIAL METABOLITES: THE COMBINATION OF LC-(HR)MS TECHNIQUES TO ELUCIDATE THE BREAKDOWN OF APPLE (POLY)PHENOLS

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Gut microbiota comprises all microorganisms found in the gastrointestinal tract, including bacteria, viruses, and fungi, with a fundamental role in many host processes. The gut microbiome exhibits plasticity and can readily adjust to various environmental and host-derived stimuli. These microorganisms digest certain foods and produce bioactive metabolites. Indeed, diet is crucial in determining gut bacterial assembly and has a significant role in shaping the human microbiota composition and function. A diet rich in phytochemicals and fiber could provide the gut microbiota with the substrate needed to produce gut microbial metabolites that may potentially promote gut health.

During my presentation, I'll pay particular attention to flavan-3-ols, the most consumed flavonoids in the Western dietary pattern. By combining static and dynamic *in vitro* models (SHIME) inoculated with human feces, we confirmed and extended the knowledge of *in vitro* degradation of these phytochemicals. The fate of these Flavan-3-ols was investigated in-depth using 48h fecal batch fermentations and long-term exposure (SHIME) of epicatechin, proanthocyanidin C1, and an apple (*Renetta Canada*). After optimizing the sample preparation, the degradation of these compounds was monitored by combining LC-QTOF and LC-QqQ. Pure compounds used, epicatechin and proanthocyanidin C1, were extensively biotransformed within 8h. In contrast, the apple polyphenol breakdown was slowed down in the food model. We also noticed interindividual differences in forming these (poly)phenol catabolites. The donors showed differences in the ability to produce specific intermediates and metabolites, such as proanthocyanidin B2, dihydroxyphenyl-γ-valerolactone, and hydroxyphenyl-valeric acids. In conclusion, the Flavan-3-ol structure and the food matrix affect the biotransformation of native compounds into gut microbial metabolites, conditioning the reaction rates and the metabolites released, which are also modulated by interindividual differences.

Keywords: *gut microbial metabolites, gut microbiota, LC-QTOF, gut health, polyphenols*

L85

MODULATION OF STAPHYLOCOCCUS AUREUS MULTIDRUG RESISTANCE BY NATURAL COMPOUNDS AND THEIR DERIVATIVES

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Antibiotic resistance is currently a serious health problem. Since the discovery of new antibiotics no longer seems to be a sufficient tool in the fight against multidrug-resistant infections, adjuvant (combination) therapy is gaining in importance as well as reducing bacterial virulence.
This work is focused on mechanisms of oxacillin and gentamicin resistance of methicillin-resistant *Staphylococcus aureus* (MRSA), which is listed as a "High priority" for discovery, research and development of new antibiotics according to the World Health Organization. Primers specific for genes conferring resistance to oxacillin (*mecA*, *blaZ*) and gentamicin (*aacA-aphD*) were designed using Primer-BLAST. The presence of these genes in the clinical isolate of multidrug-resistant *S. aureus* (strain NEM 449) was verified by polymerase chain reaction. Subsequently, the antimicrobial activity of 52 herbs extracts was tested using high-throughput screening approach. Several herbs demonstrated antibiotic-resistance modulation activity, among them, *Silybum marianum* and *Agrimonia eupatoria* showed the most promising results. Analytical procedures and correlation of biological activities identified biologically active substances in methanol extracts of herbs, which were subsequently tested.
Silybin A, 2,3-dehydrosilybin B, and 2,3-dehydrosilybin AB completely reversed antibiotic resistance at concentrations of 20 μM or less. Both 2,3-dehydrosilybin B and AB decreased the antibiotic-induced gene expression of representative efflux pumps belonging to the major facilitator (MFS), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC) families. 2,3-Dehydrosilybin B also inhibited ethidium bromide accumulation and efflux in a clinical isolate whose NorA and MdeA overproduction was induced by antibiotics. Most of the tested flavonolignans reduced cell-to-cell communication on a tetrahydrofuran-borate (autoinducer-2) basis, with isosilychristin leading the way followed by 2,3-dehydrosilybin A and AB, which halved communication at 10 μM. Anhydrosilychristin was the only compound that reduced communication based on acyl-homoserine lactone (autoinducer 1), with an IC_{50} of 4.8 μM. Except for isosilychristin and anhydrosilychristin, all of the flavonolignans inhibited *S. aureus* surface colonization, with 2,3-dehydrosilybin A being the most active (IC50 10.6 μM). In addition, the halogenation of flavonolignans significantly increased these activities. In conclusion, the selected flavonolignans, particularly derivatives of 2,3-dehydrosilybin B, 2,3-dehydrosilybin AB, and silybin A are non-toxic modulators of *S. aureus* multidrug resistance and can decrease the virulence of the bacterium, which deserves further detailed research.

Keywords: *silymarin, antibiotic, adjuvant therapy, czech herbs*

Acknowledgement: *The financial support by Czech Grant Agency (contract No. 21-00551S) is greatly appreciated.*

L86

POST TRANSLATIONAL MODIFICATION (PTM) PROFILING OF BOVINE WHEY PROTEINS BY A SEMI-UNTARGETED SHOTGUN PROTEOMIC APPROACH

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Dairy products play an essential role in human nutrition. Especially whey proteins are of very high biological value. Therefore, they are often used in specialized nutrition, e.g. dietary supplements and infant formulas. During the production of these products, various processing steps are applied that mostly include heating. The heat load leads to an increasing formation of (non-enzymatic) post translational modifications (PTMs) which may negatively influence various technological and biological properties e.g. the solubility and digestibility of the proteins. Whey proteins of infant formula can serve as the main source of essential amino acids, so that their heat induced loss must be closely monitored.
In order to assess protein damage by PTMs, a semi-untargeted shotgun proteomics approach was established which detects a wide range of modifications of the two major whey proteins α-lactalbumin and β-lactoglobulin in parallel. Protein standards as well as whey obtained from raw milk were heated in a milk simulating buffer. The samples were subsequently hydrolyzed using the endoprotease Glu-C. The resulting peptide mixtures were then analyzed by microLC-ESI-IM-QTOF in the data dependent acquisition (DDA) mode and the data was evaluated with the software PEAKS®studio. Hereby PTMs were detected according to an individually adapted and compiled database that includes 52 dairy relevant amino acid modifications such as early and advanced Maillard reaction-, oxidation-, condensation- and hydrolysis products. Downstream data analysis included multivariate statistical analysis. Reproducibility was checked by several quality assurance criteria.
The number of modified peptides proved to be a valid marker for the heat damage of proteins, since the presented method covers a wide range of modifications compared to established methods, which often include only few selected marker compounds. Thus, peptides with at least one PTM increased by a factor of 2.5 from 375 in non-heated β-lactoglobulin to 936 in the samples subjected to the highest heat load. A similar rise was observed in the α-lactalbumin samples, where the number rose from 233 to 568 by a factor of 2.4. High abundant PTMs could be monitored by label free quantification. The peak area of a peptide with a methionine sulfoxide modification, for example, increased up to 13.8-fold in the heated β-lactoglobulin samples. However, the same peptide showed only a 5.8-fold increase in the heated whey samples indicating considerable beneficial effects of the whey matrix on methionine oxidation.
Since the data acquisition was performed in the DDA mode, post hoc data analysis is possible. Thus, novel modifications can be easily included in the same set of samples without requiring additional LC-MS/MS runs.

Keywords: *whey proteins, heat load monitoring, PTM profiling, LC-MS/MS, semi-untargeted shotgun proteomics*

L87

NON-TARGETED VOLATILOMICS AND MACHINE LEARNING – FINDING FEATURES AND GETTING THE MOST OUT OF YOUR GC-IMS DATA

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Highly complex analytical tasks, such quality control or authentication of food and feed, typically require high-end instruments, among other high-resolution mass spectrometry or NMR. These techniques have a considerable demand on the laboratory infrastructure, which is particularly challenging if measurements are to be carried out directly in the countries of origin, at the distribution sites or at customs - the so-called "point of care". In this context, powerful benchtop profiling techniques, such as gas chromatography hyphenated to ion mobility spectrometry (GC-IMS), are a highly competitive and meanwhile more and more common alternative [1].
GC-IMS generates true 2D information – GC-based retention time and collisional-cross-section-based drift time – which delivers enormously complex fingerprint data. These data are optimal for non-target approaches, as they are mostly required for "diffuse" analytical questions, such as geographic or botanic differentiation of honeys, quality or authenticity of olive oils or sensory profiles of coffee. Here, there are typically no characteristic single marker compounds, but rather a plethora of different compounds in varying ratios, that carry the desired information. This approach requires intelligent and powerful signal preprocessing of raw data, as well as tailor-made chemometric tools to extract as much information as possible and to generate robust machine or deep learning models to predict class properties or quantitative information, e.g. by PLS-DA or PLS-R. These algorithms allow further the selection of potentially characteristic features or "biomarkers" based on their importance in the projection (VIP scores). This talk will demonstrate and explain use cases in food authentication and quality and discuss approaches towards non-target analysis based on GC-IMS in combination with our new Python-based, open source toolbox *gc-ims-tools* [2].

Keywords: *GC-IMS, machine learning, chemometrics, python, food profiling*

L88

AN INNOVATIVE TOP-DOWN METHODOLOGY BASED ON METABOLOMICS APPROACHES FOR A NEW UNDERSTANDING ON THE ROLE OF PLANT BIOACTIVE PHENOLIC COMPOUNDS. LOOKING FOR COMMON CIRCULATING METABOLITES BASED ON 5 BIOACTIVE PLANT MATRICES.

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Clinical evidence shows that bioactive compounds derived from a variety of edible plants exhibit potential benefits in human chronic diseases. Scientific research has attributed similar bioactive properties to a variety of compounds from different families. Traditionally, a bottom–up methodology has been carried out to discover which compounds are responsible for bioactivity. This traditional methodology consists of firstly evaluating the bioactivity of these compounds by in vitro assays. The bioactive compounds are then tested by in vivo assays in animals and humans. However, it is well known that these compounds suffer metabolic reactions before reaching the therapeutic targets. Therefore, which are the compounds responsible for bioactivity, the compounds from plants or their metabolites? Another important question is whether ther e is a c ommon mechanism amongst the different families of bioactive compounds. To resolve these questions, we performed an acute double-blinded dietary intervention study in humans using five dietary supplements based on different bioactive extracts (Hibiscus sabdariffa, Olea Europaea, Silybum Marianum, Theobroma Cacao and Lippia Citriodora). These extracts were chosen to represent a broad composition of potentially bioactive compounds. From the intervention trial, blood plasma samples were collected at different times (0, 0.5, 1, 2, 4, 6, 8 and 10 h). The biological samples were analysed by untargeted metabolomic workflow based HPLC-ESI-QTOF-MS. The data was processed using free access software (mzMine and R packages) where more than 20000 signals were detected. The fold-change (FC) ratios were determined by dividing the areas at each of the times by that corresponding to time 0 for each subject. Those variables that presented a FC greater than 20 were proposed for identification. Prelimin ary results allowed the detection of more than 60 potential signals derived from potential bioavailable metabolites. These signals were annotated using MS/MS fragmentation spectra and information available in MS databases (HMDB, FooDB, Massbanks, etc.). The current results have made it possible to annotate interesting circulating metabolites, derived from phase I and phase II metabolization reactions. Among the results, the detected common circulating metabolites coming from different matrices stand out, highlighting those that come from the Lippia and the Olive extracts (i.e. hydroxytirosol sulphate, hydroxytirosol glucuronide,...). In addition, it is also noteworthy that several of these common metabolites have their maximum absorption in plasma at different times depending on the source matrix, demonstrating different degradation/absorption mechanisms. Currently, we are working on demonstrating the bioactive properties of these bioavailable compounds isolated, which will allow us to obtain a scientific basis that will allow the development of future applications based on them such as pharmaceuticals or functional foods.

Keywords: *phenolic compounds, metabolomics, mass spectrometry, bioactive compounds, plant extracts*

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L89

MICROALGAE AS SUSTAINABLE SOLUTIONS FOR LIPID PRODUCTION: FROM LIPIDOMICS ANALYSIS TO BIOACTIVITY SCREENING

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The demand for sustainable and eco-friendly food ingredients has experienced an outstanding increase in recent years. Microalgae are crucial sources for meeting population needs in terms of valuable lipids, including omega-3 long chain-polyunsaturated fatty acids (n-3 LC-PUFA) and more complex lipids such as glycolipids and phospholipids. However, the environmentally-friendly extraction of these bioactive remains unexplored. In this work, the use of enzyme-based methods in combination with ultrasounds was evaluated as green approaches to produce high-quality lipid extracts. Moreover, the effect of eco-friendly approaches on the lipidome profiles and the bioactivity of microalgal lipids were also investigated to evaluate the potential of these valuable lipids in health and food applications. A novel lipidomics workflow was applied for the analysis of the microalgae lipidome based on liquid chromatography high-resolution mass spectrometry (LC-HRMS). Different lipids containing n-3 LC-PUFA of nutritional importance were identified, including neutral and polar lipids, such as phosphatidylcholines (PC), triglycerides (TG), as well as galactolipids including monogalactosyldiacylglycerols (MGDG), and digalactosyldiacylglycerols (DGDG). The lipid composition differed from the extraction methods applied depending on the enzymatic solution used, leading to enriching the extracts with different lipid classes. In terms of bioactivity, the cytotoxicity of the produced lipids was assessed by comparing human colon cancer cells (HCT-116) and epithelial nontumorigenic immortalized cells (HCEC-1CT). Results suggest that the lipid extracts have a selective cytotoxic effect, reducing the viability of the colon carcinoma cells but not the nontumorigenic cells. Thus, this multidisciplinary approach sheds light on the microalgae lipidome, and at the same time, provides new eco-innovative methods for extracting valuable lipids enriched in n-3 LC-PUFA from microalgae with promising biological properties.

Keywords: *omega-3 fatty acids, eco-friendly approaches, microalgal lipidome, bioactive compounds, cytotoxicity*

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L90

EU POLICY ON CONTAMINANTS IN FOOD: OUTLOOK AND ANALYTICAL CHALLENGES

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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.
The Regulation (EU) 2017/625 (the new "Official Control Regulation") contains provisions on procedures to be applied for official control, including on methods of analysis to be used. 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. In the presentation, particular attention shall be paid to the analytical requirements and analytical challenges that this entails 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, the method of analysis must be reliable, sensitive, quick and preferably cheap.

Keywords: *contaminants, EU policy, challenges*

L91

PERSISTENT AND MOBILE INDUSTRIAL POLLUTANTS IN A CIRCULAR FOOD CHAIN: AN OVERLOOKED PROBLEM?

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Within Europe, we are aiming for a more sustainable food system. One aspect of sustainability is to move towards a circular food system, according to the ambitions in Europe's Green Deal. However, it should be taken into account that food safety hazards may accumulate in circular food systems. The fate of chemical hazards in circular food systems is largely unknown. It is expected that persistent chemicals remain in the system, and to circulate in food production. Particularly in food production involving the use of water (e.g. for irrigation), Persistent and Mobile Organic Compounds (PMOCs) will stay, and over time increase. PMOCs (also referred to as PM substances) are man-made organic chemicals. They are highly polar, degrade very slowly (if at all) in the environment and show a low tendency to sorb to surfaces or to organic matter in soil and sediments (Reemtsma et al., 2016). In other words, they are very water-soluble, which give them mobile characteristics, leading to transport in surface water and groundwater.
PMOCs get into surface water through e.g. effluent from waste water treatment plants (WWTP). Re-used water (reclaimed water) may also contain PMOCs, even after purification, current water cleaning technologies are not capable of efficient removal of these chemicals. As a result, contaminated water for irrigation leads to uptake in plants, which may in turn lead to compromised food safety. Little is known about the actual risk of PMOCs in food production systems, and therefore the question is raised if we are overlooking a potential problem.
In this contribution, examples of hazards will be given, and potential risks will be addressed, as well as the regulatory aspects of water quality in relation to the agricultural production. Moreover, examples will be provided on the fate of these chemicals and the contribution of analytical chemistry to progress this field is highlighted.

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Keywords: *circular economy, contaminants, PFAS, emerging pollutants*

L92

GOING -OMICS TO UNDERSTAND PLANT RESPONSE TO MULTIPLE CHEMICALS

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Among cereal crops, increasing wheat yield is vital to meeting future global food demand. It has been estimated that we will need to produce 60% more wheat by 2050[1] and this is going to be a challenge in the facing of climate change and environmental contamination. Concerns about environmental and soil are exponentially growing as described in 2021 by FAO technical reports[2]. The matter is critical, as about 95% of the food we eat comes from it. Indeed, due to their sessile lifestyle, plants are continuously exposed to a large set of natural (i.e. mycotoxins) and man-made contaminants (i.e. pesticides, veterinary drugs, perfluoroalkyl substances). So far, an individual-contaminant approach has been employed to study wheat response to most xenobiotics. This strategy is useful to gain knowledge on the mechanism of action, but it is not representative of real-world scenarios, where wheat is simultaneously exposed to a mixture of natural and man-made contaminants. Such stress combinations can result in either specific or integrated signalling cascades that warrant further attention to gain a more realistic representation of wheat response. However, the study of the multifactorial mechanism of plant resistance against chemical mixtures is a challenging task. At the same time, it represents necessity, requiring the implementation of the most modern, cutting-edge analytical tools nowadays available such as omics in the spatial domain to correlate chemical fingerprinting and biological properties.In this talk, the major challenges but also opportunities offered by the integration of multi-omics and visualization techniques will be addressed, with the aim to improve wheat resilience and food safety.

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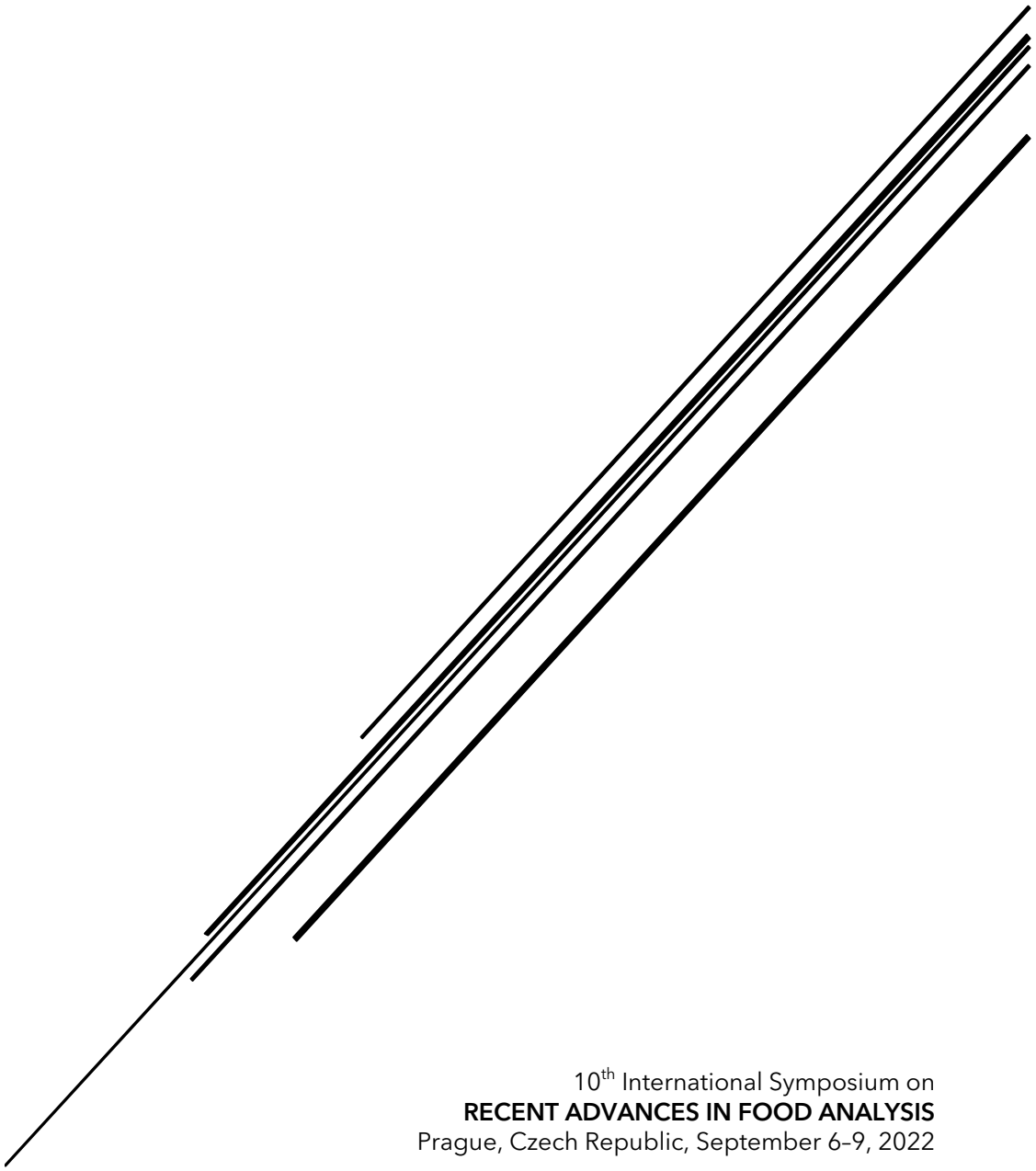
Keywords: *cereals, contaminants, omics, imaging*

POSTER SESSIONS



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Prague, Czech Republic, September 6–9, 2022

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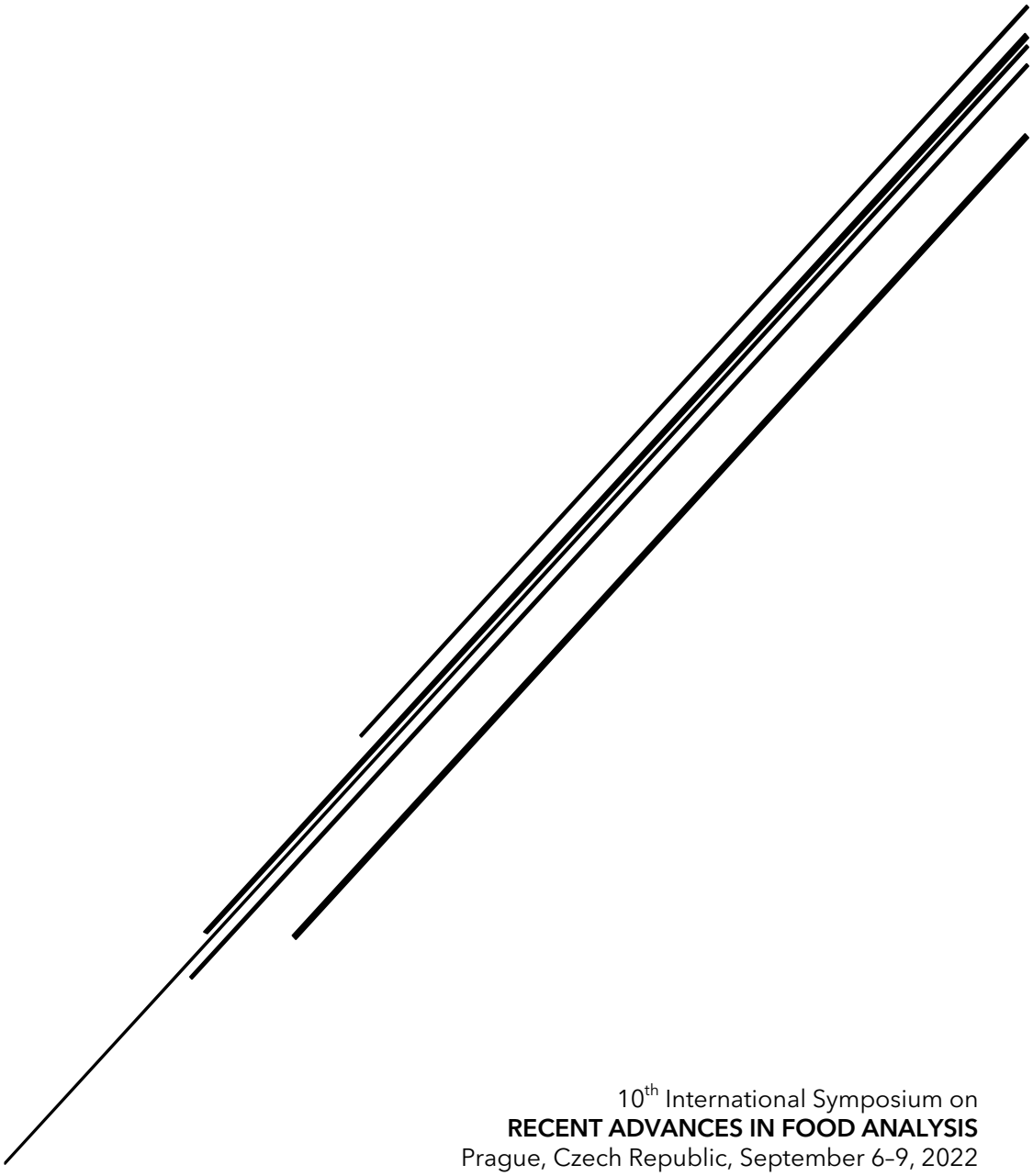

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A1

VALIDATION OF A GLUTEN 30-MINUTE ELISA SYSTEM FOR THE QUANTIFICATION OF PROLAMINS FROM WHEAT (GLIADIN), RYE (SECALIN) AND BARLEY (HORDEIN)

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Consumption of gluten might be harmful for people with gluten intolerance and wheat allergy causing a variety of symptoms. According to CODEX STAN 118/1979, food labeling is divided into, "gluten-free" and "very low gluten" products where the gluten content is lower than 20 mg/kg and 100 mg/kg, respectively. The rapid quantification of gluten in food, constitutes a challenge. An accurate, reproducible, and time-saving assay regarding sample preparation and test duration, having low LOD, LOQ and Relative Standard Deviation (%RSD), can be a valuable tool.
The aim of this study was to evaluate the recovery levels of gluten, in spiked samples (raw material and commercially available products) and reference materials, using a new and novel antibody developed by ProGnosis Biotech S.A. and a 30-minute Sandwich ELISA, implementing a one-step 40-minute extraction.
The levels of gluten were determined using the 30-minute ELISA Allergen-Shield Gluten S A1096/A1048 by Prognosis Biotech S.A. Gluten-free labeled samples were spiked with a gliadin solution. FAPAS Reference materials were also tested.
The recoveries of the spiked samples and the reference materials ranged between 70-130%. The %RSD was also within acceptable range for all samples.
The ProGnosis Biotech S.A. novel antibody and the 40-minute extraction demonstrate robust recovery and %RSD levels. The method is practical and accurate, providing reliable gluten quantification and saving time.

Keywords: *ELISA sandwich, allergens, allergen shield, gluten, antibody*

A2

COMPARISON OF DIFFERENT METHODS FOR THE DETERMINATION OF GLUTEN IN BEERS

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Cereals containing gluten, namely: wheat, rye, barley, spelt, Khorasan wheat or crosses between these species, and products thereof can lead to major hypersensitivity reactions (e.g. celiac disease, allergy). This problematic is a real challenge for healthcare systems, legislative forces but also for the related fields of food analysis. Under the Commission Implementing Regulation 828/2014, the official threshold levels for labelling the absence (< 20 ppm)/very low presence of gluten (< 100 ppm) have been established. Oats contain a distantly related prolamin called avenin. There remains some uncertainty over whether avenin from oats is harmful to people with coeliac disease. The Belgian Food Agency asked us to evaluate the methods used by the approved labs for the detection of gluten in ten Belgian beers, as National Reference Laboratory. The aim of this work was to evaluate the performances and fitness for purpose of different methods :3 ELISA kits (competitive and sandwich), a Lateral Flow Dipstick (LFD) test and a spectrometry-based method (UHPLC-MS/MS) for analysis of gluten detection in 13 beers.
The results revealed that the RIDASCREEN Gliadin Competitive R7021 ELISA (RBiopharm) was the best suited kit for the analysis of gluten in hydrolyzed samples like beer.
For this kit, data comparison between 4 different labs was possible and resulted in highly reproducible qualitative and quantitative results. The two other ELISA kits were less applicable for beer samples. The multi-allergen and grain-specific UHPLC-MS/MS method developed by CER was highly suitable to analyze the gluten concentration in beer samples (unaccredited method). Three of the four "gluten-free" beers were effectively concluded as gluten-free, based on R7021 ELISA (< LOQ) and UHPLC-MS/MS results. The fourth beer that was claimed gluten-free by the producer, delivered quantified amounts of gluten above 20 ppm with the same methods. Therefore, a notification of non-compliant results was done for this beer.

A3

SCOUT MRM TO SCREEN FOR ALLERGENS

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Introduction: Developing and maintaining large MRM assays can be challenging. Scheduling MRM transitions with retention time windows is necessary to achieve good performance as the numbers of MRM transitions increases. For methods with large numbers of MRMs or that use fast gradients, the retention time windows must be as small as possible to achieve the desired dwell and cycle times. If retention times shift, the peak may shift outside of the detection window and be missed or cut off by the acquisition window. Scout MRM acquisition alleviates this issue by removing the need to maintain retention time windows.
Methods: A research version of SCIEX OS software was modified to enable Scout MRM acquisition. In this mode, when a Scout MRM was detected, it triggered the acquisition of a later eluting dependent MRM. An existing allergen LC-MS/MS method was converted to a Scout MRM method by selecting the earliest eluting peptide as a scout for all the peptides indicative of each allergen of interest. The methods were run in SCIEX OS software on a SCIEX 7500 system. The data were processed in SCIEX OS software.
Preliminary Data: Scout MRM is a new mode of acquiring MRMs that uses specific marker transitions (scouts) to trigger elution-dependent MRMs. It eliminates the need to maintain and adjust retention time windows when running a time-scheduled MRM method. Since dependent MRMs are only acquired when the scout transition is detected above a specified threshold, confirmatory MRMs are only triggered for detected compounds, therefore enabling large screening assays
Existing allergen MRM assays typically employ 2 proteins per allergen, 2 peptides per protein and 2 MRMs per peptide. A recent method application note used 88 MRM transitions to monitor 44 peptides. The transitions were scheduled to ensure optimal cycle and dwell times. These transitions were selected carefully to maximize detection and minimize interference from background ions in a food matrix. Additional transitions would make the method more robust to different matrices but would increase the effort required to maintain the retention times. To overcome this challenge, a Scout MRM method was created to detect the same food allergens with additional MRM transitions for each allergen. This method required fewer scheduled acquisition windows, relative to the original method. The remaining transitions were triggered by the detection of their corresponding scout peptide. This Scout MRM method had similar cycle and dwell times to the original scheduled MRM method, despite the increase in transitions. This method was robust to shifts in retention time because it had fewer scheduled windows and these time windows were larger. The addition of a new allergen to an existing method was simplified, as only 1 MRM for this new allergen needed detailed characterization, such as retention time, to be used as a scout. The remaining confirmation MRM transitions for this allergen were added as dependents.

Keywords: *allergen, screening, scout MRM, quantification*

A4

STRUCTURAL AND FUNCTIONAL RELATIONSHIPS OF PLANT ALLERGENIC PROTEINS DURING GASTROINTESTINAL METABOLISM

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Food proteins frequently result in severe allergic reactions after gastrointestinal digestion. Little is known about characteristics of plant-based food allergens and their resistance against digestion on a molecular level so far.
Human gastrointestinal digestion was simulated with a standardized in-vitro digestion model (COST Infogest) for four matrices. The resulting highly complex sets of degradation products to different time points of digestion were analyzed by LC-HRMS/MS on a Q-TOF instrument in a software assisted proteomics approach.
Dealing with huge data sets (240 samples) and several thousand identified peptides we developed a multistage post-processing approach using Python, taking into account further input data such as protein assignment, secondary structure features and known epitopes of the given allergens. This approach allows detailed visualization of protein degradation and digestion product formation in the course of gastrointestinal digestion on a global scale. For the first time we were able to draw structure-function relationships between structural characteristics of food allergens, their stability to the gastrointestinal environment and immunological properties.
The development of a multistage analysis and data processing approach using Python allows to characterize plant-based food allergens during gastrointestinal digestion, providing insight into highly stable regions in context with secondary structure features and immunological functions.

Keywords: *high-resolution mass spectrometry, food allergens, in-vitro digestion model by Infogest, plant based foods, proteomics*

A5

CONTENT AND INHIBITORY POTENTIAL OF WHEAT AMYLASE-TRYPSIN INHIBITORS AS PUTATIVE TRIGGERS OF WHEAT-RELATED DISEASES

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Although wheat is one of the most important crops in the human diet, its consumption can be linked to several adverse reactions, including celiac disease, wheat allergy, and non-celiac wheat sensitivity. In addition to gluten proteins, which are considered to be the dominant triggers of these wheat-related diseases (WRDs), a group of non-gluten wheat proteins called amylase-trypsin inhibitors (ATIs) has been identified to be involved in the clinical pathogenesis of WRDs [1]. As their name implies, ATIs are able to inhibit the enzymatic activity of human and mammal amylase and trypsin leading to incomplete digestion of starch and proteins which can cause gastrointestinal symptoms such as gas production, abdominal pain and bloating [2]. To study this protein class in detail, this study comprises the quantification of ATI contents by RP-HPLC as well as the determination of *in vitro* inhibitory activities in modern wheat (*Triticum aestivum*) samples according to recently published methods [2,3]. In addition, individual samples were blended to form a sample mix that was used to validate the methods. This study revealed that ATI levels and their inhibitory potential against amylase and/or trypsin are not correlated. Consequently, both ATI concentrations and inhibitory activities need to be evaluated to help understand their role in wheat sensitivities.

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Keywords: *Triticum aestivum, wheat sensitivity, enzymatic assay, high-performance liquid chromatography*

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A6

COMPARATIVE STUDY OF MULTIPLE CELERY DNA KITS IN DIFFERENT FOOD MATRICES

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Worldwide approximately 3% of adults and 6% of children suffer from food allergies, and in the past decade the number of food allergy cases has increased by 50%. For this increasing group of allergic people safe production and correct labelling of food products are of paramount importance. However, allergen risk assessment is complex as very low levels of unintended presence in food products already can evoke a harmful physiological response in allergic consumers. Food industry is increasingly adopting self-control test methods to analyse food safety parameters in the trade or production chain, allowing them to respond quickly and avoid potential safety or quality risks. However, there are questions on the quality performance of fast self-control detection methods, especially when used with complex matrices or processed foods. In addition, when precautionary allergen labelling will be implemented based on references doses, sensitive and reliable fast allergen detection methods are required.
Here, we present the results of a comparative assessment of four commercially available test kits for the DNA detection of celery in food products. Five product groups representing different sectors of the AOAC food-matrix triangle were identified to potentially contain celery root, stem, greens or seeds. From each group two blank and two incurred (labelled to contain celery) food products were selected. Blank food products were spiked with 1, 3 and 10 ppm protein celery (derived from greens) per kg food product. Prior to qPCR analysis DNA was extracted using the by the kit manufacturers' recommended DNA extraction method. DNA and qPCR analyses were performed exactly according to the manuals in order to comparatively assess the quality performance of the kits.
Results show that three commercially available test kits can detect down to 1 ppm in four spiked product groups. The fourth test kit, specified with an LOD of 25 ppm, was not able to detect the spiked materials. For the product group defined as 'herbs and spices' presence of celery down to 1 ppm was shown with two test kits, one kit was only able to detect 10 ppm. In three of the five product groups incurred samples were shown to contain celery with three kits, samples from product groups 'Sauces' and 'Smoothies' showed inconclusive results, confirming the influence of complex matrices on the detection ability of the kits.
Quantification of celery in the different food products resulted in variable quantities between the different kits. Although trends were observed, no conclusive quantity could be assigned to any product.
In general, it can be stated that the test kits qualitatively perform according to their specifications, dependent on the complexity of the matrix. However, quantification is challenging for all kits in all food product groups.

Keywords: *allergens, celery, comparative assessment, commercial test kit*

A7

USE AND COMPARISON OF A STABLE ISOTOPE LABELLED CONCATEMER AS AN INTERNAL STANDARD FOR FOOD ALLERGEN QUANTIFICATION

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Mass spectrometry is gaining interest for the analysis of food allergens with hundreds of papers published on this topic the last few years. However, allergen quantification remains a real challenge given the high diversity of sample types to be analysed and their associated matrix effects on sample preparation and subsequent analysis.
Stable isotope dilution is commonly used in the field to compensate for matrix effects. Here, the choice of the stable isotope labelled (SIL) internal standard is crucial. SIL proteins would be ideal, but are unaffordable for routine analysis, and therefore, currently, SIL peptides are mainly used. However, food allergens are proteins and, when choosing SIL peptides, these do not undergo the whole sample preparation (extraction, enzymatic digestion) and thus, one cannot account for the inherent variability related to sample preparation.
We here propose an alternative and original strategy, based on a SIL concatemer as an internal standard. Contrary to SIL peptides, this artificial protein composed of different concatenated proteotypic peptides undergoes different steps of sample preparation. Such concatemers are known for more than a decade in proteomics but, as far as we know, have not yet been used for allergen analysis, and more broadly, for the analysis of food safety. The performance of a SIL concatemer composed of several proteotypic peptides from egg, milk, peanut and hazelnut, is compared to two other types of internal standards, SIL peptides and proteins. In a comparative analysis of three food matrices contaminated with four allergens (egg, milk, peanut, and hazelnut), our concatemer approach was found to offer the advantages of SIL proteins, that are ideal but unaffordable, and circumvent limitations of SIL peptides.

Keywords: *food allergen analysis, mass spectrometry, isotope dilution, isotope-labelled internal standard, isotope-labelled concatemer*

Acknowledgement: *The research that yielded these results, was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RT 15/10 ALLERSENS.*

A8

INTEGRATION OF SAMPLE PREPARATION AND IMMUNOASSAY BASED ON REGIOSELECTIVELY FUNCTIONALIZED ANTIBODIES: TOWARDS A PORTABLE MICROFLUIDIC PLATFORM FOR RAPID AND SENSITIVE ALLERGEN DETECTION

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Food allergy is major global issue, and children, in particular, are being affected in increasing numbers. Because there is no cure yet, the only solution for allergic patients is strict avoidance of exposure to food allergens. In response, European legislation (Regulation 1169/2011/CE) dictated the mandatory labelling of 14 potentially allergenic ingredients. Food industry therefore requires easy-to-use analytical tools to detect the presence of unwanted allergens.
Portable diagnostic systems are gaining attention in healthcare, environmental monitoring, and agro-food sectors. Bypassing traditional laboratories with rapid and on-site analysis enables time and money saving. By using centrifugal microfluidics, neither pumps nor tubings are needed to perform precise and accurate liquid handling steps creating an easy to use and portable platform. In this context, we aim to demonstrate that the integration of portable sample preparation strategies and immunological assays leads to innovative technological solutions able to lower on-site detection limits.
Soybean, one of the most common sources of dietary protein and a major food allergen, was selected as a model for the development of a prototype centrifugal microfluidic cartridge. An efficient denaturing extraction protocol was developed and optimized for complex and processed matrices. The different steps of the sample preparation and analysis are integrated in the injection moulded cartridge, including 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. The use of most denaturing reagents is not compatible with immunoassays. The dilution of the extracted sample is classically considered but leads to sensitivity loss due to inevitable associated analyte dilution. The integration of a clean-up step in the cartridge allows the elimination of extraction chemicals without undesired analyte dilution.
Anti-soybean rabbit monoclonal and polyclonal antibodies were developed in-house and were fully characterized. The combination of these antibodies with the optimized extraction protocol was validated at the benchtop level. Their integration in the centrifugal microfluidic cartridge will be the next step of the project. Particular attention will also be paid to the functionalization of the fluorescent beads with our antibodies. Regioselective modifications will be considered to prevent interferences with the analyte binding domains. This optimization will increase the immunoreactive fraction and therefore improve overall sensitivity and reduce biologicals consumption.

Keywords: *microfluidics, immunoassay, antibody functionalization, food allergen, on-site testing*

Acknowledgement: *The research that yielded these results was funded by the Public Service of Wallonia through the Cornet Program.*

A9

PREPARATION OF IMMUNOGENS FOR THE MOLECULAR MEASUREMENT OF FOOD ALLERGENS WITH ANTIBODY-BASED IMMUNOASSAYS

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Food allergy is a world-wide occurring health issue affecting 2-10% of the worldwide population. Considered a pediatric phenomenon, the majority of affected children outgrow their food allergy and develop tolerance. Nevertheless, some food allergies can be persistent and have a major impact on the life quality of allergic patients and might even cause severe allergic reactions such as anaphylaxis. The most common "treatment" of food allergy is allergen avoidance, based on food labelling. Existing labelling regulations do not indicate any threshold levels or applied detection methods, but knowing the actual concentration of allergenic material in food is crucial for allergen avoidance and prevention of allergic reactions. Among the big eight food allergy inducing foods is soybean, which is the most used vegetarian protein provider and common ingredient of industrially produced foods due to agricultural, nutritional and industrial benefits. We focus on the development of immunoassays such as enzyme-linked immunosorbent assays (ELISA) or lateral flow devices (LFD) to qualitatively and quantitatively detect soybean allergens in food, based on the availability of soybean allergen-specific antibodies recognizing and binding to the soybean allergen(s). Major soybean allergen targets are Gly m 4 (pathogenesis-related-10-protein), Gly m 5 (beta-conglycinin), Gly m 6 (glycinin) and P34 (cysteine protease). Crucial step of antibody production is the preparation of well-defined immunogens containing the allergen of interest, which are used for the immunization of Balb/c mice. Common approaches to produce immunogens based on the allergen size include cut-off filtration, size-exclusion chromatography (SEC) in combination with high performance liquid chromatography (HPLC) or recombinant allergens. We used these methods to produce well-defined immunogens from crude soy flour, verified by SDS-PAGE and mass spectrometry, to generate soy allergen-specific monoclonal antibodies, identified by indirect competitive ELISA and Western Blot screening. Those antibodies will be applied in highly sensitive and specific immunological assays for measuring soybean allergen exposure in food samples and crude material for food industry by detecting the real molecular soybean allergen amount, ideally even in a personalized manner. These immunoassays will help to improve allergen risk management of soybean allergic patients.

Keywords: *food allergy, food allergen detection, immunoassay, SEC-HPLC, monoclonal antibody*

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A10

COMPARATIVE STUDY OF MULTIPLE EGG ELISA KITS IN DIFFERENT FOOD MATRICES

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Allergen management differs considerably from other food safety issues. What makes allergen risk assessment uniquely different and complex, is that a very low level unintended presence in food products, can already evoke a harmful physiological response in allergic consumers. Using fast test methods for self-control in the food production chain has become common practice. Based on the results of these tests, producers can, when necessary, respond quickly to avoid potential safety or quality risks. However, there are issues in the quality performance of fast detection methods, especially when used with complex matrices or processed foods. Proper fast allergen detection is very important when precautionary allergen labelling will be implemented based on references doses and allergen detection methods need to be sensitive and reliable enough to reveal allergens just above the action limit.
Therefore, WFSR (Wageningen Food Safety Research) together with food industry, commercial labs and kit developers, will evaluated if commercial ELISA test kits for egg, soya, milk and celery are fit for purpose. Furthermore, the results will be benchmarked with mass spectrometry analysis. In this presentation, we want to report the comparative studies for the target allergen egg with seven commercial available test kits. Nine matrices with cognate blancs, spiked and incurred samples were measured. Moreover, samples were selected from all matrix groups and benchmarked against LC-MS. In addition, results were compared to the VITAL 3.0 reference dose for egg of 0.2 mg protein in relation to realistic serving sizes.
The results of this comparative study on EGG ELISA test kits and the benchmarking against LC-MS analysis will be presented. These results are of interest for people involved in allergen management, test development and allergen analysis.

Keywords: *commercial kits, immunoassays, LC-MS/MS, allergen detection, food matrices*

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A11

APPLICATION OF A MULTI-ALLERGEN SCREENING METHOD FOR OFFICIAL FOOD CONTROL

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5 to 8% of the population suffers from a food allergy, leading potentially to a severe reaction. Accurate labelling of allergens in prepacked foods is therefore of highest importance. Such foodstuff may contain allergens as ingredients or as contaminants, introduced accidentally during manufacturing and storage. European and Swiss legislation requires labeling the presence of the 14 most frequent allergens (peanuts, nuts, soy, sesame, milk and milk products, eggs, gluten-containing cereals, fish, shellfish, celery, mustard, lupine and sulfite), defined as the "14 majors" on the ingredient list on the packaging. We developed and used a multi-allergen screening method to identify undeclared allergens in various food matrixes.

The multi-allergen screening method uses chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the identification of peptides specific to food allergens. All major 14 food allergens with the exception of sulfite and celery are identified with this method. Briefly, soluble proteins are extracted from the food matrix, digested with trypsin, purified on SPE cartridges and analyzed by liquid chromatography coupled to tandem mass spectrometer (Orbitrap Q-Exactive from Thermo Scientific, San Jose, CA, USA). The identification of the allergen is performed on the detection of the precursor ion and three specific fragment ions of the expected peptide and a comparison of the measured mass spectrum with a peptide library built with synthetic standard peptides (Life Technologies Europe, Zug, Switzerland). The method was validated by spiking 28 different matrixes (cereals, food supplements, spreads, olive oil, spices, etc.) with known amounts of allergens. Identified allergens are quantified either using the standard addition method or with antibodies-based ELISA kits.

The method validation proved the high specificity of the method for all allergens. In certain matrixes such as spices, herbal teas and spirulina powder, some false negatives are observed. We applied this method for routine official food control on very different matrices such as pesto sauces, spreads, processed vegan foods, pasta dishes and sushi. Our analyses show that about 1% of collected prepacked foods are non-compliant regarding the legislation on allergens. Non-compliant foods include contamination of various nut species (for example almonds in cashew nut-based foods), undeclared milk in products containing dark chocolate, etc.). The multi-allergen screening approach also permits to identify unexpected undeclared allergen ingredients, such as milk proteins in sushi-style products. Such an allergen would probably never been searched in this matrix using a targeted approach with single antibody-based methods.

Keywords: *food allergens, screening method, LC-MS/MS*

A12

MASS SPECTROMETRY & EGG ALLERGEN DETECTION IN DIFFERENT FOOD MATRICES

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Food allergies are a growing global concern because even low exposure to a given food can have significant consequences to allergic people. The introduction of labelling the main food allergens when used as ingredients can be not sufficient to avoid health risk in consumers, due to unintended contamination introduced during the production of food.
Alongside antibody-based methods (ELISA) and DNA-based methods (PCR), targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) represents a useful technique to detect and quantify simultaneously multiple food allergens in complex matrices with high specificity [1]. Talking about constraints and limitations, in some situations results obtained with immunochemical food allergen detection methods may vary between kits from different suppliers, due to differences in protein extraction, reference materials and antibody selectivity [2]. However, also LC-MS approach presents some critical issues such as the choice of an optimal extraction procedure, the lack of officially approved reference standard materials and the choice of specific peptides, obtained from the enzymatic digestion of allergenic proteins, suitable for allergens detection and quantification.
During the development of Mérieux NutriSciences multi-allergen LC-MS/MS method for bakery products, resistance to thermal treatment, presence of interfering signals deriving from allergen matrices and appropriate reference materials were considered in order to optimize the selection of allergen peptide markers. The choice of LoQ (Limit of Quantification) level for each allergen was based on VITAL 3.0 references doses and referred to realistic serving sizes.
In this study, coordinated by WFSR (Wageningen Food Safety Research) inside a public-private partnership consortium [Top Sector Alliance for Knwoledge and Innovation (TKI, LWV19252, Dutch initiative for evaluation and quality assurance of fast methods in food safety testing)], the Mérieux NutriSciences LC-MS/MS method was applied to detect egg allergen in various food matrices, also different from bakery products, in order to verify the specificity, accuracy, precision and robustness of the method and consequently, the real capabilities which today make mass spectrometry a complementary technique to the more traditional and recognized ELISA and PCR approaches.

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Keywords: *allergens, mass spectrometry, LC-MS/MS, eggs, food analysis*

A13

TOWARDS A MOUSE MODEL FOR STUDYING FOOD ALLERGIES FROM A METABOLOMICS PERSPECTIVE: THE CASE OF PEDIATRIC COW'S MILK ALLERGY

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In Westernized societies, food allergies represent a growing epidemic and are considered a major cost to our health care system. Cow's milk allergy (CMA) is one of the first allergies to appear in early childhood. It can occur against various milk proteins or allergens, of which beta-lactoglobulin (BLG) and casein are the most important.
In this study, two sensitization protocols (cholera toxin (CT) or oil emulsion) and two mice strains (Balb/C and C57Bl/6; n=6 per group) were tested to determine a murine model best representative of CMA in children. For this purpose, mice were challenged orally with BLG after the sensitization phase (7 weeks) and allergy manifestations were monitored for 2 hours after the BLG challenge. Plasma and spleen cells were collected for antibody (IgE and IgG1) and cytokine (IL -10 and IFN-γ) detection and fecal samples for metabolomics analysis. Results were compared with microbiome-metabolome findings in a cohort of children with IgE-mediated CMA (n=23) and their healthy siblings (n=24).
The highest anaphylaxis scores were assigned to Balb/C mice receiving BLG and CT, whereas mild symptoms of allergic reaction were observed in C57Bl/6 mice sensitized with BLG in oil. In addition, OPLS-DA regression models based on the fecal metabolome confirmed a significant metabolic shift in the allergic mice ($R^2(Y) > 0.99$, $Q^2 > 0.7$, p-value < 0.05). The metabolites that contributed most to the model were selected based on variable importance in projection, covariance, and Spearman's correlation to immunological markers and annotated following MS^2 fragmentation.
Our results suggest that the metabolic changes in BALB/c mice sensitized with BLG and CT in PBS best mimic IgE-CMA in children of all models and sensitization protocols tested, as both exhibited the same alterations in metabolic pathways mostly associated with early dysregulation of the gut microbiome in response to BLG sensitization. Indeed, we demonstrated that microbial dysbiosis precedes allergic inflammation, as no metabolic signs of dysbiosis were measured in control mice, whereas in sensitized mice gradual significant changes were observed in multiple microbiome-derived metabolites, with most importantly bile acids, sphingosine, porphyrin and tryptophan metabolites, choline and carnitine, while the main metabolite associated with allergic inflammation, namely histamine, was most affected after the last sensitization treatment, as confirmed by clinical measurements. Our results also indicate that although sensitization is accompanied by dysbiosis, this, in turn, exacerbates sensitization, as many of these microbial metabolites and their producers have their functions in immune development in both mice and humans.
In conclusion, we have successfully applied a mouse model to study FAs and propose metabolomics as an expedient analytical strategy to follow up disease course, with the potential to elucidate novel treatments.

Keywords: *metabolomics, cow's milk allergy, food allergy, murine animal model*

A14

NOVEL LIPIDOMIC BIOMARKER PANEL TOWARDS IMPROVING OF DIAGNOSTIC ACCURACY IN PEDIATRIC COW'S MILK ALLERGY

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The prevalence of food-related allergies is increasing worldwide, especially in industrialized countries, and is considered a major public health threat. This is particularly true at a young age, where one of the first allergies to develop is cow's milk allergy (CMA), an allergic reaction to cow's milk proteins (casein or beta-lactoglobulin) that is often associated with a higher risk of other atopic manifestations later in life. However, current diagnostic methods for food allergy lack either sensitivity (atopy patch tests) or specificity (serum IgE levels, skin prick tests), resulting in patients being under- or overtreated with unnecessary dietary restrictions. In search of more accurate diagnostic/prognostic markers, a lipidomics study was performed on stool and urine samples of children with confirmed IgE-mediated CMA (n=21), non-IgE-mediated CMA (n=19), IgE-mediated food allergy to other allergens (n=11), or healthy siblings (n=21). This resulted in the detection of e.g. 65913 metabolic features for stool across the 67 to 2300 Da range. To facilitate the selection of biologically relevant metabolites, the dataset was reduced to features with an FDR-corrected p-value (t-test) ≥ 0.1 and log (FC) ≥ 1, leaving 690 biomarker candidates. In addition, classification methods including (O)PLS-DA, Random Forest (RF), and Support Vector Machine (SVM) were tested to select the best approach for building predictive models. SVM method outperformed OPLS-DA and RF with a class prediction accuracy of 89% and a multivariate ROC AUC of 0.949 (CI 0.8-1) versus 76%, 0.858 (CI 0.67-1) and 75%, 0.803 (CI 0.57-0.99), respectively. To further characterize the retained molecules, their contribution to the multivariate statistics (e.g. variable importance in the projection, importance in the model) and the univariate ROC AUC values were assessed. After filtering for adduct and isotopic peaks, the top 100 components were selected for identification based on MS^2 fragmentation spectra. Among the identified metabolites were several bile acids, including cholic acid, lithocholic acid, ursodeoxycholic acid, and hyodeoxycholic acid, as well as long-chain fatty acids, consistent with previous findings in the fecal metabolome of CMA patients and in our murine CMA model. Interestingly, the highest individual biomarker ROC AUC was 0.86 (CI 0.736-0.949), whereas a 7 biomarker combination drove the predictive accuracy to 96% with ROC AUC 0.997 (CI 0.942 - 1), emphasizing the need for multivariate assessment of the biomarker panel to account for potential interactions for optimal diagnostic performance. In summary, our study highlights the importance of multiple assessments of biomarker candidates using multi- and univariate statistical analyzes as well as wrapper-based machine learning methods to obtain the most biologically relevant molecules considering their interactions and presents a novel lipidomic biomarker panel to aid in the diagnosis of pediatric CMA.

Keywords: *lipidomics, cow's milk allergy, food allergy, biomarker*

AUTHENTICITY, TRACEABILITY, FRAUD

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B1

CROSSTOX® SPE CLEAN-UP OF PHENOLIC COMPOUNDS AS WELL AS POLYPHENOLS AS A TOOL FOR IDENTIFYING MISLABELLING IN WINE AND SPIRITS (FOOD FRAUD)

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In the general nutrition polyphenols are known for their claimed positive impact on human health. Apart from this, in alcoholic beverages some phenolic compounds are valuable parameters to evaluate the authentic vinification of wines and ageing of spirits in wooden barrels as well as to identify the additional usage of aromatic additives for flavouring.
Authentic barrique matured wines and spirits are more cost- and time-intensive in production and deserve a significant higher price and consumer appreciation. In order to create higher profits, producers could simulate the barrel maturation by adding aroma substances, e.g. vanillin to intensify the taste of wines or spirits to suggest wood-barrel ageing. In any case, the producer claims a barrel ageing of the wine or spirit, it constitutes a labelling fraud or a mislabelling, if the usage of aroma additives is not declared. Such aromatization is prohibited in the European Union for all types of wine as well as for many specified categories of spirits. In the case of permitted aromatization of spirits or alcoholic beverages, any allusion of wooden barrel ageing (e.g. the mention of *"Barrique"*) is not allowed.
Measuring the presence and composition of certain polyphenols and aldehydes in wines and spirits could be a clear indication of an authentic barrel ripening. Representative indicator molecules were selected as appropriate markers and their clean-up was tested with the newly developed CrossTOX® polyphenol cartridge. The cartridge was used in a SPE approach, where the polyphenols in the sample bind to the sorbent and elute as purified analytes. For investigation of wine and spirits, this technology was used. The composition of phenolic compounds was investigated by LC-MS/MS, for quantification and profiling purposes. A high loading capacity of the CrossTOX® polyphenol cartridge could be determined. Interfering matrix components were efficiently removed by water after the loading step. The eluate was used for LC-MS/MS analysis. The analysis of wine and liquor samples concerning the target analyte content and profile was investigated. A binding activity to other polyphenol-like substances like coumarin was observed. In addition to sensory tests, the CrossTOX® clean-up enables analysists to verify labelling of wines and spirits aged in wooden barrels and to detect the flavouring of these foods with aroma compounds (e.g. vanillin) to mimic the taste and smell of barrel aged beverages (food fraud) based on the absence or incorrect composition of the investigated analytes (e.g. the ratio of vanillin and syringaldehyde). The removal of matrix impurities and specific binding of polyphenols allows the analysis of wine and other liquors concerning their polyphenols composition and profile. Furthermore, the use of CrossTOX® cartridges for sample clean-up helps to reduce the entry of matrix compounds into the MS instrument, which reduces downtime and maintenance costs and intervals, respectively.

B2

DETECTION OF INSECT MEAL IN ANIMAL FEED BY THE USE OF NEAR-INFRARED MICROSCOPY (NIRM)

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The use of insects for feed is a new challenge for feed control, with regard to the official analytical methods for the determination of constituents of animal origin in the European Union. The yellow mealworm (*Tenebrio molitor*) and the black soldier fly (*Hermetia illucens*) are the most promising edible insects for animal feed, due to their high protein content and ease of breeding. A correct detection and identification of insect particles requires improvement in technical expertise in order to identify insect processed animal proteins (PAPs) and to differentiate insect species.
The present study investigates a new approach, as a complementary method of official ones, i.e. light microscopy and PCR, to detect insect PAPs in animal feed using Near-Infrared Microscopy (NIRM) combined with multivariate analysis. This fast and non-destructive technique allows the use of unprocessed samples. Moreover, it does not require any prior expertise and has been shown to be effective in the past for the detection and identification of PAPs. For this, different blends have been created from a pig feed adulterated at levels of 1 %, 5 % and 10 % w/w either with *H. illucens* larvae meal or with *T. molitor* larvae meal. Five replicates of each adulterated sample have been analysed and spectra have been recorded at wavelengths ranging from 1111 to 2500 nm. A discriminant calibration model has been built based on pure reference samples of insects and pig feed using PLS-DA developed on Rstudio® with the "caret" package. Based on the PLS-DA analysis, several insect particle spectra were identified in the blend. For 1% adulteration, 7 and 54 spectra were characterized as *T. molitor* and *H. illucens* particles, respectively, with 99% and 98% accuracy and a Kappa value of 0.87 and 0.47. These results allowed us to confirm the NIRM method capability to detect the presence of insect PAPs in animal feed. However, the Kappa value confirms that even with a high accuracy, the PLS-DA model shows inconsistencies in the spectra identification as shown by results obtained at 1% adulteration with *H. illucens*.
The NIRM technique seems to be a promising tool for an optimal feed control regarding the use of insect PAPs. In addition, further investigations are being carried out on other types of animal feed and on the ability of this approach to differentiate insect species, which is a crucial challenge in the light of the closed list of species authorised by the European Commission.

Keywords: *insect, NIRM, feed control, non-destructive*

B3

TO DISCRIMINATE BETWEEN VIRGIN OLIVE OILS FROM DIFFERENT SIDES OF THE SAME MOUNTAIN RANGE? SESQUITERPENE HYDROCARBON FINGERPRINTING MAKES IT POSSIBLE

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The verification of the geographical origin of virgin olive oil (VOO) is still one of the VOO hot topics for which no official method has been accepted so far. Therefore, its verification is conducted by documental review. Recently, sesquiterpene hydrocarbon (SH) fingerprint determined by HS-SPME-GC-MS has proved to be useful to verify the geographical origin of VOO both at the EU level, and at a country level[1]. Here, models based on the SH fingerprint have been developed to discriminate between VOO from various PDOs from Catalonia, using a large sample set (n=350). For this, seven Extracted Ion Chromatograms of the SH fingerprint have been aligned and concatenated conforming an unfolded matrix and that was used to develop and externally validate (3 iterations) PLS-DA models. Overall, a 93.6% of VOO samples were correctly classified into one of the 4 PDOs included in the model, leaving unassigned the 6% of the total sampling. These models even needed to discriminate between adjacent PDOs located at different sides of the same mountain range and that use Arbequina as the main cultivar, achieving a correct classification in external validation above 84%. This proves that the SH fingerprint holds information linked to specific pedoclimatic conditions.
[1] Quintanilla-Casas et al., 2022, Food Chem, 378: 132104.

Keywords: *fingerprinting, chemometrics, olive oil, geographical origin, authenticity*

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B4

RP- & HILIC-HRMS ANALYTICAL PLATFORMS INCORPORATED WITH TRAPPED ION MOBILITY MASS SPECTROMETRY FOR TARGETED & UNTARGETED 4D-METABOLOMICS: ANIMAL MUSCLE TISSUES AUTHENTICITY ASSESSMENT AS A CASE STUDY

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Metabolomics is being used to comprehensively understand a variety of food materials, such as farm animal muscles, aiming at the characterization of meat and the exploration of potential biomarkers associated with animal genetic background, sensory scores, or even feeding process treatments. So far, most of metabolomics-based approaches are lied upon the combination of separation techniques (gas or liquid chromatography and capillary electrophoresis) coupled to mass spectrometry (MS) techniques or nuclear magnetic resonance (NMR)-based approaches. These approaches are being combined with the downstream multivariate analyses, depending on the polarity and/or hydrophobicity of the targeted metabolites, providing useful information for the efficient meat quality traits, genetic background, and production system of animals.
In the present study, a novel 4D-metabolomics approach is being developed, exploiting both Reverse-Phase (RP) and Hydrophilic Interaction Liquid Chromatography (HILIC) coupled to High Resolution Mass Spectrometry (HRMS) analytical platforms to fully investigate authenticity challenges, as in case of animal muscle tissues' origin. Trapped Ion Mobility Spectrometry (TIMS) is being introduced as an additional dimension in HRMS-workflows, providing a wealth of analytical information. High-sensitivity analysis is being achieved via parallel accumulation serial fragmentation (PASEF), enabling the acquisition of high-quality spectra for reliable structure elucidation.
Through the targeted approach implemented, crucial metabolites have been successfully annotated using in-house developed databases, while potential isomers have been revealed, differentiated by their mobilities. Applying an untargeted approach, a significant number of features has been extracted, while the combination of both RP and HILIC -TIMS-HRMS analytical platforms followed by advanced chemometric tools revealed important biomarkers. This study introduces an integrated workflow that incorporates ion mobility information along with the analytical evidence generated by LC-HRMS for authenticity assessment, expanding the number of detected analytes, thus providing a clear and comprehensive metabolite coverage.

Keywords: *LC-TIMS-HRMS, metabolomics, authenticity, food of animal origin, animal muscle tissues*

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B5

EXTENDED AUTOMATION OF OLIVE OIL ANALYSIS ACCORDING TO CE REGULATION 2568/91

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The commodity characterization of olive oils, mandatory at EU level, involves the evaluation of numerous parameters aimed at determining the class to which a given oil belongs.
Many of the required analyses involve a long sample preparation, characterized by the use of large volumes of solvents and consumables, as well as massive operator intervention.
SRA instruments has developed a series of automations, validated and assessed in terms of robustness and reliability, based on the concept of green chemistry, aimed at determining Alkyl esters, Waxes, Sterols, Alcohols and Stigmastadienes. Such automations allow to:
• Drastically reduce the consumption of organic solvents. the volume of solvent required is about one tenth of that currently used
• Almost completely eliminate the use of consumables
• Hugely limit the operator's intervention
• Obtain a drastic reduction in analysis times
• Guarantee greater reliability of the analytical data.
These analytical platforms are based on HPLC/GC approach: once the sample is injected into HPLC, without any pre-treatment than dilution, the fraction containing the analytes of interest is withdrawn by the autosampler and stored in vial. Such fraction is, eventually, processed according to methods needs in a complete automatic way; once obtained the final extraxt, it is injected into a dedicated GC System.
All processes do not need any operator attendance, and analysis time is automatically optimized by controlling software.

Keywords: *evo oil analysis, sample prep automation, EU Regulation 2568/91, green chemistry, hyphenated LC/GC*

Acknowledgement: *Carmine Ventre - Centro Analisi Biochimiche, Rizziconi (RC) - Italy*

B6

HONEY CLASSIFICATION THROUGH TARGETED AND UNTARGETED METHODS BY CHEMOMETRICS

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Honey is a functional food with several nutraceutical and therapeutical properties. Besides its features, its production must be encouraged and enhanced because beekeeping is a significant sector for bee safeguarding and consequently, the environment's health. The enhancement of beehive products depends on their botanical and geographical origin, which affect their organoleptic properties, commercial value, and appeal to consumers. Therefore, honey authentication is a crucial task to achieve.
The main approach to determining honey's botanical origin is the melissopalynological analysis based on the subjective identification and quantification of pollen granules. However, this method cannot be employed for filtered honey and easily lead to misclassification, especially for unifloral honey characterized by underrepresented pollens. On the other hand, the geographical origin cannot be determined except through the analysis and comparison of some chemical markers.
For these reasons and since honey filtration is increasingly common for food safety reasons, many efforts have been made to develop reliable and fast analytical methods aimed at honey authentication and classification. Although several approaches are theoretically correct in principle, many research designs are statistically inappropriate. Therefore, this contribution aims to develop several targeted and untargeted methods, comparing different chemometric approaches for honey classification, making a critical comparison to define the pros and cons for each of them. The methods were applied to a large honey collection composed of four unifloral Sardinian honeys (i.e., asphodel, eucalyptus, strawberry tree, and thistle).
As descriptors, some unspecific physical-chemical parameters (i.e., pH, free acidity, electrical conductivity, color, total phenolic compounds, antioxidant, and free radical scavenging activity), the elemental signature (concentration and distribution of toxic and trace elements), and finally, the FT-MIR spectrum were determined and employed for the honey classification. The data acquired were submitted to Principal Components Analysis, Linear Discriminant Analysis, Random Forest, and Genetic Algorithm to develop the classification models.

Keywords: *honey, chemometrics, authenticity, ICP-MS, FT-IR*

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B7

RAPID CLASSIFICATION OF PARMESAN CHEESE WITH TARGETED AND NON-TARGETED HEADSPACE ANALYSIS COUPLED TO DIRECT MASS SPECTROMETRY

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Authentication and determination of the origin of food are of high interest for consumers and food providers, also with regard to fraud products or non-declared mixtures of ingredients. That is most important for high quality food products like edible oil or those with protected designation of origin. Automated, direct headspace coupled to SIFT-MS (Selected Ion Flow Tube Mass Spectrometry) provides rapid and economic screening of food products for flavor analysis, degradation monitoring or authentication checking. Here SIFT-MS data was processed by multivariate statistical analysis for rapid classification of genuine Italian Parmesan cheeses by product and manufacturer via either a targeted analysis of volatiles or an untargeted "fingerprint" approach.
Automated headspace analysis was carried out using a SIFT-MS (Syft Technologies GmbH) instrument coupled with a multipurpose autosampler (MPS Robotic Pro, Gerstel, Germany). Samples were first incubated in a Gerstel agitator prior to sampling of the headspace and subsequent injection into the SIFT-MS instrument through a septumless sampling head. Six different the Italian Parmesan samples were obtained from various supermarkets (Aldi, Sainsbury's, Tesco, all Cambridge, UK). For each product, ten replicates were prepared (3 g each) in 20-mL headspace vials. The analysis times were less than 40 s and 30 s for positive and negative ion scans, respectively. Multivariate statistical analysis was carried out using the Pirouette 4.5 software package (Infometrix Inc., Bothell, WA).
For the targeted approach, 17 odor-active compounds were selected. Significant differences were apparent (e.g., relative concentrations of butanoic acid and ethyl butanoate) between products, even already without the aid of statistical analysis. This approach demonstrated the rapid classification of genuine Parmesan cheese products based on the most significant odor-active volatiles. However, it was more difficult to differentiate between the various manufacturers. For the non-targeted approach, full scan mass specs were taken in both positive and negative ion mode. Class projections and interclass distances were obtained by the discriminating powers of the 10 most important variables (by m/z). Here, we could classify genuine Parmesan cheese products both individually and by manufacturer. Positively charged reagent ions perform better than negatively charged reagent ions for Parmesan, with NO+ being preferred overall because it alone classifies each manufacturer completely. However, this was based on mass spec patterns only without identifying the compounds responsible for the grouping. Automated headspace- SIFT-MS analysis determines factory origin at throughputs of 12 samples per hour, offering great potential for rapid product screening.

Keywords: *non-target, cheese, odor, flavor, high-througput*

B8

INVESTIGATING THE IMPACT OF SPECTRAL DATA PRE-PROCESSING TO ASSESS HONEY BOTANICAL ORIGIN THROUGH FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

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Honey is a valuable food commodity widely consumed around the world due to its unique organoleptic characteristics and significant nutritional value. Among the factors impacting honey market price, botanical origin plays a decisive role as certain types of honey are in a higher demand, e.g., honeydew honey [1]. To secure market sustainability and protect consumers from fraudulent acts, we developed a rapid and non-destructive Fourier transform infrared spectroscopy (FTIR) method successfully assessing honey botanical origin. To begin with, 39 honey samples originating from 9 botanical origins were collected during 3 seasons (2019, 2020, 2021). Upon measuring the samples absorbance in the mid-infrared region, a comprehensive evaluation of the spectral data pre-processing was performed, a practice commonly omitted or mispresented in the literature [2]. In detail, 16 different pre-processing methods were evaluated including both scatter-correction methods and spectral derivatives and their combinations. After developing partial least square discriminant analysis (PLS-DA) models for each pre-processing method, goodness of fit (R2Y) and goodness of prediction (Q2) were calculated. The dataset after second derivation by the GAP-segment algorithm (gap size - 3 points, segment size - 5 points) achieved the highest R2Y and Q2 values (R2Y= 0.885 and Q2=0.722) and among with 5 other pre-processing methods were further tested in a 5-classes sample set, specifically, blossom (n=15), thyme (n=7), cotton (n=5), fir (n=4) and orange (n=3) honeys. Again, the aforementioned GAP-segment algorithm obtained the best sample clustering (R2Y= 0.988 and Q2=0.880, for a 4-class model containing thyme, cotton, fir and orange honey samples) and was solely used to develop the final discriminatory models. Importantly, the blossom honey samples were excluded as the 5-classes model performance was rather unacceptable (R2Y= 0.263 and Q2= 0.063). This can be attributed to the composition of blossom honey containing nectar from multifloral sources. Six binary models were prepared for the four remaining classes and excellent sample clustering was performed with R2Y>0.98 and Q2>0.90 in every case. In conclusion, the presented study highlights the impact of data pre-processing strategies in vibrational spectroscopy and delivers a rapid and cost-efficient screening tool in honey authenticity testing.

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[2] Lee, Loong Chuen, et al. "A contemporary review on Data Preprocessing (DP) practice strategy in ATR-FTIR spectrum." Chemometrics and Intelligent Laboratory Systems 163 (2017): 64-75.

Keywords: *honey, botanical origin, authenticity, Fourier transform infrared spectroscopy, chemometrics*

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B9

VERIFYING HAZELNUT VARIETAL AND GEOGRAPHICAL ORIGIN THROUGH ITS UNSAPONIFIABLE FINGERPRINT

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Nuts are valuable components of the Mediterranean diet. Despite being fat-rich products, they present low concentrations of saturated fatty acids and high levels of unsaturated fatty acids, which have beneficial effects on health. One of the most consumed nuts are hazelnuts, which besides having a favourable fatty acid composition are also rich in proteins, sterols and tocopherols. As they are a healthy source of fat, have a rich flavour and versatility, hazelnuts have become a fundamental ingredient in the chocolate, confectionery and bakery industries. However, hazelnut sensory and qualitative characteristics are greatly influence by their cultivar and growing conditions. Additionally, market prices also differ depending on the hazelnut cultivar and country of origin. Therefore, verifying hazelnuts variety and geographical origin is relevant to protect consumers from misleading information.

The present study aims to evaluate the suitability of the unsaponifiable fraction fingerprint as hazelnut varietal and geographical authentication tool. For this study, 267 hazelnut samples from 4 countries 8 cultivars and 3 different harvest years were analysed. Hazelnut oil was extracted from the crushed samples with diethyl ether. The oil was saponified and the unsaponifiable fraction extracts were silanized. The samples were analysed with gas chromatography-mass spectrometry (GC-MS) and the chromatographic profiles of 17 specific extracted ions were aligned and used to build partial least squares discriminant analysis (PLS-DA) classification models. Two PLS-DA models were built with the exact same dataset, one to distinguish hazelnuts from different cultivars and another to classify hazelnuts according to their country of origin. Both models were evaluated by external validation. The external validation results show that PLS-DA finds different features that are characteristic of the cultivar or the geographical origin depending on the variable selected for supervising the pattern recognition analysis, proving that genetic and environmental factors exert distinct effects on the hazelnut unsaponifiable fraction, which makes it a suitable tool for the hazelnut geographical and varietal authentication.

Keywords: *hazelnut, varietal and geographical authentication, unsaponifiable, fingerprint, PLS-DA*

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B10

FOOD FRAUD DETECTION IN FRUIT JUICE BY UNIDENTIFIED MARKERS: DETECTING LOW LEVELS OF PEACH PUREE IN APRICOT PUREE BY LC-HRMS

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Adulteration is a common type of food fraud and is often hard to detect, especially if biologically similar species are used as adulterant, as is the case for our example, apricot (*Prunus armeniaca*) puree that is adulterated by peach (*Prunus persica*) puree. Fingerprinting techniques, untargeted analyses without any identification often requires the use of advanced statistics. In the case of apricot and peach juice, LC-HRMS and Principal Component Analysis alone cannot be used to detect fraudulent samples [1]. Targeted analysis is often required, but in the case of apricot and peach, peach is substantially less rich in secondary metabolites, making finding correct markers difficult.
In this study, 41 apricot purees, 46 peach purees and 82 mixtures of peach and apricot puree (containing between 2 and 50% (w/w) peach puree) were analyzed by untargeted LC-HRMS. After liquid-liquid extraction using water and tert-butylmethylether, the water phase was filtered and injected on RPLC coupled to a Q Exactive HRMS system in data-dependent MS2 mode. From the analysis of pure samples, compounds with a higher concentration in peach than apricot were selected, without any identification. Of these interesting compounds, four were selected as markers for the presence of peach.
Multiple straightforward prediction techniques were used, based on the presence of these markers above a cut off value. Various correction techniques for instrument variation were tested, including use of response factors, correction by Brix factor, re-analysis of pure apricot samples with every batch, etc. Accuracy above 95% were achieved to detect peach adulteration in apricot puree with a detection level below 5% adulteration. The procedure was tested with an independent sample set. By selecting unidentified but relevant markers, the need for advanced statistics was omitted and a successful method for the detection of peach adulteration in apricot puree was developed.

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Keywords: *food fraud, fruit juice, fraud detection, unidentified markers*

B11

WINE RECOGNITION MODEL DEVELOPMENT THROUGH THE ASSOCIATION BETWEEN 1H-NMR SPECTROSCOPY AND FUZZY ALGORITHMS

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One of the key aspects in enology and wine fraud prevention refers to the precise and accurate assessment of the geographical origin, cultivar and vintage. In light of this, the adulteration can be more or less complex and the quality of the falsification, as well as its cost, increases with the advancement of the analytical instruments applied for fraud detection. As a result, there is a constant competitive technical race between the falsifier and researchers for identifying adulterated food commodities. Besides the constant improvement of the analytical methodologies, which is directly correlated to the fast advances in the development of scientific equipment, a significant aspect is reflected by the progress made in the development of trustworthy data processing tools. In this regard, a step forward is anticipated in the application of Artificial Intelligence for developing food and beverages recognition models.
Against this background, the present work aims to highlight the potential given by the application of fuzzy logic in the development of reliable wine prediction models with respect to the geographical provenance, vintage and cultivar of the samples. For this purpose, the ^{1}H-NMR measurements corresponding to 107 authentic white wine samples, having as geographical origin two countries (i.e. Romania and France), were utilized. The sample set illustrated a high diversity in terms of the vintage (i.e. 2012, 2013, 2014, 2015, 2016 and 2017), as well as regarding the cultivar (i.e. Sauvignon Blanc, Riesling, Chardonnay and Pinot Gris). Prior to the construction of the actual classification models through discriminant analysis, two methods were applied with the aim of reducing the dimensionality of the data set, namely the classical Principal Component Analysis (PCA) and a robust method based on the fuzzy sets theory, Fuzzy Principal Component Analysis (FPCA). For each of the two techniques, the obtained scores corresponding to the first 106 principal components were further used as input data for the development of the actual discrimination models. In both cases, the performance of the discriminant analysis models has illustrated a perfect classification of the samples among the three criteria, namely according to the geographical source, cultivar and vintage. However, in some cases, the fuzzy approach conducted to a better separation of the wine samples and, at the same time, to a much more compact groping of the clusters.

Keywords: *fuzzy algorithms, Principal Component Analysis, wine differentiation, 1H-NMR, food authentication*

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B12

FINGERPRINTING TEA WITH AI AND MACHINE LEARNING

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Climate change is being addressed as one of the most urgent uprising crises of the new millennium. Issues between development and sustainability relating to vast outburst of human population growth have produced unforeseeable challenges to our future survival.
Food fraud, also known as economically motivated adulteration of food, has grown enormously under globalization as supply chains expand. Recent food fraud scandals from the past two decades have revealed the vulnerability and importance of transparency for supply chains. Tea, being the world's most popular drink, have been an indispensable part of cultures for centuries. As of 2019, tea has grown into a global market worth £15 Bn with > 10% growth rate. Because the tea supplies in developed markets rely on imports from developing countries and international trading, its quality, taste, and authenticity continue to be major concerns among stakeholders. Products from geographical indications with a higher monetary value have often become victims of adulteration, false claims, and mislabeling of the provenance. Recent changes from emerging tea plantations in African countries have also raised concerns over environmental health and worker welfares.
The principle aim of this research is to deliver a two-tier system for tea authenticity with cutting edge analytical chemistry fingerprinting and chemometrics tools. At tier one, two spectroscopic tools (NIR and FT-IR) were used to analyze those 318 black tea samples collected from 7 individual tea producing countries and regions. In tier two, a series of lab-based high performance mass spectrometry (LC-QToF, ICP-MS) based state-of-the-art instruments were used to collect their chemical and elemental fingerprints. Combined with chemometrics models and machine learning, customized solutions for different end-users and scenarios will be delivered to further promote fairness, transparency, and sustainability of the global tea supply chain.

Keywords: *tea, food fraud, machine learning, fingerprinting, geographical indications*

Acknowledgement: *We would like to thank the support for this research received from the Newton International Fellowship of Royal Society and Agilent Thought Leader Award.*

B13

DEFRA'S FOOD AUTHENTICITY RESEARCH PROGRAMME: DEVELOPING AN ANALYTICAL TOOLBOX

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The prevention of food fraud features highly on the UK Government's policy agenda. Protecting consumers, maintaining food chain resilience, and preventing fraudulent practices are significant challenges facing policy makers, regulators, and industry. Delivering high standards of traceability is vital to ensuring consumer confidence in the food chain and making the UK a globally renowned food nation.
Defra's Food Authenticity Programme develops fit for purpose analytical methods for use by official control laboratories engaged in authenticity testing. The world-leading programme has been instrumental in spearheading the development of novel scientific methods and analytical technologies such as non-targeted proteomics or novel DNA quantification methods, enabling laboratories to provide enforcement authorities with a robust set of tools and intelligence. Food fraud covers a broad spectrum of labelling misdescription issues including misleading claims about food quality, composition, and geographic origin; this presents technical challenges in terms of the analytical tools needed to verify food authenticity and support food law enforcement. The programme also supports initiatives to ensure that there is a joined-up, global response to food fraud through standardising and aligning methods, approaches, and priorities across different countries such as through Codex and CEN.
Defra continues its commitment to tackling future scientific challenges in the development of cutting-edge technology and methods that are practical, transferable, and cost-effective for enforcement authorities and industry. These methods need to overcome challenges around analytical uncertainty, quantitation, and demanding processing conditions. Better harmonisation of methods and databases and method standardisation is also needed to tackle food fraud

Keywords: *authenticity, food fraud, consumer confidence, fit-for-purpose methods, standardisation*

B14

DETERMINATION OF FURAN FATTY ACIDS IN TEA AND TEA INFUSIONS

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Furan fatty acids (FuFAs) are mostly present in low concentrations in marine oils and other fishery products [1,2,3]. However they are not formed by fish. The natural source is algae or other algae consuming organisms from the food chain. FuFAs are valuable compounds in fish oils because of their pronounced antioxidative properties, which can protect ω-3 fatty acids from oxidation [2,3]. Besides algae, FuFAs are produced by higher plants [4]. Besides their antioxidative properties, they are the precursors of the main characteristic aroma compounds in tea (*camellia sinensis spp.)*[5].
In our work we studied the FuFA profiles in teas with different grades of fermentation: green, oolong and black. For this purpose the tea matrix was subjected to alkaline hydrolysis of acylglycerols and other esters which may contain FuFAs. Another development in our work was the measurement of the FuFAs with UHPLC-MSMS equipment instead of the common GC-MS(MS) measurement of the FuFAs after methylation.
According to the literature [5], the main FuFA in diverse kinds of tea products was 11D5. Its level should be higher in fermented teas (black and oolong) than in green teas [4]. Our analyses showed higher 11D5 levels in green or unfermented teas than in fermented products. On another hand we verified the previous findings 11D5 was the prevalent FuFA in tea matrix. Therefore, 11D5 was quantified in tea infusions by means of UHPLC-MSMS equipment after mild alkaline hydrolysis. While herbal tea infusions were low or free of FuFAs, 11D5 was detectable in all samples of green and black tea infusions. Also amounts of 11D5 were higher in green tea than in black tea.
Spread over the day, regular tea consumption may contribute to the intake of valuable FuFAs.

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Keywords: *furan fatty acids, tea*

B15

HIGH THROUGHPUT AND FIELD DEPLOYABLE INSTRUMENTAL SCREENING METHODS TO GUARANTEE OLIVE OIL AUTHENTICITY

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In the context of food authenticity, it is essential to consider the detection of fraudulent cases. In 2021 the category 'Fats and oils' represents the third most notified food product in the System for Administrative Assistance & Food Fraud; this is mainly related to marketing standards and in particular to olive oil or virgin olive oil sold as extra virgin olive oil (2021 Annual Report, Alert and Cooperation Network). In this framework, there is a strong and growing demand for rapid, high-throughput, screening and easy-to-use analytical procedures to support the official analyses. Sensory analysis (Panel test) contributes to classify virgin olive oils (VOOs) in three commercial categories: extra virgin (EV), virgin (V) and lampante (L), but it consists of a time-consuming procedure. In particular, volatile organic compounds (VOCs) have a crucial role in defining VOOs organoleptic quality, since they are directly responsible for the olfactory attributes. Their rapid determination, using for example the headspace gas chromatography coupled with ion mobility spectrometry (HS-GC-IMS), can be the key to screen VOOs to pre-classify them for the following sensory evaluation. Rapid Evaporative Ionization Mass Spectrometry (REIMS) works by the analysis of lipids present in the olive oil, directly analysing samples without the need for time-consuming sample preparation or analysis. The use of a laser to instantly volatilize the sample combined with a chemometric-based data analysis, enables sample analysis to be reduced to seconds per sample. In this research work two different analytical instrumental methodologies were performed: HS-GC-IMS and REIMS technique. A set composed of 120 VOOs was collected to have a relevant and balanced variety among the commercial categories. The sensory data were obtained by the UNIBO panel. In parallel, HS-GC-IMS analyses was performed. Then, the commercial category of the VOOs was predicted by a multivariate approach (through PLS-DA classification models) on such HS-GC-IMS data and compared to that based on the sensory analysis, showing a good agreement. REIMS adopts a very similar data processing pathway, with PLS-DA classification models. Accuracy typically is lower than that achieved with HS-GC-IMS data, but analysis times are significantly reduced, perhaps enabling REIMS to be used as a rapid screening technique whilst HS-GC-IMS and sensory analysis to be then used with those samples REIMS highlights the greatest concern with. To conclude, the final goal of this investigation is to provide the most reliable instrumental, high throughput and field deployable analytical methods to guarantee olive oil authenticity.

Keywords: *authenticity, virgin olive oil, quality control, sensory analysis, screening*

Acknowledgement: *This work was developed within the project "REIMS-based analysis platform for improved traceability and consumer purchase intention of high-end food products" which is part of EIT Food projects (call Innovation, 2021) supported by EU. The information expressed in this abstract reflects the authors' views.*

B16

A PROTEIN-BASED APPROACH FOR THE INVESTIGATION OF GREEK TRADITIONAL YOGURT ADULTERATION WITH MILK POWDER VIA HIGH RESOLUTION MASS SPECTROMETRY (MALDI-TOFMS)

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The popularity of the Greek-style yogurt market has been growing vastly the last years due to its high protein content, nutritional value, and unique texture. The Greek Code of Foods and Beverages defines natural, traditional, and strained yogurt. Yogurt can be made from cow, sheep, and goat milk. The process of straining and/or the high protein content result to a natural smooth, creamy texture. However, especially for goat milk yogurt, smooth texture is a challenging task, due to the different structure of proteins. A common fraud in the dairy industry is the addition of cow milk, milk proteins or milk powder during the production of yogurt (especially goat milk yogurt) in order to achieve the proper consistency. Limited availability of goat milk combined with the lower price of cow milk, lead producers and dairy industries to economically motivated adulteration practices for profit purposes. Therefore, fraud control is vital. So far, the milk powder addition is being detected through the detection of Maillard reaction products, enzyme activity loss or sugars detection like lactulose, while for cow protein detection in dairy products, the EU reference methodology involves a gel isoelectric focusing technique (Regulation (EU) 2018/150). However, these techniques are time-consuming and present several limitations to be applied in the food industry. In the present work, a fast and sensitive Matrix-assisted Laser Desorption/Ionization Time-Off-Flight Mass Spectrometry (MALDI-TOFMS)-based methodology has been developed for the detection of yogurt adulteration with milk powder. Exploiting the intact protein profile and especially the casein content, an integrated protein-based workflow has been elaborated for the detection of milk powder addition. Several adulteration levels (1, 5, 10 and 50%) for milk powder addition were studied and characteristic m/z (species-specific markers) were detected, indicating milk's powder addition during the manufacture of cow milk traditional yogurt. Simultaneously, the yogurt samples were further classified based on the animal origin (cow, sheep, goat) and on their type traditional or strained). Statistical treatment using advanced chemometric tools, such as unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) recognition techniques were utilized for the discrimination/classification of the yogurt samples. The method developed was based on a previously reported work for feta cheese adulteration (Kritikou et al., 2022). To the best of our knowledge, this is the first study reported in the literature to rapidly detect milk powder addition in the limit of 1% in yogurt using MALDI technology, achieving substantially low detection limits and short-time analysis. The results obtained from this study clearly demonstrate that MALDI-TOF MS-based proteomics have the potential to be used as a reliable screening tool for the assessment of yogurt adulteration.

Keywords: *MALDI-TOF-MS, greek yogurt, dairy products adulteration, protein markers*

B17

VERIFYING COFFEES' ORIGIN USING UNTARGETED VOLATILE COMPOUNDS AND CHEMOMETRICS ANALYSIS

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High-quality single-origin coffee is prone to fraud and mislabelling. Verification of geographical origin is of great value to the coffee industry due to the high risk of falsification of origin. Volatile compounds in coffee are responsible for its unique aroma. The roasting of coffee produces distinctive volatile profiles individual to its production area. Furthermore, the headspace (HS) volatile collection applying solid phase microextraction (SPME)-Arrow shows excellent compound adsorption. Therefore, this research aimed to identify and classify the geographical origins of coffee based on its volatile compound profile by the novel SPME-Arrow aided by the chemometrics approach. Roasted coffee beans from four major producing Provinces of Indonesia were used in this study. The samples represented the most traded Indonesian coffee from the common species of *Coffea arabica* (Arabica) and *Coffea robusta* (Robusta). The volatile compounds from a total of 200 samples were extracted by HS SPME-arrow after equilibration and exposure at 70 °C for 10 minutes in a thermostatic system. The data acquisition on a GC/MS using an untargeted method was conducted in a mass range of 40-400 amu with a desorption temperature of 250 °C. The chromatogram data was pre-processed, deconvoluted, identified and aligned on MS-DIAL. Classification models were created using the coffee sample set dedicated for calibration and followed by validation using a separate sample set specified for verification. This study found 224 discrete volatile compounds from all coffee samples. The Random Forest (RF) classification model showed better performance compared to other discriminant analysis algorithms. RF achieved Area Under the Curve value up to 1.0 for the overall classification, with 100 correct classifications in the confusion matrix of the validation dataset. Further coffee origin classification was done by the 10 chosen important volatile compounds based on Mean Decreased (MD) Accuracy and MD Gini derived from the RF model. The result was satisfactory, as illustrated by the natural grouping of the samples in the unsupervised Principal Component Analysis score plot. Conclusively, the rapid analysis of volatile compounds in the headspace of four Indonesian coffee origins was performed with GC/MS enabling successful separation according to their geographical origins. This information and methodology will benefit the coffee industry in terms of geographical origin identification, confirmation of the coffee authentication, and detection of mislabelling or fraud.

Keywords: *coffee origin, gas chromatography, mass spectrometry, solid phase microextraction, volatile*

B18

INVESTIGATING THE OPTIMUM EXTRACTION TEMPERATURE OF THE VOLATILES IN COFFEE USING SPME ARROW IN UNTARGETED GCMS ANALYSIS FOR ORIGIN DETERMINATION

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The unique aroma of coffee can be attributed to its volatile compounds which are associated with its geographical origin. Headspace solid phase micro extraction (HS-SPME) coupled with Gas Chromatography Mass Spectrometry (GCMS) has been widely employed to analyse the volatiles of coffee. However, several factors such as extraction temperature, equilibration time, and desorption time may impact the profile of volatiles extracted from the sample. The volatile profile obtained from GCMS can be extensive. Therefore, a robust machine learning approach for multivariate analysis can be used to obtain intuitive data interpretation. This study aimed to determine the optimum extraction temperature of the volatiles in coffee using HS-SPME-GCMS for origin classification. Several machine learning approaches were used to build the classification model and to evaluate the classification performance. Roasted coffee beans were collected from four popular producing locations in Indonesia, i.e., Gayo, Bali, Lampung, and West Java. The sample was divided into training and validation datasets. SPME Arrow PDMS was employed to extract the volatiles from the coffee. Two optimisation temperatures were employed for volatile extraction, i.e., 70 °C and 50 °C. The volatile compound analysis from the coffee headspace was performed using a GCMS in a scan mode. The chromatogram was deconvoluted and aligned, followed by machine learning modelling, e.g., Partial Least Square Discriminant Analysis (PLS-DA), Random Forest (RF), Support Vector Machine (SVM), and K-nearest neighbour (KNN). The study identified 68 and 75 volatile compounds from roasted beans that employed 50 °C and 70 °C extraction, respectively. The volatile compounds data from 70 °C extraction showed higher classification accuracy compared to 50 °C. RF classification model achieved the highest accuracy (97%) compared to PLSDA (91.67%), KNN (91.67%), and SVM (75%). These results benefit the coffee industry to establish the method of volatile extraction and for further coffee origin classification.

Keywords: *coffee, GCMS, HS-SPME, machine learning, volatile*

B19

NIR SPECTROSCOPY AND MULTIVARIATE DATA ANALYSIS TO DETECT UNDECLARED MECHANICALLY SEPARATED MEAT (MSM) IN SAUSAGES

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In recent years, meat authenticity awareness has increased, related to fraudulent activities through the chain production.The European Food Safety Authority has expressed concern about mechanically separated meat (MSM) used in meat products. MSM, as defined in Reg. (EC) No 853/2004, is obtained by mechanically removing meat from flesh-bearing bones after boning or from poultry carcasses. Thus, the normal structure of the muscle fibre is mostly lost or modified in such a way that it is not comparable with regular meat, and that's why they are considered as secondary products, less valuable from a nutritional and qualitative point of view. In this context it's easy to understand why they can be used in a fraudulent way, undeclaring or minimizing their presence in products. Furthermore, there is a public health issue associated to the increasing of microbial activities caused by muscular fibre degradation and nutrients release.
This project aims at developing a fast and non-destructive approach like NIR spectroscopy coupled with chemometric models, which represents a fast, reliable and easy-to-use method that could be directly applied on site productions.
In the present study, MSM was searched in frankfurters made of meat of different species by acquiring NIR spectra with the portable laptop-controlled spectroscope MicroNIR 1700 PRO ES (VIAVI Solutions). A dataset of reference samples made by 60 frankfurters containing MSM and other 60 declared without MSM among ingredients, was collected and used as calibration set for the development of a prediction model. A discriminant model (PLSDA) was built on calibration set using the software The Unscrambler X (VIAVI).
Firstly, an exploration analysis using PCA was carried out on NIR spectra acquired on the outer side and on the inner side of samples. Since variables that mainly influenced the separation of samples were in the spectroscopic range between 1130 nm and 1350 nm, a PLSDA model to discriminate the two categories (MSM and NoMSM) of products was performed considering the reduced range. The model recognized MSM samples with a sensitivity of 100% in cross-validation and even the total accuracy was 100%. The predictive model was tested by acquiring spectra from commercial products bought in the local retailers. 64 sausages of different meat species containing MSM (32) and without MSM (32) were analysed by the PLSDA predictive model. The model sensitivity was 74,60%: poultry products (43) were mainly correctly classified, meanwhile pork products containing MSM (20 + 1 poultry product) were not correctly recognized.
The good classification results of the approach combining NIR spectroscopy and simple chemometric classification methods, especially for poultry samples, suggest great applicability directly in the marketplace by the consumers at the moment of purchase, as well as by the reselling companies when dealing with suppliers, or by authorities whenever making in site official controls.

Keywords: *NIR, spectroscopy, MSM, sausages, fraud*

Acknowledgement: *This research was funded by the Italian Ministry of Health, grant number IZSPLV 02-18-RC.*

B20

THE FEASIBILITY OF TWO HANDHELD SPECTROMETERS COMBINED WITH MULTIVARIATE ANALYSIS FOR LIME JUICE AUTHENTICITY

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Lemon is the third most important citrus species. Adulteration of lemon juice is a common problem and its common adulterants include undeclared addition of water and citric acid. Near-infrared spectroscopy (NIRS) combined with chemometrics models were used to detect lemon juice adulteration. However, rapid detection of lemon juice adulteration needs screening tools for on-site analysis at various stages of supply chain. Therefore, current study aimed to evaluate the feasibility of a handheld visible (VIS)-NIR (400-1000 nm) and a handheld NIR (900-1700 nm) spectrometer combined with multivariate analysis for the rapid detection of a combination of water and citric acid as well as quantification of water percentage in adulterated industrial lime juices. Industrial lime juice samples were collected from the market and their authenticity confirmed using a LC/MS/MS method based on the citric acid to iso-citric acid ratio. Adulterated samples were prepared through dilution of authentic lime juice samples with water at different concentrations (1, 2, 5, 10, 15, 20, 25, 30, 35, and 40%) and subsequently the refractive index (°Brix) of samples were adjusted by the addition of exogenous citric acid. NIR spectra for 24 authentic and 100 adulterated lime juice samples were recorded in triplicate using a handheld VIS-NIR (400-1000 nm) and a handheld NIR (900-1700 nm) spectrometer. Principal component analysis (PCA) and A Soft Independent Modelling of Class Analogies (SIMCA) model as a one class modeling approach were constructed for authentication of lemon juice. Quantification of water percentage in adulterated lime juice samples was achieved through construction of partial least squares regression (PLSR) model. Evaluation of the PCA scores plot revealed that PCA analysis can provide an estimation of the nature of lime juice sample when mean-centered data of VIS-NIR spectrometer and second-order derivative data of NIR spectrometer were used. In VIS-NIR spectrometer, when the SIMCA model was used, the model following smoothing and mean centering pretreatment showed 91% overall accuracy in the prediction set, while in NIR spectrometer, SIMCA model following multiplicative scatter correction pretreatment resulted in overall accuracy of 95% in the prediction set. In PLS regression, the best established model for quantification of water percentage in adulterated samples in the prediction set using VIS-NIR spectrometer (with no preprocessing) resulted in R^2p=0.85, RMSEP=8.4 and residual prediction deviation (RPD)=2.58, while the best model for NIR spectrometer (following smoothing and 1st order derivative) resulted in R^2p=0.95, RMSEP=1.3 and RPD=4.47 which showed better results in comparison with the VIS-NIR spectrometer. Our findings provided empirical evidence for the potential of both spectrometers especially handheld NIR spectrometer and the chemometrics approach as rapid, cheap, and non-destructive methods for on-site screening of lime juice authenticity.

Keywords: *lime juice, authenticity, multivariate analysis, handheld NIR spectrometer, handheld VIS-NIR spectrometer*

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B21

APPLICATION OF HANDHELD VISIBLE-SHORTWAVE NEAR INFRARED SPECTROSCOPY AND MULTIVARIATE ANALYSIS FOR EVALUATION OF SAFFRON ADULTERATION WITH SYNTHETIC DYES AND PLANT-DERIVED ADULTERANTS

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IR Iran produces more than 90% of saffron in the world. Saffron is the most valuable spice and, therefore, a candidate for the adulteration. Saffron stigmas are commonly adulterated with synthetic dyes and plant-derived adulterants. The presented work discusses the development of a non-destructive, rapid and cheap technique using a handheld visible-shortwave near infrared (VIS-SWNIR) spectroscopy (400-1000 nm) coupled with multivariate analysis for detection of adulteration as well as for identification and quantification of 4 commonly saffron adulterants including curcuma, calendula, safflower and the saffron style colored with Sudan I. Adulterated samples were prepared through mixing saffron stigma with adulterants in the concentration of 1, 2, 5, 10, 15, 20, 25, 30, 35 and 40 %. The models performance was tested using internal and external validation sets. Soft Independent Modelling of Class Analogies (SIMCA) model was constructed for authentication of saffron with 97% sensitivity and 100% specificity for each adulterant. In Data Driven–SIMCA (DD-SIMCA), both sensitivity and specificity were 100% in range of 2-40%. Partial Least Squares-Discriminant Analysis (PLS-DA) model was successfully used for correct discrimination of unadulterated and adulterated saffron samples as it showed 100% efficiency for saffron and 100, 100, 99 and 90% efficiency for saffron adulterated with calendula, safflower, Sudan I dye and curcumin, respectively, in the concentration range of 5-40%. Partial least squares regression (PLSR) models were built for the quantification of each adulterant. The performances of the model were optimized by cross-validation and examined by coefficient of determination (R^2) and root mean square error of prediction (RMSEP) in the prediction set. PLSR models predicted adulteration in saffron samples with R^2 (and RMSEP) 0.977 (6.8), 0.987 (4.8), 0.993 (4.1) and 0.961 (6.7) for curcumin, calendula, safflower and Sudan I dye, respectively. In design of experiment (DOE), 25 groups of mixtures of saffron with 4 abovementioned adulterations were made. The results showed sensitivity and specificity in both SIMCA and DD-SIMCA models were 100% and 100%, respectively. The results showed that the handheld VIS-SWNIR spectroscopy has the potential to be used by inspection officers for the non-destructive, rapid and cost-effective evaluation of commonly saffron adulterants in supply chain.

Keywords: *saffron, adulteration, handheld visible- shortwave near infrared spectroscopy, multivariate analysis, synthetic dyes, plant-derived adulterants*

B22

ANALYSIS OF SUGARS IN PHLOEM SAP, HONEYDEW AND HONEYDEW HONEYS FROM GERMAN CONIFERS BY HPLC-ELSD

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The phloem sap of plants contains high concentrations of sugars. Phloem-feeding insects (*Hemiptera*: *Sternorrhyncha*) ingest these sugars as essential nutrients, however, up to 90% of the ingested sugars are excreted via the anus. This sugar-rich material called honeydew is collected by the honeybees (*Apis mellifera*) and transformed into the dark malty taste honeydew honey. The sugar profiles of different honeydew honeys are influenced by the honeydew producers and by the ingested phloem sap. Recently, our working group developed a method for the determination of 32 sugar components by means of HPLC equipped with a VWR evaporative light scattering detector (HPLC-ELSD). The obtained sugar profiles were suitable to discriminate honeydew honeys (spruce, fir and pine) from blossom honeys and from each other [1].
Then we studied the origin and the biosynthesis of the unique sugar marker substances as well as their stability over six months under different conditions. For this purpose, phloem sap exudates, authentic honeydews from the most important honeydew producers, and honeydew honeys of the two botanical origins *Abies alba* and *Picea abies* were analyzed with the developed and validated HPLC-ELSD method [1]. In addition, a 'sugar-feeding honey' and a honeydew honey stored under different conditions were investigated. It was found that the honeydew producers and not the bees are responsible for the diversity and the high number of sugars in honeydew honeys.

[1] Recklies et al. (2021). Differentiation of Honeydew Honeys from Blossom Honeys and According to Their Botanical Origin by Electrical Conductivity and Phenolic and Sugar Spectra. *Journal of Agricultural and Food Chemistry*, 69(4), 1329-1347.

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B23

EPICATECHIN AS A QUALITY PARAMETER FOR ICED TEA BEVERAGES

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Within the group of non-alcoholic beverages, iced teas are becoming increasing popular. Iced teas are made using tea extracts from predominantly black, green and white teas, with the extract content often being less than 0.1%. Since these beverages usually also contain added flavorings and fruit juices, proof of whether tea is actually contained is often only provided by the caffeine content, which can, however, also be added. Already 25 years ago, the DIN Tea working group dealt with this problem and published in 1997 the DIN method 10811-2:1997-10: Analysis of tea and tea products - Determination of theobromine and caffeine content of fluid tea drinks - Part 2: HPLC reference method (for low theobromine contents) [1]. The caffeine/theobromine ratio should be used to justify the content of tea in the beverages. However, the method is only rarely used, since the chromatograms can hardly be evaluated when flavorings and fruit juices are added.
By using a fluorescence detector in addition to a PDA, the typical tea ingredients, especially the very stable epicatechin, can be detected and determined very sensitively and selectively. In the examined iced teas with declared green tea extract, higher epicatechin contents were determined than in those with declared addition of white and black tea. This is consistent with the substance's occurrence in black, green, and white teas. In addition to a number of different black, green and white teas, 26 commercially available iced teas were tested for epicatechin, catechin, caffeine and theobromine.
Particularly noteworthy is that in almost half of the analyzed iced teas no epicatechin or only traces of it were detected, so that it is questionable whether tea was present in the iced teas at all. This could not have been derived solely from the caffeine contents. The determination of the epicatechin with HPLC-FLD allows for the first time a clear assessment of commercial iced tea beverages.

[1] DIN 10811-2:1997-10

B24

APPLICATION OF MID INFRARED SPECTROSCOPY FOR FOOD AUTHENTICATION

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This work has been aimed at the characterization and authentication of the selected food, which were samples of cocoa powders, carob, chocolates, ketchups, barbecue and chili sauces. During experimental work spectra in mid infrared region (4000 – 400 cm^{-1}) were measured using attenuated total reflectance techniques (ATR – FTIR). In profile spectra the typical absorption bands were identified and afterwards the multivariate data analysis using statistical models applying discriminant analysis (PCA and PLS) was applied. In spectra can be observed characteristic vibration of C-H, C=O and O-H bonds typically representing fatty acids, esters, sugars and water.
For the cocoa powders, differentiation of the pure samples and mixtures with carob is demonstrated. Also, various dark and milk chocolates can be quickly distinguished. In the case of sauces, the basic differentiation according to sugar and vegetable content and kind of used sweeteners is illustrated.
Realized experiments proved the potential of the ATR-FTIR method for the fast recognition of various kinds and origin of food and also their different composition or technological processing can be distinguished.

Keywords: *mid infrared spectroscopy, food, authentication*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

B25

DETECTION OF BOTANICAL ADULTERANTS IN POWDERED SAFFRON

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Saffron is a spice obtained by drying the stigmas of the saffron flowers (*Crocus sativus* L.). Due to its high price, economically motivated adulteration occurs relatively often. For example, powdered saffron is adulterated by mixing with other plant materials or extracts thereof. Based on the spectrophotometric methods given in the international standards ISO 3632-1:2011 and ISO 3632-2:2010 dealing with the quality and classification of saffron, it is possible to detect 10-20% weight additions of safflower, marigold, and turmeric to saffron. The presented study aimed to develop an effective strategy for the detection of more potential botanical adulterants in saffron, namely safflower (*Carthamus tinctorius* L.), marigold (*Calendula officinalis* L.), turmeric (*Curcuma longa* L.), achiote (*Bixa orellana* L.), red pepper (*Capsicum* spp.), mountain arnica (*Arnica montana* L.), beet (*Beta vulgaris* L.) and pomegranate (*Punica granatum* L.). Non-target screening strategy based on ultra-high reverse-phase liquid chromatography coupled to tandem high-resolution mass spectrometry (UHPLC-HRMS/MS) was employed for the analysis of plant extracts (obtained by aqueous ethanol). By using multivariate statistical analysis of the generated ´chemical fingerprints´, specifically using the method of principal components (PCA) and partial least squares-discriminant analysis (PLS-DA), potential unique markers (metabolites) could be identified. To enable routine saffron authenticity control by target screening, the internal database was developed, currently it involves 82 unique markers (both new ones were possible reliably discovered in this study and those reported in the literature). In this way, detection of 1 – 3% (w/w) addition of all analyzed botanical adulterants was possible.

Keywords: *saffron, Crocus sativus L., authenticity, UHPLC-HRMS/MS*

Acknowledgement: *This work was supported by Metrofood-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, and from the grants of specific university research – grants No: A1_FPBT_2021_001, A2_FPBT_2021_052 and A1_FPBT_2022_005.*

B26

SPECTRAL FINGERPRINTING DATABASE – AN EXAMPLE FOR THE MANAGEMENT OF NON-TARGETED SPECTROSCOPIC DATA FROM FOOD AND FEED AUTHENTICATION STUDIES

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Non-targeted analytical approaches are frequently applied in food and feed authentication studies and the potential of these approaches has been widely demonstrated in scientific literature. However, the implementation into routine analysis is still limited, for instance in official control laboratories, and would be progressed with appropriate data management tools and infrastructure available. Non-targeted analysis of food and feed material result in a spectral fingerprint for each sample, which is subsequently processed in data analytical workflows either to establish classification models or to predict the authentication of the sample with such a model. Therefore, the spectral raw data is an essential element of the analytical process. It needs to be stored together with the meta-information in an appropriate database to enable automated and repeated processing of the data. Available tools such as certain internal laboratory information management systems or the Metabolights database revealed some limitations in dealing with non-targeted fingerprinting data in routine authentication scenarios. Therefore, a demand-oriented spectral fingerprinting database has been designed as laboratory internal management tool to deal with spectra data from different matrices and different platforms derived in the context of food and feed authentication. It is a flexible and user-friendly solution helpful to find, access and reuse valuable fingerprinting data beyond research projects and in routine workflows. Here, we will present the concept and methodology of this spectral fingerprinting database.

Keywords: *non-targeted analysis, spectroscopic database, data management, fingerprinting database*

B27

NON-TARGETED SPECTROSCOPIC ANALYSIS OF MEDITERRANEAN HONEY FOR ADULTERATION DETECTION - LOW-TECH VS. HIGH-TECH ANALYTICAL METHODS

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Honey is a natural food product that is very popular with consumers and achieves high sales prices. From the perspective of the consumer, the quality and safety of the product is related to its authenticity. A growing number of honey adulterations has been identified in recent years, raising concerns about the authenticity of honey with regard to its botanical or geographic origin as well as adulteration by the addition of sugar. Testing for honey authenticity is important for both regulatory and commercial reasons to reduce common honey fraud, such as false statements or adulteration with additional sugars. Many methods of authentication are well established and have proven to be successful for years, but are time-consuming and not sufficient for the wide range of fraud possibilities. Stable isotope mass spectrometry (IRMS) is suitable for detecting adulteration with sugars from C4 plants, such as sugar cane. The application of IRMS for adulteration with sugars from C3 plants is sophisticated, and new techniques of detection are required.
Non-targeted analytical methods are promising methods for authenticity testing of honey. They can be used to determine the geographical and botanical origin or adulteration of sugars from other sources. Non-targeted methods have several advantages over established methods: short acquisition time, non-destructive measurement, and simple sample preparation. The major benefit, though, is that adulterations or false declarations that were previously undetected and unsearched for might be found. The investigated honey samples were obtained from project partners in the Mediterranean region as part of the EU project for an Interlinked Digital Platform for Food Integrity and Traceability of relevant Mediterranean Supply Chains (Medifit). The honeys were measured and evaluated by ^{1}H nuclear magnetic resonance spectroscopy (NMR), ATR infrared spectroscopy (FTIR-ATR), dispersive Raman spectroscopy and near infrared spectroscopy handheld SCiO (NIR). The various spectroscopic techniques were taken into account separately but also merged to create models that are more significant. Multivariate statistics and machine learning methods were used to assess the recorded spectra in connection to the botanical and geographic origins as well as the addition of foreign sugars. The preliminary findings indicate that while low-tech approaches can also give suggestions in advance, high-tech methods are most suited for the detection of adulteration.

Keywords: *honey, adulteration, handheld NIR, non-targeted analysis, proton nuclear magnetic resonance*

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B28

NOVEL PIPERIDINE GLYCOALKALOIDS AS MARKERS FOR THE CLASSIFICATION OF THE CONIFEROUS HONEYDEW HONEYS FIR AND SPRUCE

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The commercial interest in honeydew honeys has been increasing due to their higher antibacterial and antioxidant properties and to their spicier and maltier flavor in comparison to that of blossom honeys. However, an accurate definition of the different honeydew honeys, especially fir and spruce, using objective methods is necessary to discover food fraud on account of their different prices in the market. The traditional microscopic pollen analysis failed for honeydew honeys due to the missing pollen. In a preliminary study we described the first objective method for the characterization and differentiation of honeydew honeys by their botanical origin (fir, spruce, pine) analyzing 32 sugar substances with a newly developed SPE-HPLC-ELSD method [1]. A 96.8% correct classification by linear discrimination analysis (LDA) for silver fir, spruce, Greek fir, pine, and non-honeydew honeys was achieved. In addition to the sugar fraction and the phenolic fraction, a further fraction containing charged nitrogen substances was analyzed by HPLC-PDA-MS/MS. Characteristic honeydew marker substances became obvious by comparing the LC-MS/MS profiles and were identified as piperidine glycoalkaloids (PGAs) by LC-QTOF-HR-MS and NMR in honey for the first time. The main derivatives are highly specific to the botanical origin of *Picea abies* (spruce), as evidenced by the detection of the aglycon in phloem exudates and glyco-compounds in the honeydew of various spruce aphid species. The marker potential for the glycoalkaloids was proved for *Picea abies* honeydew honey by comparing the relative amounts with 12 other botanical origins of honeydew and non-honeydew honeys. The best classification of the analyzed honeys was obtained by evaluating a combination of sugar and PGA markers in which the LDA resulted in a 100% correct classification rate.

[1] Recklies et al. (2021). Differentiation of Honeydew Honeys from Blossom Honeys by Electrical Conductivity and Sugar Spectra. *Journal of Agricultural and Food Chemistry*, 69(4), 1329-1347.

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B29

VOLATILES FOR THE DETECTION OF IMMATURE HARVESTED ACACIA HONEYS

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Acacia honey is produced by the honeybees by collecting nectar from the flowers, transforming and combining it with specific substances of their own, storing it, and leaving it in the honeycomb to ripen and mature. The major transformation during the maturity process is the moisture evaporation to about less than 18%, which was achieved by the hive bees by fluttering their wings continuously to circulate the air. Honey with less than 18% moisture and sealed in the honeycomb cells is considered as natural mature honey. Driven by profit, more and more beekeepers, especially in China, harvest immature honey every 2 or 3 days in order to increase their honey production. Then the watery, immature honey is artificially dehydrated in order to fulfill the relevant honey standards. Due to the lack of unambiguous methods for distinguishing between mature and immature honey we developed a GC/MS method for analyzing volatiles. It can be assumed that the removal of water also removes high volatile aroma compounds. This became visible in the aroma profiles achieved by thermo-desorption combined with GC/MS. The honey was diluted with a sodium chloride solution and then placed in a Markes® micro-chamber. After the addition of the internal standard, the flavoring substances were enriched on a tube filled with Tenax using a nitrogen stream under defined conditions. Desorption of the flavoring substances was then carried out in the Markes® thermo desorber, which was directly coupled to a GC/MS. The recorded profiles of several acacia honeys from Europe and China were compared. The Chinese honeys showed significantly lower intensities in their aroma profiles than the European ones. In addition, the furfural content of some Chinese honeys was significantly higher.

B30

ORIGIN AUTHENTICATION OF SLOVENIAN PORK MEAT

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The growing trend to identify the origin of food has positively impacted food safety, quality and consumer protection and has sparked interest in building local and regional food systems across Europe, including Slovenia. This study outlines the process of verifying if pork meat on the Slovenian market corresponds to its declaration using isotopic and elemental analysis, which is one of the most powerful and robust approaches for determining geographical origin. However, to evaluate the authenticity of commercial food samples, the isotopic and elemental data must be compared with reference data from a databank of authentic samples and evaluated in terms of their match within statistical limits. For the databank, 70 Slovenian pork meat samples from different breeds and rearing systems were collected from farms in four regions. The first authentic databank includes information on 29 elements (B, Na, Mg, Al, P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg, Tl and Pb) in meat samples, the isotopic composition of light elements (δ^{2}H, δ^{18}O, δ^{13}C, δ^{15}N and δ^{34}S) in defatted meat samples and the isotopic composition of oxygen (δ^{18}O) in meat water. Different statistical procedures (PCA, LDA, OPLS-DA) were used to distinguish between breeds and geographical origin, while data-driven soft independent modelling of class analogy (DD-SIMCA) was used to verify the correct labelling of Slovenian pork meat. The first evaluation indicates a good separation of pork meat according to the breed, region and diet. The overall prediction ability between breeds was 98.4%, with a 100% prediction ability in the case of the Slovenian breed and a 96.4% prediction ability for Krško-polje pork samples. The most powerful parameters for differentiating Slovenian and Krško-polje pork meat samples concerning the breeds were K, Mg, P, S and Na for the Slovenian breed. However, further research is needed to specify from which geographical area the pork meat originated, given the variability caused by differences in the pigs' diet. Further, the correct labelling has been verified for 18 commercial samples labelled as Slovenian obtained from different Slovenian grocery stores. The anticipated outcomes of this research will be helpful for government agencies in verifying the origin of pork meat, consumers who wish to be protected from food frauds, and farmers who would like to protect their Slovenian pork meat.

Keywords: *authenticity, IRMS, elemental composition, statistical analysis, pork meat*

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B31

BOTANICAL CLASSIFICATION OF HONEYS USING A NON-TARGTED LC-QTOF-MS METHOD

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It is reported that honey is one of the most common food ingredient that is affected by food fraud worldwide. With the increase of demand for natural sweeteners, new types of honey fraud are anticipated. The botanical origin can impact the market price of honey, and in turn, attracts lots of attention for conducting food fraud. Currently available authenticity methods for botanical classification of honey either are time-consuming (e.g. pollen analysis) or only target a few "known" types of markers (e.g. phenolic compounds). Novel tools are therefore needed to guarantee the quality, safety and authenticity of honey. Non-targeted analysis, using high-resolution mass spectrometry (HRMS) and advanced data processing tools has the potential to investigate a broad range of quality attributes simultaneously (e.g. contaminants, authenticity or freshness markers); and the resulting chemical fingerprints are virtually impossible to imitate for fraudsters due to their complexity. In this study, a non-targeted method based on liquid chromatography (LC) coupled to HRMS was optimized to explore the non-saccharide fingerprints of honeys of different floral origins in samples (350 honey samples) collected from markets in Canada. Honey samples were analyzed using a "dilute and injection" method followed by a LC-quadrupole time-of-flight (QTOF)-MS analysis. Different mathematical models were evaluated for the classification rate for floral origin. Relatively high prediction rates were obtained using a model built with the *Random Forest* algorithm. Eventually, several novel floral origin markers were identified among different types of honeys. For example, a feature *m/z* 412.2192 $[M+H]^+$ was identified as a botanical marker for blueberry honeys and its putative structure was obtained through MS/MS. The present results demonstrate that LC-HRMS-based workflows can be used to identify novel molecular markers for honey botanical classification. This workflow relies on a rapid (<20 min per sample incl. sample preparation) and simple (no extraction step) analytical method, which can be easily translated to study the authenticity of other liquid/semi-liquid food (e.g. alcoholic beverages or maple syrup).

Keywords: *honey authenticity, bio-marker, LC-HRMS, botanical classification*

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IMPACT OF STORAGE TEMPERATURE AND TIME ON THE CHEMICAL FINGERPRINTS OF HONEYS WITH DIFFERENT FLORAL ORIGINS

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Simple and effective methods are needed to monitor the quality and authenticity of commodities such as honey during their entire shelf life. In the present study, the impacts of storage temperature and time on the chemical fingerprints of honeys were investigated. Honey samples of different floral origins (including blueberry, buckwheat, clover and golden rod) were collected from markets in Canada. Individual sub-samples were created by weighing 10 g of each sample into a polypropylene centrifuge tube. Sub-samples were stored at -80 °C; -20 °C; 4 °C; 25 °C and 65 °C for 1 month, 3 months and 6 months. After storage, honeys were analyzed using liquid chromatography-quadrupole time-of-flight-mass spectrometry (LC-QTOF-MS). A "dilute and shoot" method which has been optimized in our previous study was adapted to the present study. Except heating at 65 °C, all the other storage conditions showed mild or no impact on the chemical fingerprints of honeys with different floral origins. The principal component analysis (PCA) results showed that samples stored at 65 °C were fully separated from the samples under other conditions. Samples stored at room temperature (25 °C) for 1 month did not show obvious difference from the original samples (before the experiment), while after 3 months, the difference was distinct in PCA. Results also indicated that some fingerprints (e.g. feature 107.0493 *m/z* in buckwheat honey) were impacted by the storage temperature and time (feature intensity decreased or increased), while some others were not (e.g. feature 146.1174 *m/z* in blueberry honeys). These results highlight the influence of storage temperature and time on the chemical fingerprints in honeys, which may in turn impact the botanical classification of honeys based on the non-targeted fingerprints. Thus, -80 °C and -20 °C are recommended for honey storage for honey authenticity studies, as under such conditions, honey samples show no significant difference ($p < 0.05$) from the original ones, even after 6 months.

Keywords: *honey authenticity, botanical classification, LC-QTOF-MS, storage condition*

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DISCRIMINATION OF FRESH FROM FROZEN-THAWED MEAT USING MULTISPECTRAL IMAGING

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Control of fraudulent practices is important since the increasing scale and complexity of food supply chains due to globalization leads to networks, which are more vulnerable to food fraud. An example of food fraud is the proper labelling of meat products, which is important to ensure fair-trading. Regular inspections could be a solution to control these food crime practices for the consumers' protection. A number of methods have been used for the assessment of meat fraud, such as molecular, enzymatic, spectroscopic, chromatographic, and sensory methods. The methods used for the detection of food fraud on-site, should be rapid, low-cost, and reliable, enabling regular inspections for screening the majority of samples. In that framework the discrimination of fresh from frozen-thawed chicken thigh fillets was studied using multispectral imaging (MSI) coupled with Support Vector Machines (SVM).
Chicken thigh fillets were stored in two different packaging conditions (air and vacuum) at three temperatures: 0, 5 and 10° C. Multispectral images were acquired at predetermined regular time intervals. VideometerLab system used for the acquisition of images in 18 non-uniformly distributed wavelengths ranging from 405 to 970nm. The experiment was repeated four times (i.e., experimental replicates) and each experiment was completed when the samples had a microbial population about 8 log CFU/g. The samples were frozen in -20 ° C and thawed 24 days later; the images were acquired once again. The collected images (n=534) were segmented and only the region of interest (i.e., chicken thigh tissue) was used to extract the spectral data. The mean reflectance spectrum (i.e., mean intensity of pixels within the informative area) along with the corresponding standard deviation values were calculated after the segmentation and used for further analysis. SVM was applied on MSI data and classification models were developed. The training and testing of the models was performed using the three experimental replicates, while the fourth (external validation) was used to evaluate the SVM model. The analysis was conducted four times so as each experimental replicate was used once as external validation. The accuracy for test sets ranged from 88.89% to 97.50%, while the accuracy for external validation for experimental replicate 1 (n=130), 2 (n=128), 3 (n=144) and 4 (n=132) was 88.64, 95.14, 92.19 and 80.77%, respectively.
MSI spectral data combined with SVM could discriminate fresh from frozen-thawed samples with an accuracy over 80% for all experimental replicates and regardless packaging.

Keywords: *food fraud, multispectral imaging, support vector machines, chicken thigh*

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MULTISPECTRAL IMAGING (MSI) COUPLED WITH MACHINE LEARNING FOR THE EVALUATION OF AUTHENTICITY IN SEVERAL SEAFOOD

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The expansion of Aquaculture sector enhances the need for implementing rapid and real-time technologies for their authenticity evaluation given that he detection of food fraud are among the most significant aspects in food quality and safety assessment
In brief, shellfish, finfish and seaweed samples of various microbiological quality, species, origin and form were analysed using the VideometerLab2 instrument (Videometer A/S, Videometer, Herlev, Denmark). Different machine learning models (e.g., Partial Least Square - Discriminant analysis (PLS-DA), Support Vector Machines and/or Extra Trees classification models) were generated and validated for the discrimination of the products based on geographical origin, species and/or form. Accuracy, precision and recall (%) were used as metrics for the evaluation of models' performance. Regarding finfish, accuracy, precision and recall scores higher than 90% were recorded for the discrimination of seabass and seabream fillets (with and without the skin). Additionally, mussel samples were grouped correctly based on their origin (Greek or Spanish), while the discrimination based on the different form (fresh/thawed) was also successful as 98% of the samples were grouped into the correct category. Finally, PLS-DA algorithm was efficient in discriminating brown seaweed samples based on their origin (Scottish or Irish) and the harvest year. The combination of machine learning with MSI analysis could be effectively used to discriminate several seafood products and ensure their authenticity

Keywords: *multispectral imaging, authenticity, seafood*

Acknowledgement: *This work has been funded by the project TraceMyFish (ERA-NET Cofund BlueBio) (Grant Agreement number 817992).*

B35

TRACING THE GEOGRAPHICAL ORIGIN OF FRUITS AND VEGETABLES; THE SLOVENIAN MODEL

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The complex nature of globalized food supply chain and the economic motivation to provide cheaper food products increase the possibility of fraud. Food fraud prevention is paramount to protect our consumers' trust and maintain fair, sustainable business practices. The need to protect high-quality and locally produced fruit and vegetables is becoming more pronounced. Since most agri-food products only hold a reputation and marketability at the local level, they need to be protected locally, especially from the uncontrolled import of qualitatively inferior products labelled as 'local' or emanating from a specific geographic region. The development of analytical techniques, establishing databases, and chemometric approaches allow us to assess the authenticity of products from the market.
This study demonstrates how stable isotopes of light elements and elemental profiles combined with a one-class chemometric model DD-SIMCA (Data-Driven Soft Independent Modelling of Class analogy) can be used to verify the declared origin of selected vegetables (garlic, asparagus) and fruits (strawberry, cherry, apple, kaki) on the market. Six databases of 447 authentic Slovenian samples were used to develop the verification models, while 160 imported samples were used to assess their reliability. The databases themselves can be considered high-quality since the samples were collected representative from the fields by impartial collectors and cover their natural isotope and elemental variability. Overall, all databases were applicable and have proven useful for the Slovenian enforcement agency to determine the geographical traceability of selected food crops. For verifying compliance with a given specification (geographical indications), a DD-SIMCA was used to build an unbiased verification model. Within the fruit species studied, excellent sensitivity and good specificity were only obtained for each production year separately, while for vegetable species, robust models were also created when all harvest years were combined (garlic: 98 % and 92%, asparagus: 96% and 97%). The variables important for classification within the selected crops were Sr, Ba Cs, S, Mo, Ni, Fe and $\delta^{18}O$ and $\delta^{13}C$. The DD-SIMCA models were then used to check the validity of 124 test samples from the market with declared Slovenian origin. Twenty-five per cent of garlic, 37 % of asparagus, 39 % of strawberry, 30 % of cherry, 50 % of apple and 36 % of kaki samples were potentially mislabelled.
Overall, the presented methodology represents an excellent foundation to verify the authenticity in a real-world application. Such approaches can also ensure confidence and trust in the food supply chain's integrity and can be adapted to newly identified priority food products and transferable to other countries and food commodities of interest.

Keywords: *fruits and vegetables, geographical origin, stable isotopes, elemental composition, DD-SIMCA*

Acknowledgement: *Research is financially supported by the Ministry of Agriculture, Forestry and Food, Administration for Food Safety, Veterinary Sector and Plant Protection under GA no. C2337-18-000044, C2337-19-000033 and C2337-20-000048. The financial support from Slovenian Research Agency by P1-0143 and IAEA project "Authenticity of High-Quality Slovenian Food Products Using Advanced Analytical Techniques" (Contract No. 23362) is also acknowledged.*

B36

ELEMENTAL FINGERPRINT OF COOKING SALTS MEASURED BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS AND PROCESSED BY PRINCIPAL COMPONENT ANALYSIS METHOD EXPANDED TO PROPAGATE THE UNCERTAINTY AND HIGHLIGHT THE GEOGRAPHICAL DISCRIMINATION

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The elemental composition of different cooking salts from different geographical origins were investigated by instrumental neutron activation analysis (INAA). INAA has been applied considering it is a reliable multi-element technique, suitable for the accurate determination of trace and major components in food samples, and the parameters for the evaluation of the measurement uncertainty are well known. More than 30 elements were analysed using the mentioned method. Concentration data and associated uncertainties were processed by principal component analysis (PCA) to show the diverse composition based on the origin of the samples. Monte Carlo simulation was applied as boots trapping augmentation method with the aim to propagate the uncertainty budget associated with the raw data in the PCA model.

Keywords: *instrumental neutron activation analysis, principal component analysis (PCA), Monte Carlo method, measurement uncertainty, cooking salts*

B37

UHPLC/QTOF UNTARGETED METABOLOMICS COUPLED TO MULTIVARIATE MODELLING AND ARTIFICIAL NEURAL NETWORKS FOR FOOD INTEGRITY: CASE STUDIES ON HAZELNUT, SAFFRON AND EVOO

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Untargeted metabolomics based on UHPLC liquid chromatography coupled to QTOF high-resolution mass spectrometry was used for phenolics and sterols profiling, as well as to comprehensively profile secondary metabolites. Untargeted signatures were aligned, normalized, baselined, and filtered by frequency in Agilent Profinder B.07, and then multivariate statistics were carried out in Agilent Mass Profiler Professional 14.
The combined effect of geographical origin, cultivar and blending was assessed in hazelnut, saffron and extra-virgin olive oil (EVOO). Unsupervised statistics (hierarchical clustering and PCA) and supervised statistics (OPLS-DA) allowed identifying markers specifically discriminating cultivar or geographical origin. Among others, secondary metabolites such as phenolic compounds (mainly flavonoids) and phytosterols showed the strongest discrimination potential.
Concerning saffron, the proposed approach discriminated against geographical origin (three distinct Italian PDO productions, separately from commercial products and foreign products). Moreover, the untargeted metabolomics allowed identifying the most challenging adulteration of saffron, namely the addition of other flower parts (stamens and tepals) to styles. Adulteration down to 5% of addition could be discriminated by metabolomics.
Regarding EVOO, the Taggiasca cultivar (original of the Liguria region, Italy) was used as a model because of its cost and high quality. Distinct markers could be identified for cultivar, origin, and blending (down to 5% of other cultivars). The authentic Taggiasca (cultivar within the reference area of growth) was discriminated by other samples (different cultivars and/or different origins) through Artificial Neural Network (ANN) analysis followed by transvariational analysis for markers identification. The ANN model showed good accuracy and sensitivity, allowing a proper identification of authentic Taggiasca even across different seasons.

B38

DISCRIMINATIVE POWER OF SHOTGUN METAGENOMIC AND VOLATILOME ANALYSIS FOR GEOGRAPHICAL ORIGIN AUTHENTICATION OF TYPICAL ITALIAN MOUNTAIN CHEESES

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Dairy products are among the most common products concerned by food frauds. In particular, protected land- and tradition-related labeled cheeses (e.g. PDO or produced in mountainous regions) are subjected to food fraud (mainly mislabelling and fraudulent documentation) due to their high economic value. Indeed, consumers are more willing to pay higher prices for better quality and typical mountain cheeses due to their distinctive flavors and appearance but also their more natural and animal-friendly production attributes. For these reasons, geographical origin authentication methods are used to ensure the product origin. The main methods rely on chemical analyses such as stable isotope ratios, trace elements and fatty acid profiles. Microbial ecology studies have also highlighted how the combinations of different environmental factors, and cheese-making conditions and traditional know-how select specific microorganisms. Thus, DNA-based methods applied to microbiota definition have also been suggested as potential tools to authenticate cheese geographical origins. The aim of this study was to evaluate the discriminative power of DNA shotgun metagenomics (both bacterial and viral community profiling) but also volatilome for cheese product origin authentication. To do so, a case study approach was applied to typical semi-hard raw milk Italian mountain cheeses (caciotta and caciotta-like). These methods were used to evaluate if they were able to correctly determine the origin of 42 cheeses from 5 closely located producers (51±26 km range). Sampling was performed over two years during both the hot and cold seasons in 2020 and 2021. Bacterial community profiles were significantly impacted by geographical origin based on permutational analysis of variance and non-metric multiscale dimensions. Although dominant starter lactic acid bacteria were similar among cheeses from different origins, strain-level phylogenetic analyses of the dominant and non-starter species differentiated most cheese origins. In comparison with bacterial communities, a strong origin effect was observed for viral communities (R^2=0.548 *Vs* R^2=0.452). However, preliminary data modeling through sparse partial least squares-discriminant analysis sPLS-DA showed higher accuracy in origin prediction of bacterial communities when compared to viral ones. Volatilome analyses on cheese samples using headspace-thermal desorption-gas chromatography coupled to mass-spectrometry showed a high abundance of volatile compounds mainly characterized by secondary alcohols, ketones, free fatty acids, esters and terpenes. Analyses are in progress to assess the accuracy of prediction models based on the volatilome but also on the combination of the obtained datasets.

Keywords: *geographical origin authentication, shotgun metagenomic, volatilome, geographical indications, cheese*

B39

INTERLABORATORY VALIDATION OF A DNA METABARCODING ASSAY FOR MEAT SPECIES AUTHENTICATION

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Food adulteration has become a global problem. The term "food adulteration" refers to any kind of deliberately false or misleading statement on the food label, including information on composition, geographic origin and type of food processing. Meat products are frequently discovered to contain undeclared, less appreciated meat species.
Official food control laboratories commonly apply real-time polymerase chain reaction (PCR) assays and/or DNA arrays for detecting mislabeling of meat species. In the last years, DNA metabarcoding has gained increasing attention in food analysis. In contrast to well-established methodologies, DNA metabarcoding allows the simultaneous identification and differentiation of numerous meat species in a large number of food samples, even if the samples contain meat originating from multiple species. This outstanding performance is achieved by efficiently amplifying a DNA barcode region by PCR, followed by massively parallel sequencing of the amplicons by next generation sequencing (NGS) technologies. The cost-effectiveness of DNA metabarcoding increases with the number of meat species to be covered and the number of samples to be analyzed [1].
We present and critically discuss results obtained by testing a DNA metabarcoding assay for 15 mammalian and six poultry species [2] in an interlaboratory ring trial. The ring trial was organized by the Austrian Agency for Health and Food Safety (AGES) in the framework of the §64 LFGB working group "NGS Species Identification". Each of the 15 participating laboratories analyzed 16 anonymously labelled samples (eight samples, two subsamples each). The samples comprised six DNA extract mixtures, one DNA extract from a model sausage, and one DNA extract from maize serving as negative control. Five DNA extract mixtures contained DNA from five animal species in proportions from 0.1% (v/v) to 67.5% (v/v). The sixth DNA extract mixture even consisted of DNA from seven animal species, with pig DNA as major component (94% v/v) and DNA from cattle, horse, sheep, goat, chicken, and turkey as lower components (1% (v/v) each). The model sausage was composed of 50% (w/w) beef, 40% (w/w) pig, 5% (w/w) chicken, and 5% (w/w) turkey.
Quantitative data evaluation not only focused on calculating average proportions of the meat species in the samples, but included the identification of error components within and in between laboratories. Qualitative data evaluation aimed at determining false positive rate, false negative rate, and probability of detection (POD).

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B40

LC-HRMS-BASED NON-TARGETED AND TARGETED METABOLOMICS APPROACHES FOR ASSESSMENT OF HONEY ADULTERATION WITH SUGAR SYRUPS: A PRELIMINARY STUDY

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Honey is a natural product used in food, in medicine and cosmetics. Given the great demand and the relatively high price of honey, it is among the most common targets for economically motivated adulteration. Adulteration of honey can occur directly by adding various sugar syrups to natural honey or indirectly by feeding honey bees with excessive amounts of sugar syrups. Honey adulteration techniques are constantly evolving with the use of syrups that mimic the composition and proportions of sugars naturally present in honey. More and more advanced techniques and analytical instruments are therefore required aiming at detecting honey adulteration. Metabolomics consists in the large-scale study of all the "metabolites", that are the low molecular weight molecules (<1 kDa), in a biological sample through various analytical techniques and data analysis tools. The aim of the present work is the identification of potential markers of honey adulteration with sugar syrups by using hydrophilic interaction liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS)-based non-targeted and targeted metabolomic approach.
The metabolomic profiles of aqueous extracts from ten unadulterated honeys of different botanical origin [multifloral, linden, acacia (*Robinia pseudoacacia*), alfalfa and "barena" (*Limonium narbonense*)] and from eleven sugar syrups of different botanical origin (sugar beet, sugar cane, corn and wheat) used for feeding honey bees, were obtained by using LC-HRMS in both positive and negative ionization modes. The potential markers have been selected after data processing performed by Compound Discoverer™ software, resulting in seven features for electrospray ionization (ESI) in positive polarity and eight for ESI in negative polarity whose signals are present exclusively or predominantly in syrups and not in honeys. An inclusion list has been created to obtain MS/MS spectra of such features. We analyzed fortified honey at 3 different levels (5, 10 and 20%) with a mixture of syrups, honey obtained from the overfeeding of honey bees with sugar beet syrup, and 58 honey samples obtained from the market or local beekeepers. Only one of the potential markers turned out to be a specific signal for syrups and not for honey. The targeted analysis of such potential marker showed a linear trend in fortified honeys at different levels. Honey resulted from overfeeding of honey bees with sugar syrup has a strong signal for this potential marker and the limit of quantification has been calculated around 5% of fortification. Among the analyzed honey samples, only two showed concentrations of 5 and 20% of the potential marker of adulteration respectively. The study is in progress with the aim of identifying this promising marker.

Keywords: *honey, adulteration, metabolomic, LC-HRMS*

B41

ORIGIN- AND CULTIVAR-SPECIFIC DIFFERENTIATION OF MANGO (MANGIFERA INDICA L.) PRODUCTS BY QUANTITATIVE 1H-NMR SPECTROSCOPY

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Mango purées and purée concentrates are important products in the international trade of the food industry and are in high demand in the product categories of beverages (especially fruit juice) and dairy, among others. Unlike other semi-finished products, mango products are purchased based on organoleptic properties associated with certain origins and cultivars. For example, the Indian cultivar "Alphonso" is increasingly in demand by product developers because of its terpene notes and accordingly to be priced higher than other mango cultivars.
Higher prices and limited availability always bring the risk of food fraud and many GFSI-approved food safety management systems require the food business operator to pay special attention to possible economically motivated food adulteration. The analytical toolbox for detecting food fraud must be able to detect many possible adulteration risks and so targeted methods are continuously being supplemented and expanded with non-targeted methods. Here, ^{1}H-NMR spectroscopy plays an important role. The main advantages are that (i) quantitative ^{1}H-NMR spectroscopy allows a particularly high sample throughput due to the only minimal sample preparation needed and (ii) allows the simultaneous determination of numerous parameters, representing clear advantages over many other different analytical methods that would be necessary to collect the data of 966 mango products presented in this study. We continue to see our approach as complementary to the DNA analysis used as the method of choice (isolation and purification of DNA, amplification via PCR and subsequent analysis via capillary electrophoresis): in this way, both analytical approaches can be subjected to a plausibility check.
In a univariate approach, origin- and cultivar-specific differences can be highlighted on a parameter-by-parameter basis, but the non-targeted and multivariate approach seems promising and useful in the analysis. By applying PCAs (broken down to the origin or subcontinent or country), individual cultivars can be distinguished from each other. Limitations of this approach can be observed in mango cultivars from India: the predominant cultivars "Alphonso" and "Totapuri" can be distinguished almost unambiguously, whereas "Kesar" shows high chemical similarity to "Totapuri". The cultivars processed in the Americas are not so easily distinguishable (not least because of different climatic zones). The exception is the Colombian "Magdalena River", which is different from the mangoes cultivated and processed in Brazil, Guatemala, Mexico, and Peru. When looking at the Peruvian cultivars "Criollo" and "Chato de Ica", a clear separation can be seen.
These initial results seem promising to us and promise further developments in the automated food authentication process. The next steps here will be the continuous expansion of the ^{1}H-NMR database and the development of appropriate algorithms for practical use in day-to-day operations.

Keywords: *authentication, fruit juice, targeted analysis, untargeted analysis, food fraud*

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B42

CLASSIFICATION OF IBERIAN DRY-CURED PRODUCTS ACCORDING TO BREED USING NIRS TECHNOLOGY

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Iberian pork products are officially classified into different commercial categories depending on the breed of pig according to Spanish regulation RD 4/2014, which approves the quality standard for Iberian meat, ham, shoulder and loin. Therefore, Iberian products on the market are classified according to the feeding regime and racial purity. Thus, the black label identifies the pieces that come from 100% purebred Iberian pigs fed with acorns in free-range, and is known as "Bellota 100%". The red label identifies pieces that come from acorn-fed animals but with percentages of 50% to 99% Iberian (crosses with Duroc) and is known as "Bellota Ibérico". Depending on the racial purity, the price is very different, which encourages fraud in labelling and misleads the consumer. The industrial sector also pursues the knowledge of its products to ensure the authenticity of this product, which is highly appreciated all over the world. In this work, Near Infrared Spectroscopy (NIRS) was applied to classify Iberian products according to breed. This optical method is non-invasive and is based on the interpretation of the absorption or reflection of certain wavelengths produced by the functional groups found in the sample to be analysed. It has also been applied in previous studies to classify the pieces according to the food they have received or the curing treatments they have undergone.
NIR spectroscopy coupled to a fibre-optic remote reflectance probe was used in this study by means of a Foss 5000 equipment. The analyses were carried out by applying the probe directly to the slices and on the surface fat. With this second type of sampling, the aim is to carry out a classification on the whole piece, before slicing the product. This is of great interest in the sector because hams and shoulders are marketed in whole pieces. The objective was to discriminate the samples according to their racial purity using these two types of sampling.
To do so, the NIR spectra of 120 slices of controlled pieces of ham and shoulder (54 from 100% acorn-fed Iberian pigs and 66 from 50% acorn-fed Iberian pigs) were recorded in the range 1100-2000 nm every 2 nm. On the other hand, 145 samples of surface fat were recorded from controlled pieces (68 from 100% Iberian pigs and 77 from 50% Iberian pigs). Spectral differentiation is performed using the residual RMS X method combining different mathematical and dispersion treatments. After optimisation of the pre-treatment, the percentages of correct classification according to breed were 93% in calibration and 78% in validation using the spectra of the slice sampling, while using the spectra of the surface fat the values were 83% and 75% respectively. These preliminary conclusions should be confirmed by analysing a larger number of samples, given that this is a methodology of great interest in the sector, as it has a low cost per analysis without the need to destroy the material and allows the result to be known immediately.

Keywords: *Iberian ham, NIRS, breed, pork shoulders, labeling category*

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B43

A VOLATILE FINGERPRINTING STRATEGY FOR WINE AGING AUTHENTICATION USING SPME-ARROW COUPLED TO COMPREHENSIVE GCXGC-MS COMBINED WITH ADVANCED CHEMOMETRICS

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In a global food market, Protected Designation of Origin (PDO) wines are highly appreciated for the period of aging that allows the evolution of unique organoleptic properties, and thus, the guarantee of the vintage age is a critical authenticity issue [1]. The coupling of SPME-Arrow to GCxGC/MS analysis in combination with chemometric techniques could revolutionize the field of wine authenticity with the establishment of characteristic volatile markers according to the vintage age. In this work, an SPME-Arrow-GCxGC/MS method was optimized and used for the determination of volatile markers in 24 monovarietal red wine samples belonging to the PDO Xinomavro Naoussa, produced during 4 different years (1998, 2005, 2008 and 2015), in Northern Greece. Overall, 258 volatile compounds were tentatively identified. The data matrix was further processed with multivariate techniques to establish mathematical models and reveal volatile markers for each vintage age. A partial least square – discriminant analysis (PLS-DA) model was developed and successfully classified all the samples to the proper class according to the vintage age with an explained total variance of 95.7%. Variant Importance in Projection (VIP) algorithm was used to calculate the VIP scores of the determined volatiles and distinguish the most important features that affect the discrimination, revealing markers [2]. The developed prediction model was validated and the analyzed samples were classified with 100% accuracy according to the vintage age, on the basis of their volatile fingerprint.

Keywords: *wine authenticity, 2D-GC, SPME arrow, chemometrics, volatile fingerprint*

B44

STABLE ISOTOPE-BASED AUTHENTICATION OF MEDITERRANEAN ANCHOVIES

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Seafood in Europe forms the most complex food network with multitude of middlemen and stakeholders which makes it highly susceptible to fraud. Seafood traceability thus becomes even more important to protect consumer's rights and ensure safety and trust in food chain. Origin mislabelling, species substitution and freshness concealment are the most common frauds in seafood industry. Stable isotope (SI) has been used for source detection in ecology, archaeology, environmental health & pollution, forensics, and food chemistry. The isotope composition of fish tissue is a function of biotic and abiotic factors such as geographic origin of fish, its diet and position in the food chain, isotope fractionation, isotopic signature of the water and sediments (if artificially reared in ponds), seasonal variations and migration, fish metabolism, fish organ/tissue analysed, hydrogeomorphic characteristics between the basins, etc. Most studies on use of SI for determination of geographical origin of fish have focused on differentiating between farmed and wild fishes or have differentiated between fish from widely different areas. This study has attempted to distinguish between anchovies from closely located regions in the Mediterranean Sea- Adriatic Sea, Tyrrhenian Sea, Balearic Sea, and Gulf of Hammamet (Tunisia) using SI ratios. Mediterranean Sea is oligotrophic in nature and is defined as "low-nutrient and low chlorophyll" system. Thus ideally, the SI composition of anchovies in all Mediterranean Sea should be similar, however we found this not to be true.

Our study determined a marked depletion of $\delta^{13}C$ in anchovies from Tyrrhenian Sea as compared to other areas. An opposite trend was observed for $\delta^{14}N$, where anchovies from Tyrrhenian Sea were more enriched as compared to other regions. We were successfully able to classify the anchovies according to their fishing location despite their proximity using $\delta^{13}C$ and $\delta^{15}N$ values. The differences in the SI ratios are a function of the sea-bed rock & river run-offs (contributes to dissolved nutrients) and weather conditions in the different parts of Mediterranean Sea. Tyrrhenian Sea has been proven warmer than its counterparts and this affects the food availability of the anchovies. Anchovies consume planktons and their growth depends on temperature and dissolved nutrients. We also observed a seasonal trend in changes in the SI composition of anchovies. The seasonal changes are a function in the changes in sunlight the sea receives, the temperature variations and the subsequent plankton production in different seasons.

A complete mapping of the isotopic composition of anchovies from Mediterranean Sea will aid in combatting the fish fraud against Mediterranean fish. The study will enable us to create a complete range of SI composition of anchovies in accordance with season & size (age), and finally create a model for future use in detecting fish fraud and be a reference point for further studies.

Keywords: *stable isotope, traceability, mediterranean diet, anchovy, seafood safety*

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B45

TRACING GEOGRAPHICAL ORIGIN OF ARGAN OIL USING CARBON AND OXYGEN ISOTOPE FINGERPRINTS

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Argan oil is extracted from Argan tree fruit seeds in a labor-intensive hand-made process. Argan tree (Argania spinosa) is an endemic species from south-western Morocco. Because of this, Argan oil has received a protected geographical indication status (PGI-MA-906), which assures that both local producers and consumers are protected. Such high quality and highly consumed products are also the target of economically motivated fraud. Argan oil production has tremendous environmental, social and economic importance for Morocco, and it is necessary to verify authenticity and provenance of this valuable product to ensure its reputation protected and promoted. Argan oil has an inherent isotopic fingerprint, a unique chemical signature that allows its provenance to be confirmed. Analyzing oxygen and carbon isotope fingerprints of Argan oil by Elemental Analysis Isotope Ratio Mass Spectrometry (EA-IRMS) allows the differentiation of samples from different regions and creates a framework for using isotopes as a tool for verifying Argan oil origin. Here we report on 47 Argan oil samples analyzed for authenticity control in collaboration with CNESTEN.

Keywords: *origin, EA-IRMS, fraud, authenticity, Thermo*

B46

ISOTOPE FINGERPRINTS: ADDRESSING AUTHENTICITY OF FISH OILS BY GC-MS-IRMS

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As fish oils become a popular and precious source of omega-3 fatty acids, the risk of mislabeling and adulteration has risen significantly. The fatty acid profiles of different fish oils do not often allow the discrimination between different sources and geographical origins. In this study, the compound specific multi-isotope analysis of fatty acids in 30 salmon oils and 43 cod liver oils were analyzed allowing the discrimination of fish oils from different provenance, following risk-based comparisons from market experience. In the light of emerging cases of food fraud, we present how GC-MS-IRMS advanced technology can tackle these problems for addressing authenticity of fish oils. By coupling GC-IRMS with an organic mass spectrometer (MS), the isotopic compositions and the comprehensive qualitative and quantitative sample information with high levels of selectivity, sensitivity, and confidence are accessible simultaneously from a single injection.

Keywords: *GC-MS-IRMS, Thermo, fish oils, fraud, tracing*

B47

GEOCHEMICAL FINGERPRINTING-BASED DISCRIMINATION OF MEDITERRANEAN ANCHOVIES

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Seafood is one the most complex food network in the world. Lack of transparency and reliable authentication techniques makes is susceptible to food fraud. Origin mislabelling by fish sellers to make more money (or pay less taxes) is the most common seafood fraud. The multielement (ME) composition of a food tissue is a function of the environment the food grows in and can be used to trace its origin. Plant based food products ranging from unprocessed fruits and vegetables and processed foods like wine have been successfully discriminated by ME based on their origin. This, however, gets more complicated in case of seafood, especially fish with their migratory lifestyle. The ME composition of fish tissue depends on biotic and abiotic factors such as geographic origin of fish, its diet and position in the food chain, chemical composition of the water and sediments (if artificially reared in ponds), seasonal variations and migration, fish metabolism, fish organ/tissue analysed, hydrogeomorphic characteristics between the basins, etc. This study has attempted to distinguish between anchovies from two regions of the Mediterranean Sea, Adriatic and Tyrrhenian, using their ME profile.
Anchovies were fished from both locations in the month of February 2022 and their ME composition was determined using a Thermo Fisher iCAPQ ICP-MS, with certified fish protein materials (DORM-4 and ERM-BB422) as reference. Twenty-six were the elements analysed showing acceptable recovery (±15%), i.e., Li, B, Na, Mg, P, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Sb, Cs, Ba, Hg, Tl & U. Unsupervised Principal Component Analysis (PCA) led to separation of fish from two regions with PC1 and PC2 explaining 54% and 18% variance, respectively. The separation was mostly influenced by microelements in fish (Li, Mg, Mn, Co, Rb, Sr & Mo) except K. Supervised Linear Discriminant Analyses (LDA) was performed to generate a prediction model. LDA discriminated between the anchovies from two regions with a 92.9% accuracy. Cross validation of the LDA model resulted in 97.1% correct classification with only one misclassified sample. Sodium (Na) contributed most towards the discriminatory ability of the discriminant function followed by As, B, Mo, Li, Mg & Rb. Anchovies mostly consume zooplankton with some larvae (crustacean and copepod). Cobalt, Cu, Mn, Ni and Zn are the most essential nutrients for plankton growth and none of these elements contributed very high towards the discrimination of anchovies. Thus, naturally occurring elements in the sea contributed more towards the discrimination of anchovies.

Keywords: *multielement profile, engraulis encrasicolus, traceability, geographical provenance, food fraud*

Acknowledgement: *This Project is a part of SUREFISH PRIMA project (https://surefish.eu/) Programme supported by EU Grant Agreement No. 1933.*

B48

GEOGRAPHICAL PROVENANCE OF "POMODORINO DEL PIENNOLO DEL VESUVIO" PDO BY MULTI-ELEMENT FINGERPRINTING

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The "Pomodorino del Piennolo del Vesuvio" (PPV) or Vesuvian Piennolo is a traditional tomato variety cultivated in the Campania region (Italy) on the slopes of the Somma-Vesuvius volcano complex. This product has been awarded Protect Designation Origin (PDO) since 2009. PPV is sold assembled by hand in bunches, and it is characterized by long shelf life. In addition, PPV cultivation allows the local "custodian farmers" to preserve the traditional cultivation management and biodiversity in the Vesuvian area. Due to the high typicity and high selling value, this product is susceptible to origin fraud. The main aim of this study was to test the ability of 19 chemical elements (Ca, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, K, Zn, Ba, Cd, Co, Cr, Cs, Li, Rb, Sr) to discriminate the PPV tomatoes (Acampora, Cozzolino, Patanara ecotypes) cultivated in five PDO farms from PPV tomatoes (same ecotypes) cultivated in two farms outside the PDO area. All farms were in the Campania region. The explorative PCA analysis of the multielement profile of tomatoes evidenced natural groupings of the samples according to provenance farms at the expense of different ecotypes. This was also evident by applying the PCA with the samples of each farm taken separately. This suggests that PPV tomato elemental composition does not depend strongly by ecotypes, while the natural grouping of samples by provenance suggests that mainly the features of the cultivation environments influenced the elemental profile of fruits. Consequently, all dataset was divided into calibration and validation sets (70%-30%), and Stepwise LDA was applied for discriminating the samples according to provenance. The S-LDA model gave 100% of correct classification and external validation. Furthermore, the stepwise procedure chose the 84.2% of the elements (Rb> Sr> Mo> Cs> Co> Cd> Fe> Cu> Zn> Na> Mn> Li> Ca> Mg> K> P) as discriminating variables. Further studies are ongoing to increase the robustness of the model by adding new samples from different cultivation years and farms.

Keywords: *geochemical fingerprinting, protect designation origin, custodian farmers, chemometrics, food fraud*

Acknowledgement: *This work is part of the TOMATO TRACE 4.0 project (https://www.tomatotrace.it/) financed by the Rural Development Programme of Campania Region 2014-2020.*

B49

GAS CHROMATOGRAPHY-ION MOBILITY SPECTROMETRY (GC-IMS) AS A TOOL FOR RAPID AND ACCURATE AUTHENTICATION OF GROUNDED BLACK PEPPER

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The European Commission published in 2021 the results of the first coordinated control plan on the authenticity of herbs and spices on the EU 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 describes a non-targeted method for authentication of grounded black pepper by gas chromatography ion mobility spectrometry (GC-IMS) coupled to multivariate statistical analysis. A total of 24 powdered samples were collected and analyzed. The sample set included authentic black pepper samples (from 8 different countries and four harvesting seasons) and samples spiked with non-functional material (pinhead and spent) and ten exogenous materials (green lentil, olive kernel, black mustard, sesame, garlic, corn flour, rice flour, chili and papaya). The percentage of adulteration ranged between 5% and 30%.
The samples were analyzed by using the GC-IMS (FlavourSpec® G.A.S. Gesellschaft für analytische Sensorsysteme mbH, Dortmund Germany) which allows the analysis of volatiles in the headspace without any sample pre-treatment. Each sample was analyzed in duplicate. An amount of 0.5 g of sample was incubated for 3 minutes at 60°C. Each run last 20 minutes, with a nitrogen carrier gas gradient as follows: ramping from 2 mL/min to 5 mL/min within the first 5 min and from 5 mL/min to 17 mL/min in the following 17 minutes. We applied a drift gas constant flux of 150mL/min.
The acquired data were analyzed by Python 3.9.12 using the GC-IMS-Tools package. The spectra were aligned along drift time coordinate and repeated spectra of each sample were averaged. The retention time, considered for statistical analysis, ranged between 1.3 min to 8.3 min, while only a relative drift time between 1.025 and 1.7 min was taken into account. A Pareto normalization was applied. Initially, an unsupervised principal component analysis (PCA) was attempted to discriminate the samples as authentic, exogenously-adulterated and endogenously-adulterated. Afterwards, the normalized spectra were randomly split into training and test set (80:20). While the test set was withheld for further testing of the model, the training set was submitted to partial least squared discriminant analysis (PLS-DA) for classification purposes. A good discrimination of authentic, exogenously-adulterated and endogenously-adulterated was observed in the scores plot. The model achieved high overall accuracy on test set. The model will be further validated with a new batch of 75 independent samples.

Keywords: *adulteration, machine learning, fraud, spices, herbs*

B50

MATURITY TESTING FOR AGED SIRLOIN STEAKS: A PROOF OF CONCEPT STUDY

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All fresh beef is aged for at least a few days and up to several weeks to allow enzymes naturally present in the meat to break down the muscle tissue, resulting in improved qualities such as texture and flavour. Therefore, aged beef commands a premium price on the open market, with consumers willing to pay extra for these enhanced qualities. Discussions with representatives of meat suppliers and processors in Northern Ireland suggested that determining the authentic age of beef analytically is challenging and test development within this area would be beneficial to the industry, the consumer and help in reducing waste. The requirement from the industry was for the development of a rapid test that could determine the maturity of beef in minutes. Hyperspectral Imaging is a methodology that is gaining traction within the analytical community mainly due to miniaturisation, improved optics and software. To assess whether this methodology could be used to determine meat aging, a proof of concept project was initiated with wet aged beef (sirloin steaks) supplied at various stages of maturity (Days: 10; 20; 30; 40) with spectral data generated using a hyperspectral camera from Hinelea (USA). By combining this data with chemometric analysis, models were generated to determine the age of the sirloin beef and externally validated.

Keywords: *hyperspectral imaging, beef, maturity, chemometrics*

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B51

DEVELOPMENT, VALIDATION AND PERFORMANCE OF CHEMOMETRIC METHODS FOR SPECTROSCOPY-BASED AUTHENTICITY TESTING OF SPICES

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What is all this fuss about Artificial Intelligence (AI) and Machine Learning (ML)? Is it hype? Is it really the future of food authenticity testing? We will review the outcomes of what can be achieved using conventional chemometric algorithms as well as AI / ML approaches to model spectroscopic data for detecting economically motivated adulteration in spices.
Algorithms such as Partial Least Squares-Discriminant Analysis (PLS-DA), Soft Independent Modelling of Class Analogy (SIMCA), Artificial Neural Networks (ANN) and Support-Vector Machines (SVM) will be applied to a common data set. Using cinnamon as a test case, we will explore the development, validation and performance of the various approaches exploring their strengths and limitations in detecting adulteration and substitution and hopefully move forward in reaching a conclusion on the hype vs. future question.

Keywords: *spices, chemometrics, machine learning, AI*

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B52

RAPID AUTHENTICATION OF CHINESE OOLONG TEAS USING ATMOSPHERIC SOLIDS ANALYSIS PROBE - MASS SPECTROMETRY (ASAP-MS)

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According to the US National Centre for Food Protection and Defense (Johnson, 2014), tea is among the top 18 food categories with high rates of economically motivated adulteration (EMA). Therefore, it is of paramount importance to ensure the authenticity of tea. Some forms of EMA include mislabelling, as well as substitution adulteration, whereby a portion of an authentic tea is replaced with a low-quality one (Esteki, Regueiro, & Simal-Gándara, 2019). Herein, we present the use of a non-targeted metabolomics approach using an ambient mass spectrometry technique, atmospheric solids analysis probe – mass spectrometry (ASAP-MS), to authenticate Chinese oolong teas. Ambient mass spectrometry is an emerging technique for food quality and safety analysis due to the minimal sample preparation required and the rapid acquisition time (Black, Chevallier, & Elliott, 2016). The first part of the study involves curating a database of mass spectral fingerprints for the three main varieties of Chinese oolong teas – Taiwan Dongding, Guangdong Dancong, and Anxi Tieguanyin. Then, pattern-recognition models such as Principal Component Analysis (PCA) and Principle Component Analysis – Linear Discriminant Analysis (PCA-LDA) were built, which could be used to recognise the different varieties of Chinese oolong teas and ensure labelling accuracy. In the second part of the study, using Anxi Tieguanyin as an example, the authentic tea samples were mixed with 20, 40, 60, 80% w/w of low-quality oolong tea to mimic a possible substitution adulteration scenario. The mass spectral fingerprints of the authentic and adulterated Tieguanyin teas were subjected to classification using PCA-LDA. Superior classification outcome was achieved, where authentic Tieguanyin samples were successfully distinguished from the adulterated ones with high accuracy, sensitivity, and specificity values of 92.4%, 70.4%, and 94.9% respectively. Results from this study highlight the potential of ASAP-MS as a high-throughput approach to authenticate Chinese oolong teas.

Black, C., Chevallier, O.P. & Elliott, C. T. (2016). The current and potential applications of Ambient Mass Spectrometry in detecting food fraiud. *TrAC Trends in Analytical Chemistry, 82,* 268-278. doi:https://doi.org/10.1016/j.trac.2016.06.005
Esteki, M. Regueiro, J., & Simal-Gandara, J. (2019). Tackling fraudsters with global strategies to expose fraud in the food chain. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 425-440.
Johnson, R. (2014). *Food fraud and "Economically Motivated Adulteration" of food and food ingredients. Received form USA:* http://www.fredsakademiet.dk/ORDBOG/lord/food_fraud.pdf

Keywords: *ASAP-MS, direct analysis, principal component analysis, food authenticity*

B53

TRAPPED ION MOBILITY COMBINED WITH LC-HRMS FOR HIGH-PERFORMANCE 4D-METABOLOMICS IN FOOD AUTHENTICITY: EXTRA VIRGIN OLIVE OIL ADULTERATION STUDY WITH OLIVE OILS OF LOWER QUALITY

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Extra virgin olive oil (EVOO) has been declared as food of high nutritional value across the Mediterranean zone, being associated with several positive health effects derived from its consumption. In combination with product's eminent market position, EVOO comprises a likely target for adulteration and fraud. One of the most frequent profit-driven fraudulent practices is its substitution with oils of lower quality, commonly olive oils that comes from a second-step olive processing procedure. Such characteristic substituents are refined olive oils (ROOs), previously soft deodorized or/and soft deacidified, as well as pomace oils (OPOs). Both ROOs and OPOs constitute potential adulterants of extra virgin olive oil, fraudulently mislabelled as EVOOs in the market. Fraud control is therefore vital, not only to prevent consumers' health, but also to meet the established legislative frameworks.
In the present study, Trapped Ion Mobility is combined with LC-HRMS for optimal performance. Integrated 4D-untargeted metabolomics are being developed and applied to discover potential biomarkers for the reliable detection of EVOO adulteration. Ion mobility additional dimension of analysis significantly increased the analytical depth-of-coverage, including isomers detection. Robust classification and prediction models are built using unsupervised and supervised chemometric techniques (principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) respectively) in order to discriminate authentic EVOOs from adulterated ones. Characteristic markers are detected and further identified, co-estimating CCS additional identification criterion in the HRMS workflow. Noteworthy results are also retrieved compared to relative methodology by setting TIMS dimension off (typical LC-HRMS arrangement), with the implementation of LC-TIMS-HRMS enabling the detection of more markers at the lowest adulteration levels of 1%, thanks to platform's enhanced sensitivity.
In conclusion, the results obtained from this study clearly demonstrate that 4D-metabolomics have the potential to be used as a reliable screening tool for the rapid determination of food adulteration. Owing to TIMS unique analytical capabilities, its incorporation with LC-HRMS workflows undoubtedly enhances the comprehensiveness of metabolite detection and adds significant identification evidence for metabolomic investigations in case of food authenticity studies.

Keywords: *LC-TIMS-HRMS, extra virgin olive oil, metabolomics, adulteration, food authenticity*

Acknowledgement: *The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 1308).*

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A NOVEL APPROACH FOR RAPID DETECTION OF ADULTERATION IN SAUDI WILD HONEY WITH VARIOUS TYPES OF SYRUPS USING FTIR-ATR AND CHEMOMETRICS

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Honey is a well-known natural product, not only for its deliciousness and nutritional value but also for its therapeutic properties. It is classified as a luxury product because of its pleasant flavor and taste, which makes it a high-quality and valued commodity. Saudi Arabia's honey consumption has increased significantly in recent years due to economic growth, health awareness, and social, cultural, and religious factors. According to Codex Alimentarius and Saudi Food and Drug Authority standards (SFDA), honey must be free of any food ingredient, including food additives. It is one of the most commonly adulterated foods. Since sugar syrups have low prices and are easily available, they are often added to honey during or after production. Advanced analytical techniques such as HPLC, GC, NMR can be used for honey authentication, but they are time-consuming and expensive. Furthermore, they cannot guarantee 100% accuracy in authentication. For rapid detection of adulteration, the Fourier transform infrared and attenuated total reflectance (FTIR-ATR) spectroscopic techniques have been developed. It is realized that the performance of FTIR-ATR spectroscopy can further be enhanced by combining with chemometrics such as the data compression techniques (principal component analysis PCA and agglomerative hierarchical clustering analysis AHC) which classify unknown samples on the basis of similarities. In this project, those techniques have been used to detect and quantify five Saudi honeys (Athil, Barsim, Eshir, Kina, and Samar). Samples were obtained from trusted sources through the Ministry of Environment, Water and Agriculture. In different amounts (2%, 5%, 10%, and 20%), sugar cane, brown rice, maple, and pancake syrup were spiked into the honey. Syrups were selected as possible adulterants based on their common use, availability, and based on the literature. Following the literature recommendations, the FTIR spectral region of 1180 to 750 cm^{-1} which represents mono- and disaccharides, was considered the data matrix for chemometric models. The sample-set was comprised of 95 spectra of authentic (n = 15) and adulterated (n = 80) honeys. Spectral data were compressed and analyzed using both PCA and AHC models. Surprisingly, the FTIR-ATR visually represented similar absorption bands in the MIR region 4000-490 cm^{-1} with all samples. Therefore, AHC provided detailed information on the extent of the similarities between raw honey types and their dissimilarities with syrups. PCA analysis reached 88.52–99.99% accuracy for distinguishing pure from adulterated honey samples, indicating excellent predictive power. The developed and designed method successfully identified and classified adulterated honey at concentrations as low as 2% (w/w). With such a simple, rapid and powerful method for detecting and quantifying adulteration in honey, it provides a promising start for detecting food adulteration, as well as improving consumer protection worldwide.

Keywords: *honey, adulteration, syrup, FTIR-ATR, PCA, AHC, chemometric*

B55

VERIFYING EU AND NON-EU IDENTITY OF VIRGIN OLIVE OIL BY SESQUITERPENE FINGERPRINTING

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According the EU regulation (29/2012), the geographical origin of Extra virgin (EVOO) and Virgin Olive Oils (VOO) must be label-declared, referring to the EU, to the EU member state or to the third country of origin, as appropriate. In the case of olive oils produced in more than one EU or non-EU countries, or in a mixture of both EU and non-EU countries, the corresponding blend should be mentioned. However, the lack of official analytical methods to verify the declared origin triggers falsification in this sense, leading to a high rate of non-compliances related to false declaration of origin. For this reason, stakeholders consider that verifying the compliance of EU and non-EU olive oils should be addressed with the highest priority[1].
Given the high potential of sesquiterpene hydrocarbon (SH) fingerprint for olive oils geographical authentication[2], the present work addresses this issue by developing a classification model (PLS-DA) based on the SH fingerprint of 400 samples obtained by HS-SPMEGC–MS to discriminate between EU and non-EU olive oils. A 92% of correct classification was achieved in external validation. Subsequently, multi-class discrimination models for EU and non-EU countries were developed and externally validated with successful results (average of 92.2% of correct classification for EU and 96.0% for non-EU countries) [1] Casadei et al. 2021. Food Control, 121: 107902. [2] Quintanilla-Casas et al. 2020. Food Chemistry, 307: 125556.

Keywords: *virgin olive oil; geographical origin; sesquiterpene hydrocarbons; fingerprint; SPME-GC–MS*

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B56

AGING DISCRIMINATION OF GRANA PADANO PDO CHEESE WITH AN NMR-BASED METABOLOMIC APPROACH

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In recent years, the interest of consumers in high-quality food products has been increased remarkably. One of the most imitated products is the Grana Padano PDO cheese, one of the most renowned Italian cheeses. Grana Padano PDO cheese can be mislabeled regarding its aging, because the quality and consequently the price of the cheese increases accordingly to this parameter. Thus, younger cheeses can be mislabeled for economic gain as more aged products. In this study, we tried to study Grana Padano PDO cheeses to discriminate them based on the aging. After a simple extraction, the samples were analyzed with NMR spectroscopy and an untargeted metabolomic approach was developed: the fingerprints of the samples obtained from the NMR spectra were studied and treated with multivariate statistical analysis. The results demonstrated that this approach can be used to distinguish Grana Padano of different ages and therefore can be interesting for industry and authorities in charge for controlling food quality and labels.

Keywords: *NMR, cheese, authenticity, metabolomics*

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METABOLOMICS: AN EFFECTIVE TOOL FOR AUTHENTICATION OF SPELT FLOUR?

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The food authenticity, the confirmation of declaration on the packaging, or the detection of adulteration, is a challenging process for modern analytical chemistry. One of the commodities that can be a target for fraudulent practices is spelt wheat (*Triticum spelta*), especially in the form of spelt flour. The cultivation of spelt wheat is more demanding, compared to the well-known common wheat. Due to the more problematic cultivation, mainly lower yields, spelt wheat is a more expensive commodity than other common wheat varieties of the genus Triticum (e.g. *Triticum aestivum L.*). This fact may tempt fraudulent producers to partially replace spelt wheat with cheaper wheat variety in milling products, i.e. flour.
There are several analytical approaches to detect these dishonest practices and thus control the authenticity of spelt flour. However, the situation is rather complicated because of the significant genetic relatedness of both types of wheat (*Triticum spelta and Triticum aestivum L.)*, and therefore methods based on the analysis of genetic information are less effective. One of the promising possibilities is the use of a metabolomic approach employing liquid chromatography coupled to high-resolution mass spectrometry as a very effective tool and subsequent processing of the obtained data using advanced chemometric analyses.
In the initial phase of this pilot study, 10 flour samples were analyzed, including 6 authentic spelt flour samples and 4 authentic wheat flour samples. Two approaches were chosen for sample extraction, the first aimed at the isolation of rather non-polar substances using a mixture of dichloromethane:methanol (50 : 50, v / v), and the second aimed at the extraction of polar substances with a mixture of methanol : water (80 : 20, v / v). The separation was carried out by liquid chromatography on a reversed-phase system coupled to high-resolution mass spectrometry. The data were acquired in positive and negative ionization modes and processed by principal component analysis and partial least square discriminant analysis. Chemometric data processing showed very promising results in this initial phase, when the samples were separated and classified into two different groups and at the same time, the characteristic compounds for both types of wheat were identified. These markers will be further verified on a larger set of samples and the smallest possible detectable addition of wheat (*Triticum aestivum L.*) to spelt flour will be determined.

Keywords: *authenticity, spelt, wheat, flour, metabolomics*

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SESSMENT OF AUTHENTICITY OF BEESWAX USING NUCLEAR MAGNETIC RESONANCE (NMR)

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Beeswax has unique physicochemical properties and is therefore used in various technological industries. Due to high demand, the risk of adulteration increases. Waxes with the addition of various adulterants appear on the market more and more frequently. Within the presented study, nuclear magnetic resonance (using Bruker Avance III HD 500 MHz) was used to evaluate changes in the composition of beeswax due to the addition of the most common adulterants (paraffin, stearin, carnauba wax, and palm wax). Authentic beeswax, adulterants, model samples with the addition of adulterants, and real samples (from beekeepers, and wax foundations) were measured to assess the NMR method settings and performance characteristics. The presence of paraffin and stearin was detected mainly based on signal intensity in the chemical shift region of 2.56-2.20 ppm and 2.14-0.29 ppm. Adulteration using carnauba and palm wax was assessed based on signal intensity in the chemical shift region of 8.0-6.0 ppm and 4.4-4.1 ppm. Commercial samples suspected of adulteration were subjected to further analyses by reference methods: fatty acid profile (GC/FID), hydrocarbon profile (GC/MS), acid value, and saponification value, which proved the nonconformity of the products. The NMR method was found to be suitable for the screening of authenticity and purity of the beeswax. Based on the analysis of authentic samples, the limit intensities of selected signals have been determined as such (limit) intensities that can serve for evaluation of unknown samples.

Keywords: *beeswax, NMR, paraffin, stearin, adulteration*

Acknowledgement: *This work was supported from the grant of Specific university research - grant JIGA 2022 (Detection of adulterants in beeswax (Apis mellifera)).*

B59

SALMON ORIGIN AUTHENTICATION ANALYSIS WITH INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICP-MS) AND CHEMOMETRICS

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The demand for high-quality salmon is increasing. However, the product suffers greatly as a result of high sales volumes and prices that vary by region. It is critical to improve the authentication of geographically iconic salmon using discrimination science and technology. Approximately 500 salmon samples were analyzed in this study, originating from Alaska, Iceland, Scotland, and Norway. A screening method was established on an ICP-MS system. The method was capable to determine 47 elements in salmon from 4 different countries of origin. In combination with chemometric evaluation, both unsupervised and supervised pattern recognition techniques, principal component analysis (PCA) and Partial Least Squares algorithms (PLS-DA) respectively were performed for the whole data set in order to discriminate and classify salmon samples according to their origin and detect corresponding elemental markers.
The PCA score plot produced shows the elements of each group and outlined the differences between each. R^2X and Q^2 values of 0.979 and 0.85, respectively, were obtained, indicating that the PCA model was both robust and stable. The PLS-DA results revealed that there were high level separations across Alaskan salmon, Icelandic farmed salmon, Icelandic wild salmon, Norwegian salmon, and Scottish salmon. The PLS-DA resulted in all element components with $R^2X = 1$, $R^2X = 0.757$, and Q2 (cum) = 0.739. This suggests that the PLS-DA result has a strong capacity to explain sample differences. Thus, we have concluded that the distribution of elements in salmon vary largely between five groups.
Li, B, V, Fe, Co, Zn, Se, As, and Cd were identified as significant elements for origin determination. These large variances in mineral element levels were capable of discriminating the geographical origin of salmon, according to multivariate analysis. The results of ICP-MS also found that the salmon purchased in Iceland (including farmed and wild) had higher arsenic content than that purchased in Norway and Scotland. Cadmium was also detected in wild Alaskan salmon and wild salmon purchased in Iceland. This project has set the basis for assessing the origins for salmon and has very high potential to become a routine method for salmon authenticity analysis.

Keywords: *salmon, origin authentication, ICP-MS, multielement profiling, food analysis, chemometrics*

B60

COMBINING CLASS-MODELLING AND DISCRIMINANT APPROACHES FOR AUTHENTICATION OF SIMILAR CLASSES

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Honeybush is a popular herbal tea endemic to South Africa, highly valued for its taste, aroma, and medicinal properties such as antioxidant, anti-diabetic or anti-inflammatory activity. In the European Union, honeybush is protected as Geographical Indicator, which means that its unique characteristics are related to its geographical origin. Honeybush is produced from number of *Cyclopia* species, and most commonly used are *C. intermedia, C. genistoides* and *C. subternata*. Recently, the demand for species-specific honeybush teas instead of their mixtures has increased thus a methodology for authentication of these three species is required.
The process of authentication of certificated food products can be presented as follow: firstly, the samples of the original products from the known source are prepared and analysed with the selected analytical technique. The obtained data are pre-processed and subjected to class-modelling, also known as one-class classification. The aim of the class-modelling is to construct an individual mathematical model based on the similarities among samples of class modelled only. The class-model is used to predict whether new samples of unknown origin belong to the class studied. Class-model is based on similarities among samples of the classes studied, thus in the case when the goal is authentication of several similar classes, the individual class-models can lead to poor classification results. In such situations, the discriminant model, which is based on differences between classes studied, can lead to better classification than class-models. However, the classical discriminant model always assigns new sample to one of the classes studied, even if in reality it belongs to none of them. For this reason, discrimination cannot be applied for authentication purposes.
In this study, the two-step approach is presented that combines the class-modelling and discriminant approaches in order to authenticate similar classes that overlap in the feature space.
The first step is the construction of the class-model for the training set consisting of samples from all authentic classes studied. At this step, samples belonging to none of the classes studied are supposed to be identified and neglected in the next step, whereas samples assigned by the class-model as belonging to one of the classes studied are in the second step discriminated into specific classes with a discriminant model. The performance of the two-step approach is compared with two so-called soft discriminant methods and ROC (Receiver Operating Characteristics) based SIMCA (Soft Independent Modelling of Class Analogy) method. The methods were tested against different scenarios of authentication of three *Cyclopia* species. It was revealed that the two-step approach, soft discriminant method proposed by Calvini et al. and ROC-based SIMCA [1] allowed successful authentication of three *Cyclopia* species.

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Keywords: *authentication, class-modelling*

Acknowledgement: *The authors acknowledge the financial support of the bilateral project PL-RPA2/04/DRHTeas/2019, co-financed by the National Research Foundation (NRF), South Africa, (grant nr 118672 to DdB) and the National Centre for Research and Development (NCBR), Poland. Z. Małyjurek acknowledges the financial support from the project PIK, POWR.03.02.00-00-I010/17.*

B61

THE ROLE OF MASS SPECTROMETRY AND CHEMOMETRICS IN FOOD CHARACTERIZATION AD AUTHENTICATION

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The evaluation of food authenticity and traceability plays a pivotal role in ensuring food safety and quality at all levels of the production process, from raw materials to finished products. The most widely used analytical strategies applied in this field rely on both chemical and biochemical approaches, based on the use of biochemical markers, component profiling or chemical fingerprinting of a product and DNA or proteins, respectively [1].
Mass spectrometry (MS)-based techniques are widely applied to ensure food quality and safety throughout the supply chain, as well as traceability of food production processes [2]. Being able to provide the unambiguous identification of the compounds investigated, high resolution MS (HRMS) is the most versatile analytical tool for characterization and authenticity assessment. HRMS coupled to ultra-high- performance liquid chromatography (UHPLC-HRMS) is a very powerful combination for identification purposes, as recently demonstrated in the separation and identification of fatty acids of nutritional interest in a dietary supplement sample [3]. On the other hand, rapid HRMS methods can be also proposed as useful tools for screening purposes with minimal or no sample preparation, thus avoiding chromatographic separation, reducing sample handling, costs and analysis time. In this context, the use of a risk-based and systematic approach, i.e. the so called Quality by Design, should be promoted to ensure that all sources of variability affecting a process are identified, explained and properly managed [4].
Taking into account that large amount of information is provided by MS-based techniques expecially when untargeted approaches are applied, multivariate statistical tools are required to turn complex data set into useful information, thus increasing reliability in decision-making [5]. Examples highlighting the power of chemometrics for both exploration and prediction purposes in ensuring food control across the supply chain for characterization and authentication purposes will be presented and critically discussed.

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Keywords: *chemometrics, mass spectrometry;, food authenticity and traceability*

B62

INVESTIGATION OF THE INFLUENCES OF EDAPHIC FACTORS ON HYDROGEN STABLE ISOTOPIC COMPOSITION OF FATTY ACIDS IN VEGETABLE OIL – CASE STUDY RAPESEED HARVESTED IN HESSE, GERMANY IN 2017-2019

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Compound-specific stable isotope analysis (CSIA) is an important tool for the investigation of authentication of vegetable oil, as the isotopic composition of fatty acids (FAs) could reflect the environment, in which oilseed plant grows[1-3]. However, the factors that specifically affect the isotopic composition are rarely studied. In this study, we investigated the correlation between the hydrogen stable isotopic composition (δ^2H) of FAs in rapeseed oil and edaphic factors such as surface soil moisture, soil temperature, and soil texture of the origin of the rapeseed samples.
50 rapeseed samples (winter type *Brassica napus*) harvested from 2017 to 2019 in Hesse, Germany, were analyzed. The δ^2H values of individual FAs, namely C18:1, C18:2, C18:3, and C16:0, were prepared and measured by the gas chromatography/pyrolysis/isotope ratio mass spectrometry system. The data of surface soil moisture and soil temperature of every sampling location were extracted from Copernicus data using remote sensing techniques and from the model at Deutsche Wetterdienst, respectively. The other edaphic factors were determined by laboratory measurements.
Our results showed that the δ^2H values of all FAs in rapeseeds strongly positively correlated with the surface soil moisture in July (r=0.6 to 0.7; $p<0.001$) and strongly negatively correlated with the soil temperature in July (r=-0.6 to -0.7; $p<0.001$). These two factors could affect the root system absorbing the soil water from different depths with various δ^2H values. Furthermore, there was a negative correlation between the silt content and the δ^2H values of FAs in 2017 and 2019 (r=-0.4; $p<0.05$). The electrical conductivity of soil didn't affect the δ^2H values of FAs of rapeseeds. These correlations suggest that these edaphic factors influence the hydrogen isotope fractionation of FAs in rapeseed oil. Accordingly, the H-CSIA can be an effective tool in the geographic traceability study of rapeseed oil.

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Keywords: *hydrogen isotopes, edaphic factors, geographical traceability, rapeseed oil*

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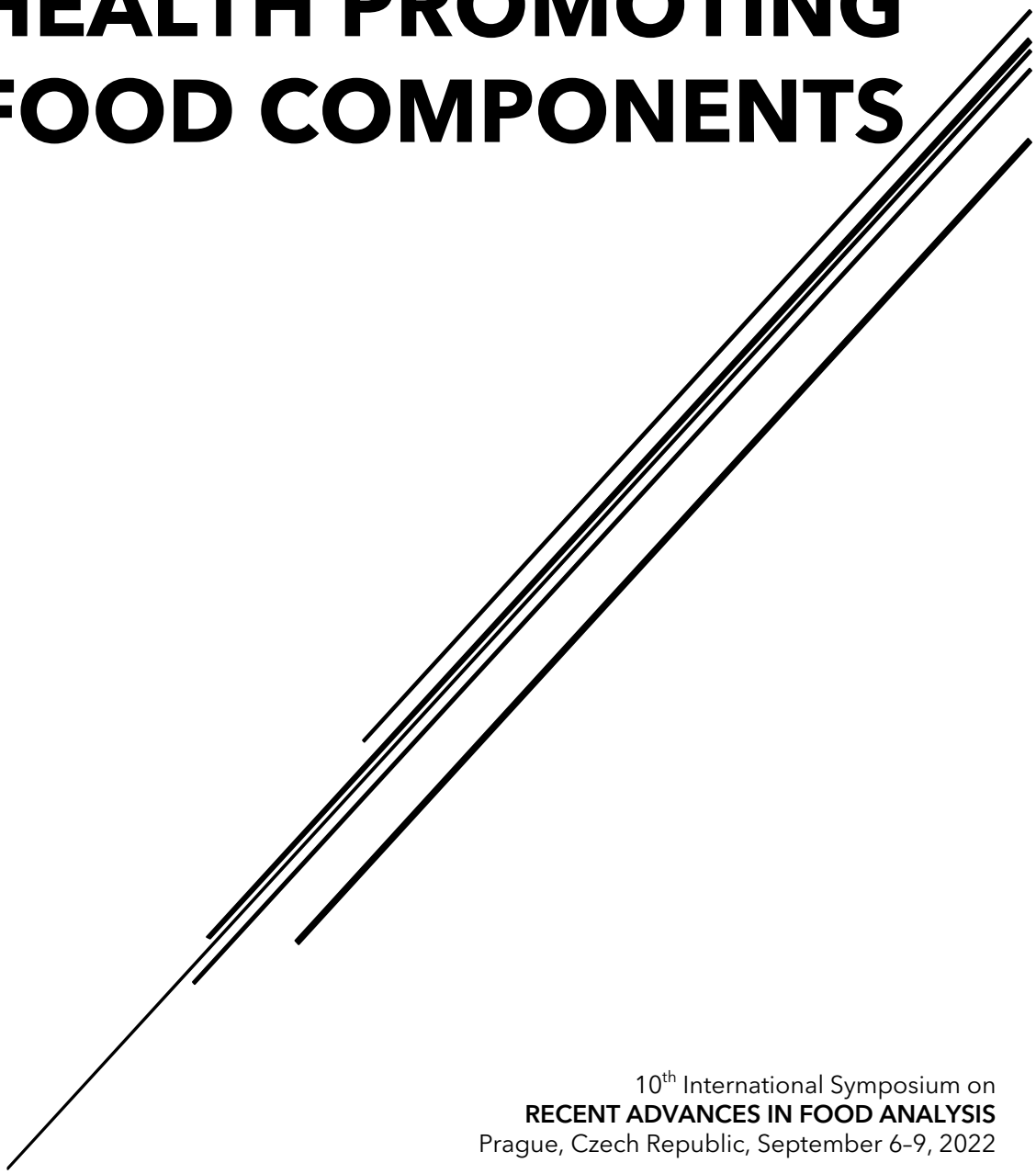
BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS



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C1

BIOFISH7000. NEW PORTABLE AND CLOUD CONNECTED DEVICE FOR RAPID AND ACCURATE QUANTIFICATION OF HISTAMINE IN FISH SAMPLES

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Histamine is an important and toxic biogenic amine which may be present in food and may cause food poisoning in humans when contained at high levels. It is produced during bacterial decarboxylation of histidine in fish muscles. Therefore, the levels of histamine have been suggested as indicators of fish bacterial spoilage. In fact, it is the unique biogenic amine with regulatory limits for fishery products. According to the European Commission, at least seven of the nine samples taken from a batch of fresh fish must be less than 100 mg/Kg of histamine, but none of the samples tested can exceed 200 mg/Kg of histamine. Traditional analytical methods for histamine testing in fish include HPLC-FD or HPLC-MS, being the official method HPLC-UV. Despite of their high accuracy, precision and possibilities of automation, they are labour intensive methods, requiring skilled personnel and expensive instruments.
In this work we present a new portable, rapid, easy to use, accurate and cloud connected system for the analysis of histamine in fish, composed by a portable electrochemical reader and disposable histamine biosensor test strips. The developed biosensor combines the selectivity of enzymatic reactions with the accuracy, high sensibility and speed of electrochemical biosensors offering results in less than one minute with minimum sample pretreatment. The biosensor, a pre-calibrated and ready to use enzyme-modified screen-printed electrode, allows the quantification of histamine in fish at any point of the value-chain. Biosensors are disposable, so no long cleaning procedures are needed between analysis. Additionally, the only sample treatment required is to mince the fish sample, weight an appropriate amount of it and dilute it in the provided extraction buffer. The measurement device, is a highly intuitive and ultra-light portable electrochemical reader connected to the cloud, allowing to securely store, visualize and manage the analyses data in order to keep analysis traceability. The analytical validation for this device has been carried out for raw, boiled and canned in oil tuna samples with good results. The biosensors are able to quantify histamine levels in fish with a limit of quantification as low as 5 mg/Kg and covering a range from 5 to 50 mg/Kg, with an imprecision lower than 15%. Accuracy of the measurements is ensured between 80% and 110% in all the histamine concentrations of the covered range. Additionally, biosensors are stable for one year when stored refrigerated in its sealed package.

Keywords: *food safety, histamine, in-field detection, cloud-enable biosensing*

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C2

BIOMILK7000. A PORTABLE AND CLOUD CONNECTED DEVICE FOR THE ACCURATE QUANTIFICATION OF LOW AMOUNTS OF LACTOSE IN DAIRY PRODUCTS

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Lactose is the major disaccharide found in dairy products. As an example, in cow's milk it can be found at a concentration of 4-5%. Lactose concentration in milk is important for the evaluation of raw or processed milk quality. Low lactose levels in raw milk can be an indicator of mastitis infection. Moreover, the effective analysis of lactose is also significant during the processing of milk to different dairy products. Despite there is no regulation on the maximum amount of lactose that a dairy product should have to be labelled as low in lactose, some countries have established conditions for use of the phrases "Lactose-free" and "Low in lactose" in dairy products labelling. Thus rendering quite important the analysis of residual lactose content in dairy products. Currently available methods for determination of lactose are infrared spectroscopy, polarimetry, gravimetry, HPLC, etc., all of which are time-consuming, expensive, or difficult to automate. However, they are not able to detect low amounts of lactose, and by the moment, only two methods have been validated by the AOAC for the analysis of lactose in low lactose products, both of then based on enzymatic methods, facilitating a rapid performance. Particularly, enzyme-based amperometric biosensors have been found to be versatile analytical devices with high selectivity that can be operated by unskilled personnel.
This work describes an easy to use, rapid, and accurate method for the determination of lactose in dairy products using a portable and cloud connected system composed by a portable electrochemical reader and pre-calibrated disposable enzymatic biosensors. The determination involves a simple dilution of the sample with the provided dilution buffer, and the system returns the results of the analysis in less than one minute. The measurement device, is a highly intuitive and ultra-light electrochemical reader connected to the cloud, allowing to securely store, visualize and manage the analyses data in order to keep analysis traceability. The method has been validated for the quantification of lactose in various dairy matrices with very different compositions such as milk, cream, yogurt, and growth milk, showing no matrix effect and the validity of the method in terms of linearity, recovery and precision in a range from 60 mg/L to 2500 mg/L. The calculated limit of quantification of the method is 60 mg/L for milk matrices and 80 mg/L for the rest of dairy products evaluated. The precision of the method is higher than 85% in all the cases tested while the accuracy of the results is kept between 80% and 110%. Additionally, biosensors are stable for six months when stored refrigerated in its sealed package.

Keywords: *food safety, lactose, in-field detection, cloud-enable biosensing, dairy products*

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C3

VALIDATION OF BIOMILK 3000 LAC FOR THE QUANTIFICATION OF LACTOSE IN LACTOSE-FREE AND LOW LACTOSE DAIRY PRODUCTS. AOAC OFFICIAL METHOD 2020.09

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Increasing demand for lactose-free and low-lactose dairy products has led the dairy industry to need new rapid methods for accurate and precise lactose quantification for regulatory compliance. The BIOMILK 3000 LAC is an enzymatic electrochemical digitalized biosensor for the quantification of lactose in free and low lactose dairy products developed by BIOLAN Microbiosensores. The test method is based on the direct enzymatic recognition-electrochemical detection of residual levels of lactose by means of the BIOMILK 3000 biosensor reader, in less than five minutes and without intricate sample pre-treatments.
The BIOMILK 3000 LAC method was validated according to AOAC INTERNATIONAL policies and procedures against performance requirements as outlined in the SMPRS® for Lactose in Low Lactose or Lactose-Free Milk, Milk Products, and Products Containing Dairy Ingredients, AOAC SMPR 2018.009. A Single Laboratory Validation was performed to evaluate the method performance over various different matrices including milk, sugary plain yogurt, fruit plain yogurt, flavoured liquid yogurt, greek yogurt, cream, soft cheese, infant formula, café latte, chocolate milk and high protein milkshake. Moreover, a Multilaboratory Validation was also conducted to assess the reproducibility of the method. A collaborative study was organized with fifteen laboratories, where nine different samples were sent as duplicate blind coded and analysed in terms of internal repeatability and external reproducibility.
The linearity and matrix studies demonstrated linear dose responses from 5 to 600 mg/100 mL of lactose, with recoveries between 85-115%, and precise lactose quantification for all the evaluated matrixes (RSDr < 10%). The LOQ was validated at 5 mg/100 g of lactose for all matrixes tested. The method proved to be robust, consistent, and stable. Results from the collaborative study demonstrated a very high internal precision of the technique (RSDr < 8%), as well as a good external reproducibility (RSDR < 14% for concentrations up to 100 mg/100 g and RSDR < 8.2% for higher concentrations), meeting requirements from the AOAC INTERNATIONAL. Taking into account these results, the BIOMILK 3000 LAC method was granted with Final Action Official Method status by AOAC INTERNATIONAL in July 2022.

Keywords: *lactose, biosensor, official method, food safety, dairy products*

Acknowledgement: *This work has received funding from Torres Quevedo Program: Grants for the hiring of R&D personnel in companies, technology centers and business associations - PTQ-2019-010390.*

C4

VALIDATION OF BIOFISH 300 SUL FOR THE QUANTIFICATION OF TOTAL SULFITE IN SHRIMPS. FIRST ACTION 2020.09

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Sulfites are the oldest and most widely used additive in food and beverage sector with antioxidant and preservatives properties. However, they are recorded as allergens by the main international regulatory bodies on food safety due to their adverse health effects, and the maximum concentration permitted must be ensured by the agro-food processing industries as well as regulatory laboratories. In shrimp aquaculte sector, sulfites are used to inhibit melanosis, a natural browing effect caused by postmortem enzymatic oxidation of polyphenols, which diminish the market value of the product. In Europe, the limit in crustaceans is restricted from 150 to 300 mg/kg SO2, according to the size of the crustacean. The most widely used method for the quantification of sulfites in food is the Optimized Monier-Williams method (AOAC Official Method 990.28), which consist of a tedious and time-consuming titration with the usage of toxics solvents. Due to these drawbacks, the industry demands for more straightforward technologies which drastically reduce the analysis time and procedure without compromising the accurary of the results, allowing a faster traceability throughout the shrimp production chain.
In this study we present the BIOFISH 300 SUL, a simple, fast and accurate method based on a digitalized bench-top biosensor as an alternative for the quantification of total sulfites in shrimps. The BIOFISH 300 SUL method is based on the direct enzymatic recognition-electrochemical detection of SO2 by means of a biosensor reader. The method mainly consists of the extraction of sulfite from the solid matrix in an aqueous solution, and its subsequent quantification by the device in less than 3 minutes, covering the quantification range of 7-150 mg/g of total SO_2. A Single Laboratory Validation was performed according to AOAC INTRNATIONAL policies and procedures to evaluate method performance, which included comparatives studies between BIOFISH 300 and OMA 990.28 over naturally contaminated and spiked samples of raw and boiled shrimps to assess the accuracy of the method. The linearity and matrix studies demonstrated linear dose responses from 7 to 150 mg/Kg of shrimp and precise sulfite quantification for all the evaluated matrixes, with RSD_r < 10%. Comparisons between BIOFISH 300 SUL and OMA 990.28 demonstrated the accuracy of the method, with recoveries ranging from 85 to 110% for all matrixes over the whole quantification range. The calculated and validated LOQ was 7 mg/Kg of SO_2, which is lower than that of the OMA 990.28 set at 10 ppm, making it attractive to regulatory labs. The method proved to be robust, consistent, and stable. Taking into account these results, the BIOFISH 300 SUL was adopted as First Action Official Method℠ by the AOAC Expert Review Panel for Sulfites in Seafood Methods in February 2021 after rigorous review

Keywords: *sulfite, biosensor, food safety, AOAC - first action*

Acknowledgement: *This work has received funding from Torres Quevedo Program: Grants for the hiring of R&D personnel in companies, technology centers and business associations - PTQ-2019-010390.*

C5

STABILITY, BIOACCESSIBILITY AND ENZYME INHIBITION POTENTIAL OF ANTHOCYANINS PIGMENTS FROM AMELANCHIER LAMARKII BERRIES

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Introduction. Anthocyanins are water-soluble pigments responsible for red, purple and blue color in fruits and vegetables. Due to their intense and eye-catching hues they have been traditionally used as natural pigments in food products. Unfortunately, the food applications of anthocyanin-rich products are limited by their reduced stability and bioavailability. *Amelanchier lamarkii* juneberries are a great source of anthocyanins, understudied in scientific literature.
Aims. Our aim was to identify anthocyanin profile of fresh juneberries and evaluate their individual stability at different pH solutions and temperatures, their inhibitory activity on selected enzymes and the bioaccessibility following *in vitro*digestion.
Materials and Methods. The anthocyanins were extracted with methanol (0.03% HCl), the solvent was removed. The extract was characterized by C18=HPLC-PDA and LC-ESI-MS and further used for stability, enzyme inhibition and bioaccessibility studies. Samples were exposed to various pH buffers (1, 5, 8) and temperatures (4, 24, 37, 60, 100°C), at different times (0, 1, 4, 8, 24h). The inhibitory potential of the extract on alpha-glucosidase, tyrosinase and cholinesterase was tested using *in vitro* assays, compared with specific inhibitors. *In vitro* simulated gastrointestinal digestion for the oral, gastric and intestinal phase was performed using the INFOGEST protocol, and the samples were analyzed by C18-HPLC-PDA in order to calculate the bioaccessibility.
Results. Three major anthocyanins were identified in the juneberries extract: cyanidin-3-galactoside, cyanidin-3-arabinoside and cyanidin. After exposing the methanolic extracts to different pH and temperatures, it was found that the maximum stability of the anthocyanins was reached at a strong acidic pH and a temperature of 4°C. The stability decresed with the increase of temperature and pH, dependent of time. The inhibitory potential of the extract on the three enzymes was relatively modest, with the highest inhibitory activity on tyrosinase, IC50=8.843mg/ml. Regarding the stability during *in vitro* digestion, the higher amount of anthocyanins was found after the gastric phase. The bioaccessibility of total anthocyanins was low (1.083%), probably due to their metabolization and low stability at the slightly alkaline pH of the intestinal fluid. These results are in line with studies performed on bioaccessibility of anthocyanins from other sources. anthocyanins.
Conclusions. Given the importance of anthocyanins in terms of health benefits, the results of this study could be useful for the nutraceutical and food industries.

Keywords: *anthocyanins, juneberry, pH stability, bioaccessibility, enzyme inhibition*

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C6

READY-TO-USE GREEN EXTRACTS ENRICHED WITH CAROTENOIDS USING HYDROPHOBIC DEEP EUTECTIC SOLVENTS

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Carotenoids are a group of pigments recognized for playing an important role in human health for their antioxidant properties. Carotenoid extraction is an important step for food industry in order to concentrate them for their inclusion in food products in proper doses for human consume. Development of greener solvents for carotenoid extraction avoiding the expensive downstream process of solvent elimination is a challenge for food industry. Recently Hydrophobic Deep Eutectic Solvents (DES) have been studied for the extraction of non-polar compounds and comply with the principles of green chemistry of being non-toxic for human consume and the environment. The aim of this study was obtained ready-to-use extracts enriched with carotenoids from orange peels (OP) using hydrophobic DES, that can be directly included in food products. Orange peels were obtained from orange fruits (*Citrus sinensis*, Navel cultivar). Three hydrophobic DES prepared were Menthol: Camphor (Me:Cam), Menthol: Eucalyptol (Me:Eu) and Lauric Acid: Octanoic acid (C12:C8). Extractions were performed with 1 g of milled OP using different DES (1:10) for 30 min in Ultrasounds at 50W. The obtained extracts were characterized, and total carotenoid content (TC) was determined spectrophotometrically. In order to evaluate the biological activity, the antioxidants were determined by ORAC assay, and the cytotoxicity was evaluated *in vitro* by antiproliferative assay in tumor cells (HeLa) using MTS reactive. Additionally, to evaluate the human absorption of the obtained extracts a Parallel Artificial Membrane Permeability Assay (PAMPA), was used. TC in the extracts was not significative different between the 3 extracts, Me: Cam (161.52 ± 13.9 mg/100g_{fw}), Me: Eu (166.67 ± 16.41 mg/100g_{fw}) and 12:C8 (151.24 ± 7.04 mg/100g_{fw}) and in all the cases were higher compared with hexane extract (112.14 ± 0.044 mg/100g_{fw}). ORAC values were higher in C12:C8 extract (2650.94 ± 25.15 µmolTE/g_{fw}). The *in vitro* assay showed an antiproliferative effect of Me:Cam extract on tumor HeLa cells with 26.7% of cell viability. These results are probably related to the antioxidant effects of carotenoids and also the anti-inflammatory effect of Menthol and camphor compounds. The PAMPA assay demonstrated that the carotenoids present in Me:Cam extract have higher apparent permeability (102.543 ± 9.80 x 10^{-8} cm/s) compared with ethanol extract (22.157 ± 2.19 x 10^{-8}cm/s) after 24 hours which means than can be absorbed easily thought intestinal human membrane and have good bioavailability. The use of solvents with inherently safe components as Menthol and Camphor is mandatory for developing "bioactive compounds-DES" formulations for food product application. The carotenoid green extracts obtained could be considered as ready-to-use in food products avoiding the downstream process of solvent elimination and can add biological value to the final product.

Keywords: *carotenoids, bioactivity, deep eutectic solvents, green extraction, orange by-products*

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C7

LIPPIA CITRIODORA AND HIBISCUS SABDARIFFA SEMI-INDUSTRIAL EXTRACTS AS POWERFUL INGREDIENTS FOR CHRONIC DISEASES

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Lippia citriodora and *Hibiscus sabdariffa* have traditionally been used as infusions due to their beneficial health properties. Much of these properties are due to their composition rich in phenolic compounds [1]. Among the bioactive properties of phenolic compounds, their antioxidant power stands out, which is directly related to the ability of these compounds to alleviate oxidative stress when dealing with different reactive oxygen species (ROS). Two other remarkable bioactive properties of these compounds present in these plants are the anti-inflammatory and anti-aging capacity [2]. In this sense, there are different enzymes involved in aging and related to inflammation in which phenolic compounds can modulate their action. However, the obtainment of bioactive compounds is a key step in the development of innovative high added value products. For this reason, it is important to incorporate extraction methodologies near large-scale industrial design instead of lab-scale experiments.
Thus, our objective is to obtain semi-industrial extracts from *L. citridora* and *H. sabdariffa* and to study their bioactive compounds and biological potential for developing new bioactive ingredients. For this purpose, the characterization of the phenolic profile was performed using a high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-ESI-QTOF-MS). The measurements of the total content of polyphenols and antioxidant capacities were carried out by Folin-Ciocalteu and by means of different techniques based on the transfer of protons and electrons. In addition, the free radical and ROS scavenging potential have been evaluated using different methods. Finally, the power of the extracts to modulate the activity of enzymes such as acetylcholinesterase, tyrosinase, xanthine oxidase, elastase, hyaluronidase, and collagenase has been evaluated.
Among the remarkable results, a different phenolic profile between the two plant matrices was observed. A high content of phenylpropanoids, iridoids and secoiridoids was detected in *L. citriodora*. However, phenolic acids and flavonoids were more abundant in *H. sabdariffa*. This deference in the phenolic profile may be related to the different bioactivity results. In any case, both plants showed high antioxidant and anti-aging capacities, which makes them good choices for the prevention and treatment of different pathologies related to oxidative stress, chronic inflammation, and aging.

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[2] Villegas-Aguilar, M. del C. et al. (2020); Molecules.

Keywords: *bioactive compounds, phenolic compounds, Lippia citriodora, Hibiscus sabdariffa, HPLC-ESI-QTOF-MS*

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C8

COMPREHENSIVE ASSESSMENT OF BIOACTIVE SECONDARY METABOLITES IN A SET OF AQUEOUS EXTRACTS FROM AGRIMONY (AGRIMONIA EUPATORIA L.)

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Agrimony (*Agrimonia eupatoria* L.) is a medicinal plant widely used in traditional medicine. Nowadays, homemade herbal extracts are gaining growing popularity. Aqueous agrimony extracts are mainly used for rinsing wounds, alternatively, dried plant is used as tea. These products have positive effects on human health and body (antioxidant activity, anti-inflammatory effect or hepatoprotective effect) thanks to the content of several bioactive compounds such as flavonoids, phenolic acids or tannins. In this study, "homemade" aqueous extracts from agrimony herbs collected at different locations, differently processed (dried, fresh) and picked in various vegetation states were prepared and targeted screening of polyphenol compounds with the reversed phase ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) was carried out. For the purpose of aqueous extracts screening and interesting bioactive metabolites identification, an in-house spectral library of more than 500 polyphenolic compounds occurring in plants was created. In total, 62 compounds were identified. The samples did not differ significantly in the type of bioactive compounds, differences were only in the occurence of some individual polyphenols depending on a geographical origin of respective sample. In the samples from the last vegetation state (senescence) was identified significantly less compounds and their profiles differed in comparison with other samples.

Keywords: *agrimonia eupatoria, bioactive compounds, polyphenols*

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C9

UTILIZATION OF HIGH-RESOLUTION MASS SPECTROMETRY TO INVESTIGATE THE METABOLOME OF CARROT TREATED BY PULSED ELECTRIC FIELD (PEF)

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The pulsed electric field (PEF) represents a mild non-thermal processing technology that utilizes short, high-intensive electrical pulses that lead to either temporary or permanent pores of cell membranes. This principle is applicable in food pasteurization; however, it presents potential in juice production, including increased juice extractability. With increased juice yield, nutrients and biological active compounds such as vitamins, minerals, or polyphenols can be extracted more effectively.
Vegetable juices have become popular in healthy diets, and therefore the aim of this study was to contribute to the knowledge of PEF on the extractability of bioactive compounds. For this purpose, carrot (*Daucus carota*) was used since this root vegetable is widely used in the production of juices either separately or in vegetable mixtures. The carrot was treated with different numbers of pulses (20, 60 and 100, per batch) in a constant electric field of 3 kV/cm and then juiced. Ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UPLC-HRMS/MS) and supercritical fluid chromatography coupled to high-resolution mass spectrometry (SFC-HRMS/MS) were employed to investigate the metabolomes of treated and untreated carrots. Data were processed by multivariate analysis and visualised by principal component analysis (PCA). Furthermore, the fate of carotenoids during PEF treatments was also investigated. The suitability of analytical methods for assessing carrot treatment by PEF will be discussed.

Keywords: *PEF, carrot, metabolome, UPLC-HRMS/MS, SFC-HRMS/MS*

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C10

OPTIMIZATION OF PROCESSING METHODS IN A POLYPHENOL-RICH SMOOTHIE TO ENSURE FOOD SAFETY AND NUTRITIONAL QUALITY

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Mediterranean dietary pattern presents a preventive effect against several chronic diseases related to oxidative stress, such as cardiovascular diseases, type II diabetes, cancer, etc. Among bioactive compounds present in Mediterranean products, polyphenols stand out for their multiple bioactivities such as antioxidant activity or anti-inflammatory, among others. Fruit and vegetable-based products are a convenient way to increase the fruit and non-starchy vegetables intake. However, these beverages have a very short shelf-life which makes them difficult to market and distribute from an industrial point of view. So, a processing method is required to extend the shelf-life of these products, reducing the enzymatic activity and spoilage microorganisms.The aim of this work was to optimize the preservation treatments in a fruit and vegetable based-smoothie rich in polyphenolic compounds to ensure food safety, sensory quality and polyphenolic content.
Apple, celery, chicory, mint and lemon juice were used to formulate the smoothie. This beverage was submitted to two different processing methods: high hydrostatic pressure (HHP) and ultra-high temperature (UHT) treatment. A response surface methodology (RSM) was used for the optimization of HHP processing. A Doehlert experimental design was performed with 2 factors (pressure and holding time). Pressure was studied from 300 to 600 MPa and holding time from 2 to 10 min. A total of 15 experimental runs were carried out. Desirability functions were used to optimize at the same time the different response factors dealing with microbiological and quality properties. UHT equipment was used to apply the thermal processing. Three different temperatures were assessed at a flow rate of 31 L/h: 78, 80 and 82 ºC, corresponding to F_{70}≈1.5-2.1 min, F_{70}≈3.1-4.6 min and F_{70}≈6.6-9.5 min, respectively. The responses measured to assess the effect of pressure/temperature and holding time on safety and quality were aerobic mesophiles, yeast and mould, pH, total soluble solids (°Brix), chromatic parameters (CIE scale), antioxidant activity (DPPH free radical scavenging assay) and total phenolic content (Folin-Ciocalteu Reagent method).
The optimal conditions proposed were 529 MPa and 10 min for the HHP processing using the desirability function. It was carried out to maximize the reduction in all microbial counts, Chroma chromatic parameter, antioxidant activity and total phenolic content and to minimize a*chromatic parameter. As regards UHT processing, all treatments achieved the required microbiological inactivation and maintained the quality. No significant differences were found between the different UHT pasteurization treatments with a 95% confidence level. In summary, both technologies showed to be suitable for the preservation of the smoothie product and led to a safe and stable product, maintaining the bioactive target compounds and the sensory profile.

Keywords: *polyphenols, high hydrostatic pressure, thermal treatment, biactive compounds, smoothie*

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C11

THE EFFECT OF VEGETAL OILS ADDITION ON THE BIOACCESSIBILITY OF CAROTENOIDS FROM CARROTS AND BABY SPINACH

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Introduction: Carotenoids can be defined as natural lipid-soluble pigments which can be found in many types of fruits, vegetables and algae, but also in animal organisms. Numerous studies have demonstrated that these compounds can exhibit pro-vitamin A activity, and antioxidant activity and their consumption can be associated with a lower incidence of some diseases. However, their beneficial effects are limited by low bioaccessibility and low bioavailability, especially from raw/unprocessed food sources.
Aim: This study aimed to determine the possible increase in carotenoid bioaccessibility from raw carrots and baby spinach leaves, by adding cold-pressed vegetal oils with different fatty acid profiles and unsaturation degrees.
Materials and methods: Fresh vegetable samples were subjected to a simulated *in vitro* gastrointestinal digestion, using the INFOGEST protocol. Samples, with and without vegetal oils addition, were subjected to a static *in vitro* digestion protocol consisting of three phases, i.e. oral, gastric and small intestinal phases. The digesta obtained after the intestinal phase, was centrifuged and the micellar phase was obtained by filtration. An aliquot of the micellar phase has been used for determination of carotenoids bioaccessibility. Carotenoids were extracted from fresh carrots, baby spinach, and from the corresponding digesta, using solvent mixtures, evaporated and stored at −80°C prior to chromatographic analysis. All samples were analyzed by C30-HPLC-PDA and carotenoid quantification was done using external calibration with commercial standards.
Results: The main carotenoids identified in samples and corresponding digesta were α-carotene for carrots, and β-carotene and lutein for carrots respectively, baby spinach. Three types of vegetable oils (olive, sunflower, flaxseed) were characterized by GC-MS and added to food samples, at two different concentrations (5% and 10%). Significant changes in the bioaccessibility of β- and α-carotene were observed after the addition of oils, but there were no major changes in the bioaccessibility of lutein.
Conclusions: The addition of vegetable oils increased significantly the bioaccessibility of nonpolar carotenoids (carotenes) but had a lower effect on bioaccessibility of the more polar xanthophylls (lutein). The effect of oil addition varied with the concentration of oil but was also dependent on the fatty acids profile of the vegetable oil.

Keywords: *lutein, β-carotene, α-carotene, bioaccessibility, fatty acid*

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C12

USE OF KIWIFRUIT EXTRACT IN THE PRODUCTION OF SICILIAN CANESTRATO CHEESE: NUTRITIONAL AND HEALTH ATTRIBUTES

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Actually, in the cheese production there is an increasing demand for alternative sources of milk coagulants to religious and dietary, or to the ban on genetically modified foods. Plant-derived coagulants are interest substitute of animal rennet in cheese production. Since actinidin, a protease derived from kiwi (*Actinidia deliciosa*) forms clots in milk under typical conditions of cheese production [1], the kiwi extract could be a valid alternative replacing animal rennet. The use of Kiwi extracts as a coagulant are reported in literature [2], but little information exists on the nutritional composition of obtained products. In this study, Sicilian Canestrato cheese samples produced using traditional calf rennet (C_T), Halal-certified commercial rennet (C_H) and vegetable rennet by kiwifruit extract (C_K) were evaluated. The contents of moisture, protein, fat, total polyphenols, tocopherols and sterols were determined in all samples for human consume. Moisture content (g/100g cheese) was significantly higher ($P < .0001$) in C_K (41.76±0.18) than in C_H and C_T cheeses (24.72±0.03 and 27.84±0.08, respectively). Conversely, protein and total fat (g/100g cheese) contents were significantly higher ($P < .0001$) in C_H (23.19±0.48 and 32.72±0.45, respectively) and C_T (24.04±0.11 and 31.86±0.44, respectively) than in C_K (20.17±0.09 and 27.86±0.54, respectively). The different protein and fat content is probably due to proteolysis and lipolysis processes in the cheeses with kiwi extract. C_K cheese contained cholesterol (mg/100g cheese) at 54.37±0.98, while C_T and C_H at 112.25±1.26 and 132.12±1.78, respectively. The lower value can be linked to the higher water content of C_K. Furthermore, C_K cheese contained also some phytosterols such as stigmasterol, campesterol and β-sitosterol. The last in C_K was the most abundant (0.47±0.15 mg/100g cheese). The polyphenols content (mg/Kg) in C_K (450.83±0.19) was significantly higher ($P < .0001$), than in C_H (163.70±0.53) and C_T (131.92±0.37). Polyphenols and phytosterols in C_K reflects the use of kiwi extract as a coagulant [1] α-tocopherol was the most abundant tocopherol in all samples. C_H and C_T cheeses had α-tocopherol (mg/Kg) at of 20.87±0.44 and 21.62±0.56, respectively, while C_K has significantly lower value (4.16±0.12; $P < .0001$). The loss of α-tocopherol in C_K is probably due to the cheese making. Principal components analysis (PCA) also highlights that C_K can be discriminated from others by higher values of moisture and polyphenols. The results suggest that the use of kiwi extract improved the nutritional characteristics of cheese. Finally, these preliminary results show the possibility of using kiwi extract as an excellent alternative to animal rennet in milk coagulation.

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Keywords: *kiwi extract, canestrato, cheese, polyphenols, phytosterols*

C13

CHEMICAL CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF DRUPES OF RHUS CORIARIA L. GENOTYPE FROM SICILY (ITALY)

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This study reports a full characterization of the Sicilian sumac, Rhus coriaria L. This fruit represents a potential source of fiber (33.21 ± 1.02%) and unsaturated fatty acids, being the contents of linoleic and α-linolenic acids, 30.82 ± 1.21% and 1.85 ± 0.07%, respectively. In addition, the content of phenolic and total anthocyanin was 71.69 ± 1.23 mg/g as gallic acid equivalents, and 6.71 ± 0.12 mg/g as cyanidin-3-O-glucoside equivalents, respectively. The high content in mineral elements, consisting mainly of potassium, calcium, magnesium, and phosphorus, followed by aluminum, iron, sodium, boron, and zinc, was detected by inductively coupled plasma mass spectrometry (ICP-MS). Moreover, its antimicrobial activity was evaluated against multidrug resistant (MDR) microorganisms, represented by Escherichia coli and Klebsiella pneumoniae strains isolated from poultry. The activity of seven different sumac fruit extracts obtained using the following solvents-ethanol (SE), methanol (SM), acetone (SA), ethanol and water (SEW), methanol and water (SMW), acetone and water (SAW), water (SW)-was evaluated. The polyphenol profile of SM extract, which showed better activity, was analyzed by ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The major component identified was gallic acid, followed by quercetin, methyl digallate, pentagalloyl-hexoside, and kaempferol 3-O-glucoside. The non-toxicity of Sicilian R. coriaria was confirmed by testing the effect of the same extract on zebrafish embryos.

Keywords: *Rhus coriaria, polyphenols, sumac, antibacterial activity*

C14

DEVELOPMENT AND VALIDATION OF HPLC-MS/MS METHOD FOR SIMULTANEOUS ANALYSIS OF B-VITAMINS IN FRUIT JUICES AND INVESTIGATING THE INFLUENCE OF AMMONIUM FLUORIDE AS AN ELUENT MODIFIER

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There are only a few studies in the scientific literature that explored the topic of multi-vitamin analytical methods using HPLC-MS/MS and most of them were limited to a few selected vitamins. In this study, an HPLC-MS/MS multi-vitamin analysis method with a run time of 20 minutes is developed for nine major water-soluble B-vitamins: Thiamine, Riboflavin, Nicotinic acid, Nicotinamide, Pantothenic acid, Pyridoxine, Biotin, Folic acid, Cyanocobalamin. An Agilent Poroshell EC-C18 (3.0 mm x 150 mm) reversed-phase column with ammonium formate buffers in methanol and water as mobile phases under gradient elution mode is used to accomplish chromatographic separation among the analytes. A simple dilute and shoot method is used for sample preparation. Considering the importance of a calibration study in method validation, it is conducted as a separate stage which includes linearity assessment (in-line and on-line linearity) and linear range determination using standard solutions of target analytes in the concentration range of 0.1 µg/L – 1000 µg/L. Possible matrix effects are also investigated with the standard-addition method (SAM) using strawberry and multi-vitamin juice as reference matrix material. An accuracy study with the use of accuracy profiles is conducted in validation standards (i.e. spiked samples of strawberry and multi-vitamin juice). The accuracy profiles, as a part of the accuracy study, are estimated from the uncertainty measurement obtained from the validation data according to the ISO/DTS 21748. The limits of quantification were determined to be in the range of 0.5 µg/L – 8 µg/L. The proportional bias due to the matrix effect for both the matrices under study were ranging between 70 % - 110 % for all the target analytes. This study is the first of its kind that investigated the effect of ammonium fluoride as an eluent modifier on the response signals of B-vitamins with HPLC-MS/MS. The use of 0.5 mM of ammonium fluoride led to a multi-fold increase in the signal intensity i.e., over a 5-fold increase for nicotinic acid, pantothenic acid, and cyanocobalamin, and a 4-fold increase for folic acid. The response signal for Thiamine is suppressed by the use of the use 0.5 mM of Ammonium fluoride. Using 1.5 mM of Ammonium fluoride did not significantly increase the response signal compared to when using 0.5 mM concentration and resulted in significant suppression of the response signal in the case of Folic Acid and Thiamine. This study concludes with a simple and quick multi-vitamin analysis method along with an outline for an accuracy study of an analytical assay from a contemporary and holistic perspective. The knowledge can be used to establish vitamin analysis protocol in the fruit juice industry and quality control laboratories.

Keywords: *accuracy profile, validation, simultaneous, vitamins, fruit juice*

C15

DEVELOPMENT AND VALIDATION OF HPLC-MS/MS METHOD FOR SIMULTANEOUS ANALYSIS OF ASCORBIC ACID AND DEHYDROASCORBIC ACID IN FRUIT JUICE AND INVESTIGATING THE INFLUENCE OF AMMONIUM FLUORIDE AS AN ELUENT MODIFIER

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Simultaneous analysis of Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA) with HPLC-MS/MS is described as an analytical challenge in literature due to the difficulty associated with the analysis of DHA with mass detectors. In this study, an HPLC-MS/MS method with a run time of 10 minutes is developed for AA and DHA. An Amaze HA bi-modal column (3.0 mm x 150 mm) designed to retain compounds by a combination of anion-exchange and hydrogen bonding mechanisms is used in method development. A mix of 20 mM ammonium acetate buffer and acetonitrile is used as a mobile phase under gradient elution mode to accomplish chromatographic separation among the analytes. A simple dilute and shoot method using 10 mM ammonium acetate buffer with 0.05 % EDTA is used for sample preparation. A goodness of fit test is conducted as part of the calibration study using standard solutions of analytes in the range of 30 – 1000 µg/L and 10 – 150 µg/L for AA and DHA respectively to assess the determined regression models. Potential matrix effects are also investigated with the standard-addition method (SAM) using strawberry, multi-vitamin juice, and mixed berries juice as reference matrix material. An accuracy study with the use of accuracy profiles is conducted in validation standards (i.e. spiked samples of strawberry, multi-vitamin, and mixed berries juice). The accuracy profiles are estimated from the uncertainty measurement obtained from the validation data according to the ISO/DTS 21748. The proportional bias due to the matrix effect for the matrices under study were ranging between 85 % - 105 % for the target analytes. This study is the first of its kind that investigated the effect of ammonium fluoride as an eluent modifier on the response signals of AA and DHA with HPLC-MS/MS. The use of 0.5 mM of ammonium fluoride led to a triple-fold increase in the signal intensity for AA. However, the use of ammonium fluoride in the eluents significantly suppressed the signal for DHA. Using 1.5 mM of Ammonium fluoride did not significantly increase the response signal for AA compared to when using 0.5 mM concentration. This study describes an easy and quick analysis method for AA and DHA along with an outline for an accuracy study of an analytical assay from a contemporary and holistic perspective. The knowledge can be used to establish HPLC-MS/MS method-based protocol for routine analysis in the fruit juice industry and quality control lab, consequently allowing them to avoid time-consuming AA and DHA analysis that typically involves reduction or derivatization reactions.

Keywords: *validation, ascorbic acid, dehydroascorbic acid, matrix effects, accuracy study*

C16

ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPOUNDS OF DIFFERENT COMMERCIAL NON-TRADITIONAL FLOURS

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In recent years, there has been a growing interest in the potential use of plant-based proteins in human nutrition. In this context, non-traditional flours, obtained from different raw products, have attracted the interest of researchers and the food industry (Mikulec et al., 2019). These types of products are suitable for use in vegan or vegetarian diets and for consumers who are aware of the need to reduce the consumption of animal products. In addition, these flours can be used to meet the need for gluten-free (GF) formulations (Gambus et al., 2009). Among them, there has been a growing interest in the use of gluten-free cereals, pulses and, more recently, hemp. A wide range of these products is now available in supermarkets or online, but their nutritional composition can be very different depending on the raw material and the production process itself. The aim of the present study was to evaluate the bioactivity of ten commercial flours from: rice, pea, chickpea, soya and hemp.
For this purpose, after extraction of the bioactive compounds in a 70% ethanol aqueous solution in an ultrasonic bath for 8 minutes and centrifugation, the following parameters were measured Total phenolic compounds (TPC) according to the Singleton method, Total flavonoid content (TFC) according to the method of Valencia et al, (2012), Total flavanones and dihydroflavonols according to the method of Popova et al., (2004) and Total equivalent antioxidant capacity (TEAC) using the ABTS method described by Chen et al., (2003). Significant differences were determined by ANOVA and LSD-Fisher test.
All the studied parameters TPC, TFC, flavanone and dihydroflavonol content and TEAC varied significantly in the different samples studied, depending on both the plant species and the commercial brand. Thus, TPC ranged from 6.33 in rice flour to 139.31 mg GAE/100 g in soybean flour; TFC ranged from 1.47 for rice to 66.65 mg rutin/100 g for hemp, flavanones and dihydroflavonols showed values from 328.31 for rice to 638.45 mg pinocembrin/100 g for pea, while TEAC ranged from 1.08 for pea to 8.69 nmol Trolox/100 g for soybean meal. Then, soybean flour showed the highest TPC and TEAC values, followed by hemp flour, which showed the highest values for TFC and flavanones and dihydroflavonols. Pea flour showed similar values to hemp for this group of phenolic compounds, but the lowest TEAC values. On the other hand, rice showed the lowest values for all bioactive compounds. Strong inter-brand variations were observed in TPC and TFC for chickpea, pea and soybean and in flavanones and dihydroflavonols for pea and soybean, while TEAC did not show strong inter-brand variations.

Keywords: *bioactive compounds, antioxidant activity, gluten free flour, commercial brand*

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C17

BIOACCESSIBILITY OF APPLE POLYPHENOLS DURING IN VITRO AND EX VIVO ORAL DIGESTION

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Polyphenols (PP) are a structurally diverse group of water soluble secondary plant metabolites. For different structures a wide range of various health benefits are postulated, e.g. positive effects against different cancers and diabetes mellitus type II.[1] To exhibit these bioactivities, the PP must first become bioavailable. Bioavailability strongly depends on the bioaccessibility, which is defined as the fraction of a substance that is released from the matrix and available for absorption during digestion.
Apples are an important source of PP, vitamins and fibers in the western diet due to being on offer throughout the year.[2] However, around 70% of patients suffering from a birch pollenosis develop a cross allergy against fresh apples due to the structural similarity between apple allergen Mal d 1 and Bet v 1 in birch.[3] The allergenicity differs among varieties and, in particular, commercial breeds, with a general low total phenolic content (TPC), are described as highly allergenic. Therefore, a correlation between the TPC and the allergenicity is proposed.[4] Since Mal d 1 is proteolytically labile and symptoms of this allergy are limited to the mouth and throat area, the bioaccessibility during oral digestion must be taken under consideration when evaluating this hypothesis. So far, the knowledge about the bioaccessibility of apple phenolics during oral digestion is limited. Therefore, we studied the release of PP from lyophilized samples for 19 traditional and commercial apple varieties under *in vitro* and *ex vivo* digestion conditions. Simulation of the oral digestion phase was performed according to Minekus et al. with simulated saliva fluid (SSF)[5] and centrifuged and non-centrifuged saliva of two probands.
The oral bioaccessibility differed between the varieties. A release of 68 ± 16% and 48 ± 7% of the TPC was determined in flesh and peel, respectively. The different phenolic classes present in apples varied in their release behavior. For example, hydroxycinnamic acid derivatives were significantly better bioaccessible than flavonol glycosides and flavanols. For procyanidins a decreased release with increasing molecular weight was observed. No significant differences between SSF and centrifuged and non-centrifuged saliva were obvious. Therefore, our data support the substitution of human saliva in bioaccessibility studies with SSF, removing possible ethical and hygienically concerns during experiment set up. Furthermore, our data does not indicate any correlation between the TPC, bioavailable polyphenols and the Mal d 1 content with the reported allergenicity.

(1) Boyer & Liu *Nutr J* 2004;3:5.
(2) Wolfe et al. *J Agric Food Chem*. 2003;51(3):609.
(3) Gao et al. *BMC Plant Biol* 2008;8:116.
(4) Kschonsek et al. *Nutrition* 2019;58:30.
(5) Minekus et al. *Food Funkt*. 2014;5(6):1113.

Keywords: *phenolics, release, apple allergy, malus domestica Borkh.*

Acknowledgement: *Sven Richter is acknoledged for his help freeze drying the samples.*

C18

LIGNANS IN WINE

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Among compounds beneficial for human health, wine contains certain amounts of lignans, namely lyoniresinol, isolariciresinol, secoisolariciresinol, and to a lesser extent matairesinol, lariciresinol, and syringaresinol. Lignan concentrations can vary depending on the particular wine type from negligible concentration to more than 3 mg/L. Generally, the source of lignans in wine may be wood used in oenology in the form of chips or wooden barrels or the grape bunch itself. Our results based on LC-MS analyses show that lyoniresinol originates from oak wood, isolariciresinol can be released into wine from all parts of the grape bunch, secoisolariciresinol comes from the grape seeds and possibly also from the berry skins, low amounts of matairesinol may be released into wine from the grape skins, and syringaresinol comes from the wooden parts of both the grape bunch and the barrel. Therefore, the lignan content in wine can increase with maturation in contact with grape berries, seeds, or stems or with wood. Furthermore, the content of syringaresinol can be affected by the type of wooden barrel used, the content of matairesinol is affected by the grapevine cultivar, and the amounts of isolariciresinol and secoisolariciresinol are affected by both the cultivar and the year of growing.

Acknowledgement: *This paper was supported by the project "Study of polyphenolics compounds in wines and parts vines" IGA-ZF/2021-SI2009, by the project CZ.02.1.01/0.0/0.0/l6_0l7/0002334 Research Infrastructure for Young Scientists, co-financed by Operational Programme Research, Development and Education, and by the Grant Agency of Masaryk University: Support for biochemical research in 2021, grant number MUNI/A/1604/2020.*

C19

EFFECT OF OLIVE BY-PRODUCT INCLUSION IN THE DIET OF BULLS ON BIOACTIVE COMPOUNDS CONTENT IN MEAT

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Bioactive compounds also called nutraceuticals are substances present in small amounts in certain foods and have the ability to provide health benefits. The interest on formulating diets of ruminant with feeds source of high-value bioactive compounds has increased over the last years, but it would be necessary to evaluate if these expected animal health benefits are also highlighted in animal products such as meat. Olive Pomace (OP) is a by-product derived from the olive oil industry and it is rich in several high-value bioactive compounds, in particular polyphenols. The present study was carried out to assess whether food supplementation with olive pomace in bulls would increase the bioactive compound contents in their meat. A total of 45 Limousine young growing fattening bulls born in the same season, weaned and reared under the same management and feeding systems were chosen. After a 2-week adaptation period, the animals were randomly divided into three homogeneous groups for numbers and according to body weight (350 ± 15 kg): CTR (Control: 0%), LOP (Low OP: 10%), and HOP (High OP: 15%). At the final weight average of 578 ± 65, animals were slaughtered and 7 days later, individual samples of *Longissimus dorsi* muscle (between the 7° and 8° thoracic vertebrae included) were collected, vacuum packed and stored at -20 °C till the following analysis. For each group of animals, a pool of 5 g of meat (obtained from 3 bulls/group) was homogenized in methanol. The homogenates were then wrapped in laboratory filter paper (Qual Pia D.150 mm) and extracted with Soxhlet for 5 hours to fall, using 150 ml of pure methanol. The obtained solutions were then evaporated with a Rotavapor (Buchi B-490). The extracts of meat samples have been characterized for their profile in phenolic compounds (hydroxytyrosol, tyrosol, oleuropein, ligstroside, pinoresinol, carnosol, myricetin, luteolin and apigenin) through HPLC-DAD, for *in vitro* antioxidant properties ($ABTS^+$ and DPPH assays), and for total phenol content and reducing activity (Folin-Ciocalteau assay).
Among the different phenolic compounds investigated, only hydroxytyrosol and tyrosol have been found in the extracts. However, significant differences ($p<0.05$) among groups were obtained. Indeed, both hydroxytyrosol and tyrosol increased linearly by increasing the levels of OP inclusion in the diet and HOP (2.20 ± 0.004^c and 1.46 ± 0.024^c mg/100gr meat, respectively) showed greater levels than LOP (1.31 ± 0.013^b and 0.97 ± 0.064^b mg/100gr meat, respectively), in turn greater then CTR (0.8 ± 0.001^a and 0.05 ± 0.003^a mg/100gr meat, respectively). Accordingly, different antioxidant activities among the three different extracts were observed. These results seem to confirm that an olive pomace supplementation to bulls' diets is capable of improving the antioxidant properties and meat phenol content in a dose dependent manner.

Keywords: *olive pomace, bioactive compounds, meat, polyphenols*

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C20

THE EFFECT OF THE PROCESSING OF SPECIALTY COFFEE BEANS ON BIOACTIVE AND VOLATILE SUBSTANCES

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Coffee is one of the most frequently consumed beverages in the world, and its consumption is increasing every year. Coffee beans contain a mixture of chemical (bioactive) and volatile compounds that play important roles in the final beverage and can be influenced by the beans' processing method. Processing is one of the most important processes leading to the elimination of damaged beans and the increase in bean quality, including the adjustment of flavour and aroma. Basic processing methods have recently been supplemented by new innovative processing methods, e.g. anaerobic fermentation and carbonic maceration. Anaerobic fermentation and carbonic maceration rapidly change the coffee fruit, resulting in a flavour much different than traditional fermentation methods.The aim of this study was to determine the effect of various methods of processing, such as natural, washed, honey, anaerobic fermentation, and carbonic maceration, on the antioxidant activity and bioactive and volatile compounds in green and roasted specialty coffees from various countries of origin. High-performance liquid chromatography analysis of bioactive compounds and gas chromatography-mass spectrometry analysis of volatiles after solid-phase microextraction was performed. Total flavonoid contents in roasted Peruvian coffee beans were significantly higher ($P < 0.001$) for natural processing than carbonic maceration, and antioxidant capacity was significantly higher ($P < 0.001$) for carbonic maceration than natural processing. Burundian coffees had the highest antioxidant capacity, with the highest values for naturally processed beans compared to beans processed by anaerobic fermentation. 2-Furanmethanol, a volatile compound, was a major compound in all roasted coffees. The presence of volatile 2-methylbutanoic acid that produces a desirable fruity, acidic, and fermented aroma of specialty coffees was found in Burundian and Ethiopian coffees processed by anaerobic fermentation and Nicaraguan coffee processed washed methods. Volatile 2-methylbutanoic acid was found in the roasted coffees with a relative proportion from 0.45 to 1.04%.In conclusion, the type of processing affected the content of bioactive and volatile compounds in specialty coffees. The beans processed by anaerobic fermentation were able to maintain bioactive compounds at high levels and contributed to the antioxidant activity of the roasted coffee.

Keywords: *coffee procession, antioxidant activity, arabica coffee, anaerobic fermentation, volatile compounds*

Acknowledgement: *This study was supported by funds from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0073/22) and from the METROFOOD-CZ research infrastructure project (MEYS Grant No.: LM2018100).*

C21

REDUCING BLOOD PRESSURE BY ANTI-HYPERTENSIVE NATURAL COMPOUNDS: NEW SOURCES FOR THE ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITOR PEPTIDE LKPNM

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Background: According to the World Health Organization (WHO), cardiovascular diseases are the number one cause of all deaths in Europe. One of the major risk factors is hypertension, also known as elevated blood pressure. Hypertension is a chronic medical condition in which the arterial blood pressure is above 140 mmHg (systolic) or 90 mmHg (diastolic). Angiotensin converting enzyme (ACE) plays an important role in regulating blood pressure by converting the inactive angiotensin I into the potent vasoconstrictor angiotensin II. Angiotensin II has an impact on the excretion of antidiuretic hormones (ADH) and aldosterone as well as on the tubular reabsorption of Na^+ / Cl^- and the K^+ reabsorption. Furthermore, Angiotensin II features an important role in the physiological regulation of the blood pressure. Since ACE inactivates the catalytic function of the vasodilator bradykinin, it plays an important role in the regulation of blood pressure especially in hypertension ACE inhibitors (so-called beta blockers) traditionally used to treat hypertension are often associated with serious side effects. This has generated a greater focus on naturally occurring ACE inhibitors derived from different food sources as a possible alternative to the traditional treatments.
Aims: Well known and already marketed as dietary supplement, is the natural ACE-inhibitory peptide LKPNM. This peptide is currently sourced from "Bonito" *(Katsuwonus pelamis*). Therefor, the aim of this study was to discover new natural sources which show inhibitory effectiveness and might be a better and sustainable source for the ACE-inhibitory peptide LKPNM.
Material and Methods: The Angiotensin-converting enzyme inhibition assay was performed by HPLC-DAD, measuring the end product hippuric acid after an enzymatic reaction between ACE and the substrate hippuryl-histidyl-leucine (HHL) [1]. Synthetic LKPNM was used as internal and external standard for identification and quantification. Species such as different algae, jellyfish, mealworms, yellow pea and buckwheat were investigated for their ACE-inhibitory effectiveness. Furthermore, LC/MS and LC-MS/MS were used to detect, identify and quantify the LKPNM in muscle tissues and deposits of different fish species such as:
Atlantic salmon (*Salmo salar),* cod *(Gadus morhua),* turbot *(Scophthalmus maximus),* whiting *(Merlangius merlangus*), coalfish *(Pollachius virens),* Atlantic redfish *(Sebastes norvegicus*) and herring (*Clupea harengus).*
Results: All species investigated showed ACE-inhibitory effectiveness and muscle (tissues) and deposits from all fish species investigated contained LKPNM and showed ACE-inhibitory activity after thermolysin digestion. The highest LKPNM level was found in fillets and deposits of Atlantic salmon.

[1] Cushman DW, Cheung HS. Spectrophotometric assay and properties of angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971; 20, 1637–48.

Keywords: *antihypertensive peptides, angiotensin-I-converting enzyme, LKPNM, LC-MS/MS, dietary supplements*

C22

TARGET SCREENING OF BIOACTIVE COMPOUNDS OF DIFFERENT IRIS SPECIES CULTIVATED IN AEROPONIC AND HYDROPONIC SYSTEMS USING UHPLC-HRMS/MS

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Iris belongs to the Iridaceae family and currently, over 300 recognized species distributed across the Northern hemisphere have been described. The *Iris* plants have a long history of use in traditional medicine and modern research reveals many bioactive properties of the extracts, including antibacterial, anti-inflammatory, antioxidant, anticarcinogenic, and more. Responsible for these activities are the secondary metabolites, represented mainly by isoprenoid and phenolic compounds. The study of the phytochemical composition of the *Iris* plants is vital to understand the causes and means of their action and propose optimal applications in medicine, pharmacology, and the food industry. The current research of the *Iris* generally focuses on analysis of a small number of compounds or one chemical class. Moreover, the impact of growing conditions has not been investigated. In most studies, liquid chromatography employing fairly non-specific UV detection was employed. To get a deeper insight into bioactive secondary metabolites profile of *Iris* species grown in aeroponic and hydroponic systems, target screening strategy using ultraperformance liquid chromatopraphy coupled to high resolution tandem mass spectrometry (UHPLC-HRMS/MS) was optimized. Spectral database of secondary metabolites of the *Iris* containing 295 compounds was created for the screening of extracts of different *Iris* species and varieties. Variation in the metabolic profile was observed between different species but showed to be insignificant between varieties of the same species. Considerable difference in the phytochemical profile between the leaves and roots/rhizomes was observed, especially in terms of the content of xanthones, specific for leaves, and terpenoids, characteristic for roots/rhizomes. A substantial effect of the cultivation mode (aeroponics/hydroponics) with regard to the number and type of detected metabolites was noted. The findings are useful in terms of better understanding the metabolic pathways in different *Iris* species and provide basis for the efficient optimization of growing conditions and use of *Iris* plants in various industries.

Keywords: *LC-MS, iris, bioactive compounds, metabolic profiling, secondary metabolites*

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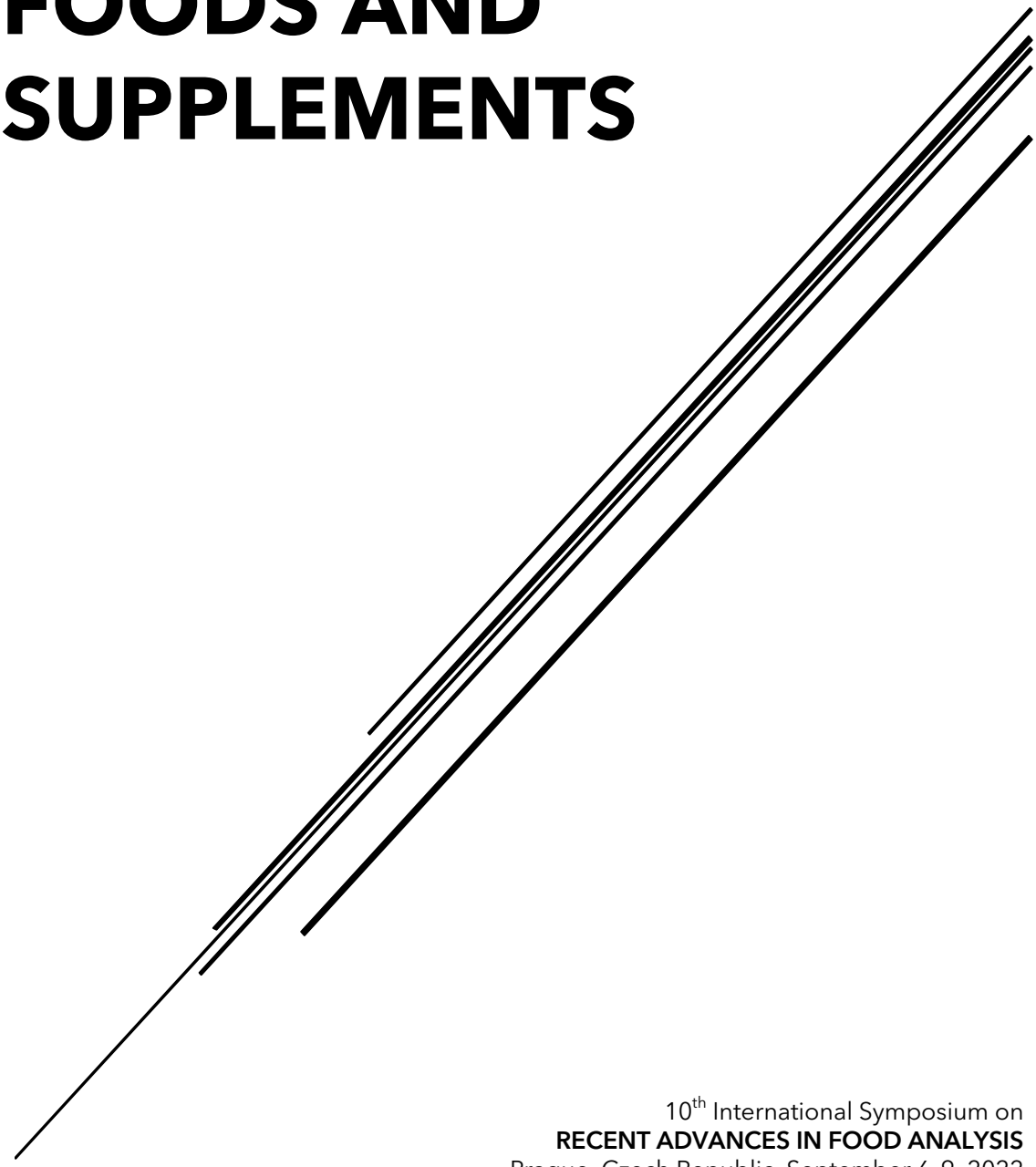
CANNABINOIDS IN FOODS AND SUPPLEMENTS



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D1

ICP-MS ANALYSIS OF CANNABIS SATIVA CONTAINING FOOD PRODUCTS USING A CRM HEAVY METAL MIX (AS, CD, HG AND PB)

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Cannabis (marijuana) and (industrial) hemp are known to accumulate heavy metals and have been used for the remediation of contaminated soil. Due to potentially hazardous effects of these metals, this property can hinder the use of hemp in food or medical industries. As a consequence, food or pharma products containing hemp must be tested for their heavy metal content.
This report describes the application of a premixed blend of arsenic, cadmium, mercury and lead (the "big four") plus eight individual heavy metal CRMs in the inductively coupled plasma mass spectrometry (ICP-MS) analysis of various hemp containing food samples.

Keywords: *cannabis, heavy metals, ICP-MS, cannabis foods*

D2

CANNABINOIDS ANALYSIS OF HEMP DERIVED PRODUCTS- DEVELOPING METHODS THAT ARE ROBUST AND DEPENDABLE

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Legalization and use of hemp, recreational and medical cannabis is expanding globally. Cannabidiol (CBD) and Tetrahydrocannabinol (THC) containing cannabis products are consumed in various forms such as flower to vape pens, edibles, concentrates, tinctures, beverages, topicals, capsules, etc. These hemp, CBD and cannabis products need to be tested to ensure accurate label of contents and consumer safety. A research study found that only 17% of edible products were accurately labeled when 75 different cannabis infused edible products were tested.[1] Due to the complexity of cannabis product matrices, sample preparation for cannabinoid testing is very challenging. Accurate extraction and analysis procedures are required to ensure proper regulation of these products. In this study, we explored simple and accurate sample preparation methods for analysis of cannabinoids from several matrices.

Cannabinoids from hemp bud, hemp oil, chocolate, hard candy, gummy, cream, and beverage matrices were extracted with methods such as liquid extraction, QuEChERS,[2] etc. Extraction efficiency and repeatability with different solvents and extraction methods were studied using HPLC/DAD, and HPLC/MS analysis methods. HPLC methods capable of separating more than 18 major cannabinoids with good resolution within ten minutes were developed. Different varieties of each matrix type were also tested to evaluate matrix effects on extraction efficiency. Multiple injections of filtered and unfiltered extracts were tested to assess column robustness. Fused-Core® particle C18, C8, and monolithic columns demonstrated accurate, precise and robust quantitation of cannabinoids. The challenges of sample preparation and the methods to overcome them will be discussed during the presentation.

Keywords: *certified reference materials, cannabinoids, cannabis, hemp, HPLC*

D3

FAST ANALYSIS OF PHYTOCANNABINOIDS IN PLANT MATRICES AND PRODUCTS THEREOF BY SFC-HRMS/MS

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Due to their high nutritional value, the interest in hemp products such as seeds and cold pressed oil is rapidly growing. Although hemp seeds themselves do not naturally contain phytocannabinoids including psychotropic delta-9-tetrahydrocannabinol (Δ9-THC) or its precursor delta-9-tetrahydrocannabinolic acid (Δ9-THCA), contamination may occur during harvest and/or processing. To prevent consumers´ health risks, the presence of this phytocannabinoid in hemp-based foods should be monitored. Until recently, no EU regulation (maximum limits) was available, nevertheless, at present, the determined concentration of total THC (sum of free Δ9-THC and that bound in Δ9-THCA) can be assessed against ´new´ EU limits: specifically, 7.5 mg/kg set for hemp oil and 3.0 mg/kg for dry foods (flour, proteins, seeds, snacks...). In response to the urgently needed effective control tool, ´new´ rapid, specific, and sensitive employing supercritical fluid chromatography coupled to high resolution mass spectrometry (SFC-HRMS) was developed and validated for altogether 19 phytocannabinoids. ACQUITY UPC2TM Torus 2-PIC (3.0 x 100 mm, 1.7 µm) was used as column and Isopropanol:acetonitrile (8:2; v/v) with 5% de-ionized water (v/v) and 5 mM ammonium formate and 0.1% formic acid (v/v) as modifier of mobile phase. The recoveries ranged from 71 % to 105 %. The repeatability, expressed as relative standard deviation (RSD), ranged from 5 % to 15 %. The linear range of the analytical method for individual phytocannabinoids was from 0,05-1,0 mg/kg (seeds) and 0.25-5.0 mg/kg (oil). The limit of quantification (LOQ) ranged from 0.05-0.25 mg/kg (seeds) and 0.25-1.25 mg/kg (oil). Furthermore, the method was used for the analysis of 10 hemp seed samples and 10 samples of hemp oil collected at the market. The total phytocannabinoid content of the seeds ranged from 4 - 49 mg/kg and 2 seed samples exceeded the legal limit for 'total' THC by 30 and 250 %. For hemp oils, the phytocannabinoid content ranged from 13 - 1400 mg/kg, and 3 samples exceeded the limit for 'total' THC by 60, 610, and 620 %. In one oil sample, all analytes determine were below the LOQ.

Keywords: *supercritical fluid chromatography, cannabinoids, hemp oil and seed, mass spectrometry*

Acknowledgement: *This work was supported by the grant of Specific university research – A1_FPBT_2022_005 and also supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

D4

ACCURATE MASS LIBRARY FOR NATURAL PRODUCTS BASED ON COMPOUNDS IDENTIFIED IN HEMP OIL

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Introduction: The use of hemp and CBD oils has become increasingly popular in many parts of the world as a direct use product and incorporating into another product. One of the important directions of the chemical analysis of these goods includes a broad overview of the chemical composition for different strains of CBD products and hemp to identify compounds with bioactive properties. Concentrated hemp oils typically produce between 350 - 560 chromatographic peaks under a simple 1D configuration. Here we describe the development of the Retention Index based accurate mass library for these types of samples with the idea that it would reduce the overall data analysis and allow for a focus on the unique components of the individual sample.
Methods: Five different hemp oil samples were analyzed using a high-resolution GC/Q-TOF in either 1D or comprehensive GCxGC configuration using the ZOEX ZX2 thermal modulator. A 5% phenyl, 30m column was used for the 1D data while the GCxGC configuration was a 5% phenyl 30m column coupled to a 2.8m DB-HeavyWAX. The data were acquired at 70eV. The retention indices were calculated based on the alkane ladder to assist compound identification and library curation. The GC/Q-TOF data were processed using the Unknown Analysis tool of MassHunter Quantitative Analysis Software, MassHunter Qualitative Analysis Software and GC Image.
Preliminary data (results): The goal of the present study is to create a comprehensive accurate mass PCDL (Personal Compound Database and Library) based on hemp oil samples for higher confidence quick screening in 1D GC configuration. In order to achieve adequate chromatographic separation of these complex samples, the data were collected using GCxGC configuration. The GCxGC data were visualized using GC Image software and compounds were identified using NIST17 and NIST20 libraries. Compound spectra identifications were supplemented using accurate mass, accurate isotopes, as well as retention indices (RI). The spectra of the identified compounds were annotated with fragment formulas using MassHunter Qualitative Analysis Software and exported to the PCDL after curation and automatic conversion of the measured m/z values to the theoretical values from the elemental compositions of the individual ions. Whenever a precise identification of an isomer was impossible, a compound would be assigned an indexed molecular formula instead of a name. The current PCDL contains approximately 350 compounds. The PCDL has been validated using the cannabis extracts and hemp oils data acquired using 1D GC and compound metadata has been crosschecked for accuracy. Despite the complexity of the samples, the majority of the PCDL hits generated the library match scores of over 80.

Keywords: *QTOF, hemp oil, GCxGC, natural products*

D5

CANNABINOIDS IN FOOD AND NOVEL FOOD: BEYOND THC AND CBD

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The glandular hairs of Cannabis sativa produce a resin consisting of 80-90% cannabinoids. More than 100 cannabinoids are known, some of them are known to be psychoactive, most notably Δ9-tetrahydrocannabidiol (THC). Analytics of cannabinoids have gained importance as more and more hemp containing products are being introduced into the market. In the early 2000s, the determination of "total THC" (= sum of THC and THC-acid) after gas chromatographic separation was common. At that time the most common published limits were reference values for "total THC" published by the former German Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV) in 2000. Food produced from hemp seeds was the only analyzed matrix and there was neither a market need for the differentiation between the contents of THC and its precursor THC-acid nor for the analysis of other cannabinoids like cannabidiol (CBD).
Due to the publication of a scientific opinion by the European Food Safety Authority (EFSA) about Delta-9-THC including an ARfD-value in 2015, there was a first call for methods which were able to differentiate between single compounds. Also in 2015, the European Commission published a recommendation for a monitoring program on several cannabinoids. Because of the wide variety of methods and instrumentation applied for the investigation of cannabinoids, there is a great need for harmonization and standardization. Here we present a sensitive and robust LC-MS/MS method which allows specific quantification of several cannabinoids. Depending on the matrix (e.g. hemp seeds, hemp seed oils, CBD oils, soft drinks, alcoholic beverages) we established and validated different sample preparation protocols. Because the needs are different depending on the analyzed matrix we offer different tailored analyte packages: The basis package consists of THC, the only analyte in the EU which is regulated by toxicological limits, THC-acid as its precursor and potential THC, CBD and Cannabinol (CBN). To improve robustness and accuracy we use isotope-labeled internal standards.
Especially in the case of CBD products we strongly recommend the additional analysis of CBD-acid. Here we also apply the labeled substance as internal standard. As in fresh plants the major parts (not rarely up to 90%) of many cannabinoids are present as their precursors carboxylic acids the CBD-acid content must not be neglected when analyzing CBD, just like THC-acid must not be neglected when analyzing the THC content. The establishment of maximum levels which will include the content of THC-acid is also planned. The application of elevated temperatures, for example during storage, processing or smoking leads to a decarboxylation of these carboxylic acids and can alter the final contents of THC and CBD. Finally, we are able to quantify 9 analytes in total which contain the analytes mentioned above and in addition cannabidivarin, tetrahydrocannabivarin, cannabigerol and delta-8-THC.

Keywords: *cannabinoids, hemp, cannabidiol, THC, novel food*

D6

LOW LEVEL LC-MS/MS DETERMINATION OF CANNABINOIDS IN HEMP SEEDS, HEMP SEED OIL, AND HEMP EXTRACTION BY-PRODUCTS

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In the last few years, the popularity of hemp and hemp-based products has significantly increased. Commercial consumer products with cannabidiol (CBD) and other cannabinoids, such as dietary supplements, cosmetics or vape liquids have become subject of high demand. Additionally, hemp plant, hemp seeds, seed oil and their processing/extraction by-products represent valuable assets to livestock and poultry industries with a high potential for use as feedstuff. Considering the above, reliable and sensitive methods are required for analysis of hallucinogenic and other cannabinoids in these matrices. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed and validated for quantification of fifteen cannabinoids including Δ^9- and Δ^8-tetrahydrocannabinol (THC), tetrahydrocannabinolic acid (THCA), tetrahydrocannabivarin (THCV), and cannabinol (CBN) in hemp seeds, oil, meal and protein matrices. Sample preparation and chromatographic separation were based on AOAC Official Method of Analysis AOAC 2018.11. The method was thoroughly validated to demonstrate adequate performance at a target limit of quantification (LOQ) of 1 mg/kg. Acceptable accuracy and precision with recoveries within 70-120% and RSDs ≤20% was achieved. Additionally, the applicability of the method to real samples was demonstrated through analyses of hemp meal and seed hull samples. The presented method was shown to be suitable for routine quantification of relevant cannabinoids at low levels in wide range of matrices.

Keywords: *cannabinoids, LC-MS/MS, hemp oil, hemp seed*

D7

CHARACTERIZATION OF CANNABIS METABOLOME USING A GAS CHROMATOGRAPHY HIGH-RESOLUTION MASS SPECTROMETRY (GC–HRMS) TECHNIQUE

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Cannabis (*Cannabis sativa* L.*)* is a complex polymorphic plant species that has recently seen a tremendous resurgence of interest because of its application mainly in the pharmaceutical industry and medicine. In terms of chemical composition, this plant is a complex mixture of thousands of different substances; which together are responsible for unique biological effects on the human body. Therefore, it is necessary to study cannabis not only by means of target analysis to determine dominant phytocannabinoids but also to characterize the entire set of metabolites. The purpose of this study was to develop and optimize a method for the characterization of the cannabis metabolome using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). The method development included testing different organic solvents and reagents for derivatization. Different derivatizing agents were tested with the aim to increase the volatilization/detectability of more polar substances with a higher boiling point and low thermostability and thus enable a more comprehensive evaluation of the entire metabolome. Finally, the most suitable combination (ethyl acetate – N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA)) was chosen. The newly developed approach was applied to the real set of cannabis samples (n=19) of different varieties. The obtained data (metabolic fingerprints) was subjected to a chemometric analysis PCA followed by PLS-DA, which enabled the samples classification according to the varieties. Characteristic metabolites ("markers") for individual cannabis varieties were selected and subsequently identified.

Keywords: *Cannabis, GC-HRMS, metabolomics*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities and a grant for specific university research – A1_ FPBT_2022_005.*

D8

TRANSFER OF CANNABINOIDS FROM HEMP TEAS INTO THEIR INFUSIONS

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In recent years, the range of hemp-based foods on the market has grown and some adverse health effects are being discussed for the cannabinoids they contain. Maximum levels for the psychoactive tetrahydrocannabinol (THC) in hemp seeds and products derived therefrom are in preparation by the European Commission. The leaves and flowers of the hemp plant can have higher cannabinoid content, but this has not yet been regulated. Considering the high interest of consumers on hemp-based food e.g. hemp tea, usually declared as consisting of hemp leaves and/or hemp flowers, a broad analytical spectrum must cover the cannabinoids contained in hemp tea. Therefore, our study focused the investigation of cannabinoid profiles and contents of different hemp tea samples and on the transfer of cannabinoids from hemp teas into their infusions.
In this work, 23 hemp tea samples were analyzed for their cannabinoid composition and content using liquid chromatography coupled to tandem mass spectrometry to distinguish cannabinoid acids, e.g. tetrahydrocannabinolic acid (THCA), from their corresponding neutral cannabinoids, e.g. the psychoactive THC. The analytical scope of the method includes a total of 16 cannabinoids namely cannabichromene (CBC), cannabichromenic acid (CBCA), cannabidiol (CBD), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabidivarinic acid (CBDVA), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabicyclol (CBL), cannabicyclolic acid (CBLA), cannabinol (CBN), cannabinolic acid (CBNA), THC, THCA, tetrahydrocannabivarin (THCV), tetrahydrocannabivarinic acid (THCVA).
The total content of these 16 cannabinoids in hemp tea (n = 23) averaged 14,956 mg/kg. The average THC content was 221 mg/kg and the THCA/THC ratio ranged from 0.1 to 2.6 (average of 0.9). Despite differences in cannabinoid content, all hemp teas had similar cannabinoid profiles, with CBDA (62 %) and CBD (24 %) accounting for the highest proportion, and THC and THCA each accounting for 1 % of total cannabinoid content. The averaged total cannabinoid concentration in the tea infusion was 32,704 µg/L. The THC concentration ranged from 1 to 64 µg/L and the averaged THCA/THC ratio was 8 due to the higher transfer rate of THCA into the tea infusion.
Generally, a tenfold higher transfer rate of cannabinoid acids compared to their corresponding neutral cannabinoid was observed. The transfer rates of cannabinoid acids ranged from 6 % (CBLA and THCA) to 70 % (CBDA) and for the corresponding neutral cannabinoids from 0.3 % for CBG to 4 % for CBDV. During tea infusion at 100 °C, no conversion of THCA to THC was observed. The transfer rate of the psychoactive THC was 0.5 %.

Keywords: *cannabinoids, THC, hemp tea, transfer*

D9

DEVELOPMENT AND VALIDATION OF A FAST LC-MS/MS-METHOD FOR THE DETERMINATION AND QUANTIFICATION OF 11 CANNABINOIDS IN FOODSTUFF

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Cannabinoids are C_{21}-terpeno-phenolic substances and occur naturally in the cannabis plant (*Cannabis sativa*). So far, there are more than 100 different substances known. The best-known substances are Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). These substances, especially CBD are very trendy since a few years and widespread. They are added to many products like oils, cosmetics, confectionery as well as beverages. The producers declare their products with different concentrations of cannabinoids. It should be noted that Δ9-THC, due to its psychoactive effect, is subject to the Austrian Narcotic Drugs Act and a limit of 0.3% Δ9-THC must not be exceeded in foodstuff.
The method presented here is an LC-MS/MS method, for the determination of Δ9-THC, Δ8-THC, CBD, Δ9-THCA (Δ9-tetrahydrocannabinolic acid), CBDA (cannabidiolic acid), Δ9-THCV (Δ9-tetrahydrocannabivarin), CBG (cannabigerol), CBGA (cannabigerolic acid), CBC (cannabichromene), CBDV (cannabidivarin), CBN (cannabinol). The minimum detection limit for all cannabinoids is 1 µg/L in beverages with a high precision and selectivity.
To get a first overview about products on the Austrian market, 10 cannabinoid-containing oils, which were advertised with a content of 5 % CBD, were analysed.A content of 5% CBD could be confirmed in all samples examined; in addition, Δ9-THC (1.20 - 36.7 mg/kg) and Δ9-THCA (Δ9-tetrahydrocannabinolic acid) (0.529 - 6.28 mg/kg) could be detected in 6 samples, although these samples were declared as „THC-free".

Keywords: *cannabinoids, LC-MS/MS, tetrahydrocannabinol, cannabidiol*

D10

THE DETERMINATION OF CANNABINOIDS CONTENT WITHIN GUMMY BASED CONFECTIONARY

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The growth of the Cannabinoid industry has led to a wide variety of nutraceutical products being commercially available. The leading products within this range are confectionary in nature, typically gummy based sweets. This work looks at the extraction method from the confectionary utilizing dedicated cannabinoid method for the analytical analysis.
The extraction method is tested using standard spiking addition techniques using non cannabinoid containing gummy confectionary, as well as using commercially available CBD gummy products. This combination of testing procedures ensured a robust and accurate extraction method was developed for a variety of gummy based confectionary products. The standard spiking tested out the methods precision, accuracy across differing spiking levels and specificity. Investigations were also carried out to ensure the method is suitable for gummy based confectionary that is suitable for vegans, which does not contain gelatin.

Keywords: *cannabinoids, HPLC, gummy based confectionary*

D11

DEVELOPMENT OF UHPLC-MS/MS METHOD FOR STUDIES OF PHYTOCANNABINOIDS FATE IN EXPERIMENTAL ORGANISMS

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Phytocannabinoids are unique biologically active metabolites of hemp (Cannabis sativa L.) with a wide therapeutic potential. Besides psychotropic Δ9-tetrahydrocannabinol (Δ9-THC), exponentially growing attention has been paid to its non-psychotropic isomer, cannabidiol (CBD). Phytocannabinoids are used to alleviate the symptoms of various diseases, in the form of isolated substances or complex hemp extracts, alternatively or in the form of dietary supplements such as CBD oils. Needed to emphasize that Cannabis plants contain dozens of other phytocannabinoids (large range of isomeric structures) and non-cannabinoid biologically active substances (phenols, terpenoids, etc.), thus the detailed analysis of obtained extracts becomes an analytical challenge. Due to their lipophilic nature, phytocannabinoids are mainly absorbed by the tissues of the heart, lungs, liver, and brain, while their distribution from the blood to the tissues is relatively fast. To understand dose-effect relationships, investigation of parent compound distribution within the tissue of exposed organisms and monitoring of their further transformations is of high concern.
In the presented study focused on the transfer of phytocannabinoids into the brain tissue of 200 exposed animals administered a CBD preparation encapsulated with various carriers (oil with free CBD as a reference), an analytical method was developed, optimized, and validated for the target analysis of 18 phytocannabinoids, 3 Δ9-THC metabolites, and 2 CBD metabolites using reversed phase ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The recoveries of phytocannabinoids ranged from 61 % to 121 %. The repeatabilities of measurements expressed as RSD were within a range of 1 % to 20 %. The linear range of the analytical method for individual phytocannabinoids was from 1 ng/ml to 250 ng/ml in extract and the limit of quantification (LOQ) was determined from 1 ng/ml to 20 ng/ml in the extract as the lowest point of the matrix calibration series.

Keywords: *phytocannabinoids, metabolites, biological material, UHPLC-MS/MS*

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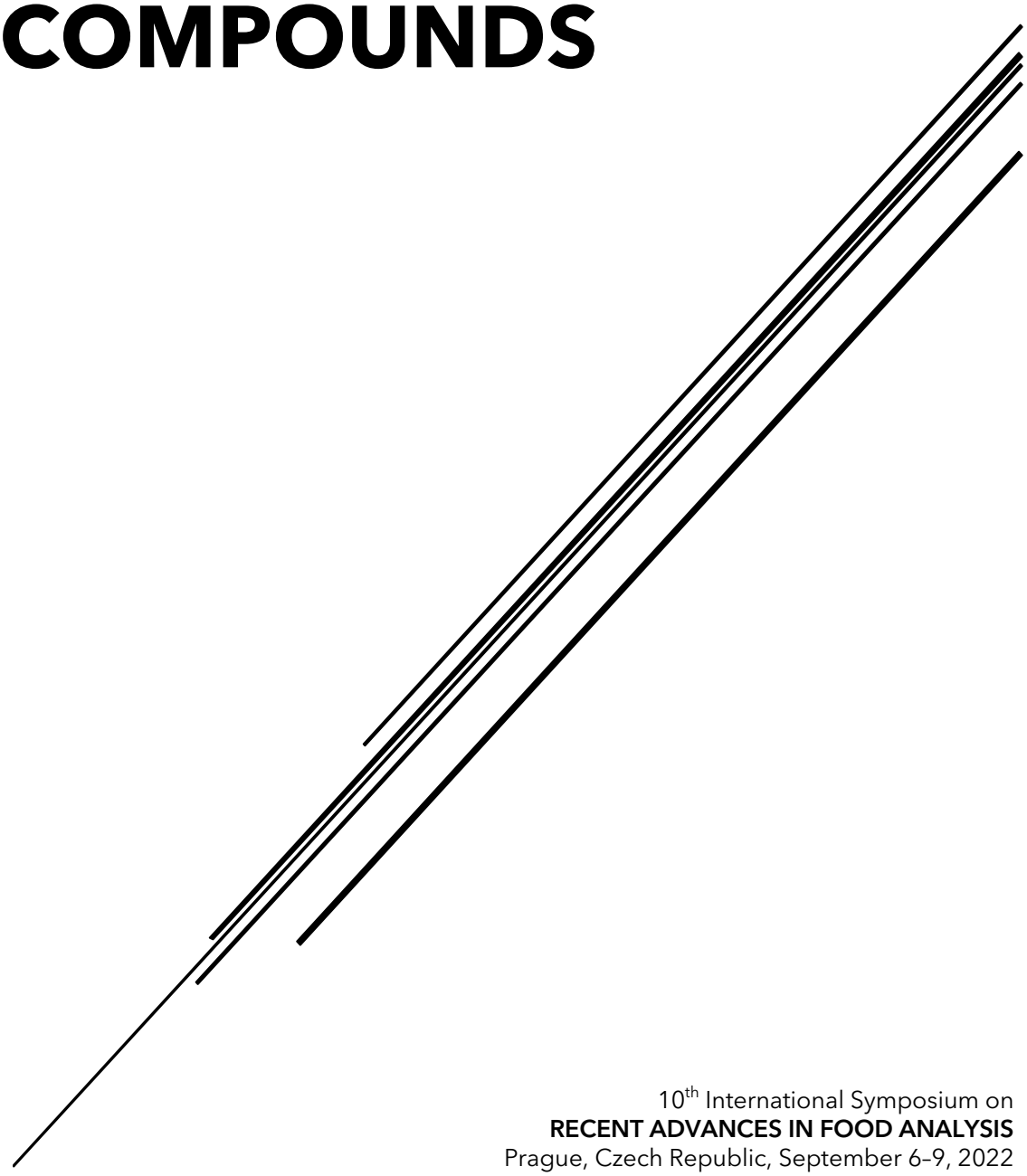
FLAVOUR SIGNIFICANT COMPOUNDS



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E1

SIMULTANEOUS QUANTIFICATION OF MAJOR OAT AND PEA SAPONINS IN PLANT-BASED PRODUCTS

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Despite the ecological and health benefits of plant-based diets, the specific long-lasting bitterness perception is one of the important constraints of a wider application of some plant-based proteins in the product development of nutritious plant-based foods. This specific off-flavour of oat and peas has been often associated with saponins. For food industries, it is important to evaluate objectively the raw materials bitterness and to apply technologies to reduce bitterness during the manufacturing process. Besides their bitter taste, there is also a doubt whether saponins have a positive impact on humans' health or if they exert antinutritional effects.

Saponins are widely spread compounds in the plant kingdom. Avenacoside A and B, 26-desglucoavenacoside A are specific bisdesmosidic steroidal saponins found in oat (*Avena sativa*) leaves and grains. Peas (*Pisum sativum*) contain monodesmosidic triterpenoid saponins: saponin B and 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) saponin. These compounds are linked to the proteins bodies of legumes and cereals, and therefore during dry milling and air classification the saponin content increases in the resulting protein concentrate – the primary ingredients of many protein-rich plant-based food products.

The hydrophilic interaction chromatography coupled to mass spectrometric detection (HILIC-MS) methodology was developed and validated for simultaneous quantification of major oat and pea saponins in powder samples and liquid food products. Effective chromatographic separation of analytes was achieved using BEH Amide column with gradient elution using ultrapure water with 0.1% formic acid and acetonitrile with 0.1% formic acid as mobile phases. Linear range of avenacoside A and saponin B was determined to be from 0.02 to 2.5 mg/L. The powder and liquid sample methodology was validated separately for oat and pea matrices. The total recovery of avenacoside A in oat protein concentrate was 104%, in oat drink 104% and in pea drink 100%. The total recovery of saponin B in pea protein isolate was determined to be 93%, in pea drink 105% and in oat drink matrix 93%.

E2

PREDICTING VIRGIN OLIVE OIL SENSORY TASTE ATTRIBUTES BY MEANS OF EXCITATION-EMISSION MATRICES: A FEASIBILITY STUDY

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Unlike any other food product, virgin olive oil must undergo a sensory evaluation that determines its quality degree and, accordingly, it is graded into one of the commercial categories namely extra virgin, virgin or lampante olive oil. The current official method for the organoleptic assessment of virgin olive oil relies on a trained human panel, following the International Olive Council procedure [1]. Although it has been responsible for improving the quality of virgin olive oils in the last years, many authors still agree on the controversies about the sensory panel test. In fact, it still presents some drawbacks linked to the method's nature that might affect the method's efficiency and robustness. Therefore, disposing of instrumental screening methods to support the sensory panels is of paramount importance. In this context, sensory parameters involved in commercial classification are generally linked with the aroma profile of virgin olive oil (fruitiness and off-flavours), therefore analytical methods based on volatile organic compounds have been developed to become useful screening tools [2]. Nonetheless, the previous method is not suitable to measure positive taste-related attributes such as bitterness and pungency since they are related to non-volatile compounds.
Therefore, the present work aims to explore the application of excitation-emission fluorescence spectroscopy (EEFS) in virgin olive oil to predict bitter and pungent attributes. Both organoleptic properties are known to be related with phenolic compounds, which are fluorophores [3]. Bitterness and pungency intensities of 250 virgin olive oil samples were provided by an official sensory panel and used to build and compare partial least squares regressions (PLSR) with the excitation-emission matrix (EEM), after proper pre-processing. Both parallel factor analysis (PARAFAC) scores and two-way unfolded data led to successful PLSR, given that errors in prediction were always close to the error of the sensory reference method. According to PLS regression vectors, the most relevant PARAFAC scores for both attributes agreed with virgin olive oil phenolic spectra. This fact evidenced that EEFS would be the fit-for-purpose screening tool to support the sensory panel in this regard.

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Keywords: *virgin olive oil, EEM, taste attributes, PARAFAC, PLSR*

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E3

QUANTITATIVE DETERMINATION OF VANILLIN AND ETHYLVANILLIN IN FOOD BY LC-ESI-MS/MS

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Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most important natural or nature_x0002_identical flavourings worldwide. Natural sources of vanillin are mainly the capsule fruits of the spice vanilla. In addition, vanillin can be obtained biotechnologically or from the wood component lignin. Out of more than one hundred identified vanilla flavour compounds, vanillin is the most important. In addition to vanillin, there is a synthetic, non-naturally occurring variant, ethyl vanillin. Ethyl vanillin is used today as an artificial flavour in e.g. ice cream and baked goods.
At national level (German food law), different terms are used in the list of ingredients depending on the source of the vanillin. According to Regulation (EC) No. 1334/2008 (Flavour Regulation), the designation natural vanilla flavour may only be used for extracts made from vanilla pods. If biotechnological processes were used to produce the aroma, its designation may only be declared as a natural aroma. If, on the other hand, synthetic compounds (methyl vanillin, ethyl vanillin) are used, the designation may not be used in the declaration. Accordingly, such compounds are referred to as vanilla flavouring or aroma. In Germany, vanilla extract and biotechnologically produced (nature-identical) vanillin are not considered as additives but food ingredients and may therefore be used in food without quantity restrictions. However, according to Chinese Regulation GB2760-2015 (Chinese Standards for Food Additives), the addition of flavours, including vanillin and ethyl vanillin, is completely prohibited in infant formula food for infants aged 0–6 months.
All processing steps for the determination of vanillin and the analogous compounds are carried out in just one plastic tube. The weighed sample is first mixed with a defined volume of an isotope_x0002_labeled (internal) standard. The extraction is then carried out by adding defined volumes of acetonitrile (protein precipitation), water and n-heptane (degreasing). After ultrasonic-assisted extraction at approx. 55 °C, a salt mixture is added to force a phase separation of water and acetonitrile, with highly polar impurities (e.g. sugar) remaining in the aqueous part. After centrifugation, an aliquot of the acetonitrile phase is fed to the measurement using LC-ESI-MS/MS. The quantitative determination is carried out by an external calibration series and by correction of the internal standard carried along.

Keywords: *vanillin, HPLC, MS, infant formula*

E4

PTR-MS AS ANALYTICAL STRATEGY FOR THE RAPID AND GREEN EXPLORATION OF FLAVOURING POTENTIAL OF THE OENOLOGICAL SPACE

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Wine production represents a relevant added-value sector in the framework of the food and beverage industry. A huge number of variables can affect the final quality of oenological products, such as, among others, pedo-climatic characteristics, grapevine varieties, microbial resources, oenological processes, technological solutions, additives, technological adjuncts, and ageing conditions. Often these variables are interdependent, increasing the complexity of the systems to be analyzed in research and development programs. The high number of parameters capable of impacting the properties perceived by the consumer leads to a complex oenological space that must be explored in order to undertake innovative paths.
The sensory quality of wine represents an aspect closely related to the added value of the finished product, as it can influence the associated hedonistic significance. In the framework of the overall sensory quality, the qualitative-quantitative variability of the volatile organic compounds (VOCs) associated with an oenological matrix plays a fundamental role in shaping the unique properties of a given bottle. From literature evidence, all the variables mentioned above can affect the presence/absence and abundance of volatiles. From this point of view, the classification of aromas into primary, secondary and tertiary aromas is functional, respectively associated with the varietal, fermentation and ageing.
Direct injection mass spectrometry (DIMS) techniques are conceived and designed to permit online and real-time studies of VOCs associated with a given headspace, avoiding extraction and difficult sample preparation and/or complex chromatographic separation. In other terms, DIMS approaches allow direct and green volatile analyses, offering low-cost, time-saving, and low-impact solutions to drive and bear sustainable research in the wine sector. In particular, here, we provide insight concerning the potential of Proton Transfer Reaction Time-Of-Flight Mass Spectrometry (PTR-TOF) coupled with an autosampler and tailored data analysis that contributes to the production and management of big data in this specific field of wine chemistry.
We provide information *i)* on the development of methodological strategies to improve the potential of PTR applications on oenological matrices and *ii)* on case studies concerning the oenological variables explored in the literature using PTR-TOF, from production to consumption. The evidence indicates this strategy is insightful in studying specific productions' sensory properties and unique traits. Volatile fingerprinting can be crucial to carrying out pre-feasibility assessments in the presence of a high number of experimental variables/conditions to be explored. All this concretizes opportunities to speed up research and development in the oenological field following sustainability criteria, improving massive screening and enhancing the effectiveness of segmentation in the sector.

Keywords: *wine, direct injection mass spectrometry, proton transfer reaction time-of-flight mass spectrometry, volatile organic compounds (VOCs), flavour*

E5

BATTLE OF THE BRANDS: SORPTIVE EXTRACTION AND GC×GC–TOF MS TO COMPARE THE FLAVOUR PROFILES OF SOFT DRINKS

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Unlike counterfeit goods, replica and imitation products are legal, as they do not use the branded product's trademark. In the food and beverage industry, imitation products attempt to mimic the taste experience of popular branded products. However, flavour profiles are extremely complex and consist of a broad range of chemical classes – the combination of which ultimately determines the consumer's preference for a particular brand. It is important to be able to confidently identify these volatiles during product development, as well as in quality and authenticity studies.
Traditional sample preparation methods, such as headspace and SPME, are widely used, but often limited in terms of sensitivity. We demonstrate the use of high-capacity sorptive extraction with novel trap-based focussing to provide enhanced sensitivity and improved chromatographic performance. This improved performance, coupled with improved separation by GC×GC and highly-sensitive detection by time-of-flight mass spectrometry (TOF MS), gains greater insight into sample composition. However, sampling, separation and detection is just the beginning – the resulting datasets must then be reduced to discover significant differences and ultimately allow meaningful conclusions to be reached. Here, we will demonstrate the use of a new chemometrics platform to transform complex data sets into useable results. Firstly, alignment of the raw data is applied, to account for potential retention time drifts. Next, advanced feature discovery identifies key differentiators across sample classes using all of the raw data. This innovative approach ensures that trace peaks are not ignored and enables automated workflows to be adopted, minimising laborious pre-processing steps and accelerating analytical workflows. Here, we will show this efficient end-to-end workflow in action for the comparison of brand and imitation soft drinks.

Keywords: *drink, flavour, immersive sampling, extraction, GCxGC-TOFMS*

E6

DETERMINATION OF SIMILARITIES AND DIFFERENCES IN BAIJIU SPIRIT SAMPLES BY MEANS OF GAS CHROMATOGRAPHY TIME OF FLIGHT MASS SPECTROMETRY (GC-TOFMS) USING A NON-SUPERVISED STATISTICAL APPROACH

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Baijiu is a traditional Chinese spirit with a complex pattern of volatile organic compounds (VOCs). The rich aroma results from the relatively complex and multilayered manufacturing process, including fermentation and various technological processing steps. In this study, gas chromatography coupled to time-of-flight mass spectrometry (GC-TOFMS) was used to differentiate Baijiu samples in terms of their aroma and origin (region). In total, 10 Baijiu samples were investigated: volatile compounds were extracted by SPME with a subsequent analysis by GC-TOFMS. The individual Baijiu profiles showed a wide range of concentration levels, challenging the proper detection and identification of trace level compounds. In fact, the correct determination of trace compounds can be crucial for a comprehensive aroma profiling in food analysis, as they might contribute greatly to the overall aroma. The optimized analytical parameters allowed for a comprehensive characterization of the individual Baijiu samples. The comparison of the aroma profiles and the data analysis was facilitated by the means of a non-supervised statistical analysis tool. Group type separation according to their respective class in terms of aroma-type and origin was obtained using this approach.

Keywords: *Baijiu, GC-TOFMS, differential analysis, aroma profiling*

E7

COMBINING GC, MS, AND OLFACTORY DETECTION FOR CHARACTERIZATION OF FOOD FLAVORS

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Characterization of food flavor is important for quality control (QC), to drive product development, and it can lead to a better understanding of a sample. Effectively achieving this characterizing often requires identifying the individual chemical components of a sample and then linking these features to their aroma or sensory impact. The combination of gas chromatography (GC), mass spectrometry (MS), and olfactory detection provides an analytical method that is well-suited to achieve these goals.
In this study LECO's Pegasus BT GC-MS was used to separate and identify the individual analyte components in a variety of complex food, beverage, and aroma samples. Olfactory detection (GL Science Phaser Pro) was incorporated by splitting a portion of the effluent to an olfactory port. The olfactory descriptors allowed for directly connecting the identified features with their contributions to the overall aroma or flavor and for determining which of the separated and identified analytes were most important for contributing to the characteristics of the sample.
A variety of samples were analyzed by GC-MS-O and this poster presents the benefits of acquiring and analyzing this data together. The individual components of each sample were primarily separated by GC, but MS (in particular, time-of-flight (TOF)-MS) detection allowed for additional resolution with deconvolution that mathematically separated many instances of chromatographic coelution. The identifications of the isolated analytes were determined by matching the observed spectral patterns to commercial library databases such as the NIST mass spectral library. GC retention order information further supported these identifications by matching observed Retention Index (RI) information to library database information. The incorporation of olfactory detection was particularly helpful for connecting the identified features with their contributions to the overall aroma or flavor. This type of sensory directed analysis highlights regions of interest and allows for a focused review of the data ultimately providing specific analytes of interest. Examples highlighted include: a nutmeg essential oil analyzed to determine the most characteristic nutmeg notes in the sample, an analyte responsible for an off odor in a beer sample was determined, and sensory differences amongst a panel were explored with the analysis of cilantro. Each component of the instrument played an important and complementary role in separating, identifying, and determining the sensory impact of the individual analytes within these complex food matrices.

Keywords: *GC-MS, VOC, olfactometry, aroma profiling, beverage*

E8

REAL-TIME ANALYSIS OF FOOD FLAVOURS BY VOCUS CI-TOF: THE EXAMPLE OF OXIDATION BYPRODUCTS

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Food spoilage due to oxidation processes is a compelling problem. For example, fish oil contains abundant quantities of long-chain, unsaturated fatty acids that are extremely susceptible to oxidation. Within the food industry, it is often common to use ozone treatments to control biological growth of unwanted organisms. However, ozone is also a potent oxidant that can lead to the generation of oxidation byproducts. Oxidation process may happen on short timescales.The Vocus Chemical Ionization - Time of Flight (Vocus PTR-TOF) represents a cutting-edge technique which advanced Proton Transfer Reaction – Mass Spectrometry (PTR-MS) for the online monitoring of volatile organic compounds (VOCs), such as flavours in food.
This work demonstrates use of a Vocus CI-TOF for on-line monitoring of volatile fish oil oxidation byproducts in capsuled fish oil. This method, which requires no sample preparation or pretreatment, can be used to non-invasively assess fish oil degradation levels during production processes or for high-throughput characterization of product samples. In a first experiment, fish oil capsules were exposed to high concentrations of ozone to simulate atmospheric oxidation. Oxidation products emerged seconds after ozone infusion. Dominant products in the headspace of ozone-treated fish oil included acetaldehyde, acetone, hexenal, propanediol, butanal, and others. The oxidation produced many high molecular weight functionalized compounds that can serve as unique traces for diagnosing fish oil degradation. In a second experiment, fish oil capsules were monitored for several days during aging at ambient temperature in closed vials. The concentrations of several species were positively correlated with the aging time. Some VOCs plateaued earlier than others, likely due to various degradation pathway involved. Different external factors such as radiation, ozone, temperature, and humidity are believed to heavily influence the headspace composition of aged samples. A systematic study involving all experimental factors could likely pinpoint qualitative and quantitative VOC markers for oxidation of unsaturated fatty acids in fish oil.

Keywords: *PTR-MS, real-time MS, food flavours, volatile organic compounds*

E9

REAL OR FAKE? AROMA PROFILING OF GROUND MEAT AND PLANT-BASED BURGERS

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Plant-based meat substitute products have increased in popularity in recent years, and a goal for food producers is to create a product that mimics the sensory experience of cooked meats. However, ground meat may release hundreds of volatile organic compounds (VOCs) during cooking, many of which are odour-active and cover a wide range of chemical classes; therefore, capturing a comprehensive VOC profile to estimate quality or to reverse engineer the aroma is a challenging task.
Firstly, high sensitivity is required to ensure that trace compounds are detected because even a trace amount of a compound with a low odour threshold can have a large impact on the overall aroma. Here, high-capacity sorptive extraction probes were used to provide a larger volume of stationary phase (65 µL) compared to traditional solid-phase microextraction (SPME) (~0.5 µL) for higher sample loadings. When used in combination with trap-based focusing this provides enhanced sensitivity and improved chromatographic performance. Secondly, the diverse range of chemical classes requires advanced separations to resolve co-elutions and provide confident identification of the analytes present. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC–TOF MS) can tackle this challenge by coupling two columns of different selectivity to separate the analytes based on two different chemical properties (e.g., volatility and polarity). Finally, sophisticated data analysis software is required to quickly identify subtle differences between aroma profiles. In this case, an automated untargeted comparison was performed using a novel data mining and chemometrics platform. Firstly, chromatographic alignment accounts for retention time drift over the course of the study and minimises the risk of false hits. Next, feature discovery is performed on the raw data to find significant changes across sample classes. In this study, we demonstrate the performance of the described workflow to compare the aroma profiles of ground meat and plant-based substitutes from different manufacturers.

Keywords: *vegan, vegetarian, beef, GCxGC-TOFMS, headspace*

E10

IMPACT OF FERMENTATION ON AROMA-ACTIVE COMPOUNDS OF COFFEA CANEPHORA

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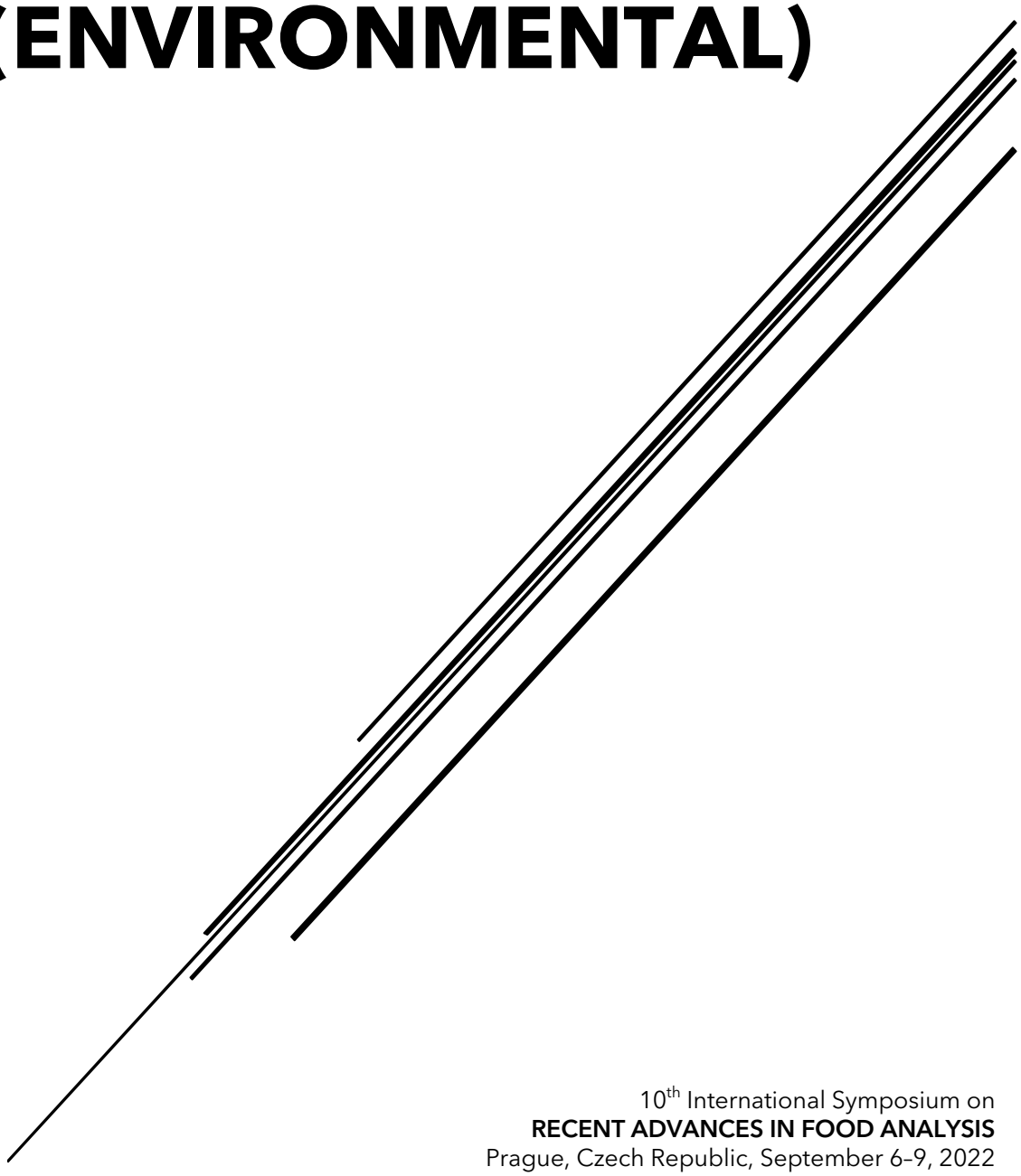
Arabica coffee is more popular than Robusta coffee, probably due to its pleasant sensory characteristics, dominated by caramel and sweet roast flavours. By comparison, Robusta coffee is characterised by earthy, nutty, spicy, and roasted flavours, which might be a reason for its lower preference by consumers. Because of this, different methods have been carried out to influence and modulate the sensory properties of the Robusta coffees. One approach is fermentation. However, the impact of the sensory changes caused by fermentation requires further investigation. The present work addressed this need by investigating changes in the composition of aroma-active compounds of fermented Robusta coffees in comparison with Arabica and Robusta coffees obtained by natural processeses. The aroma-active compounds were analysed in three different Robusta obtained by fermentation and natural process, and one Arabica coffee sample obtained by natural process. The compounds were isolated from 3 g of each sample by solvent-assisted flavour evaporation followed by micro distillation for sample enrichment. The sample extract was then subjected to gas chromatography-olfactometry (GC-O) and aroma-extract dilution analysis (AEDA), with olfactory assessments performed by individual trained panellists. The results were expressed as flavour dilution (FD). Identification of the individual compounds was performed via additional analysis with mass spectrometric detection (GC-O/MS) using the same chromatographic conditions, with identifications based on characteristic mass spectra in comparison with a reference library, linear retention indices and odour qualities. Although more odorants were identified in Arabica coffee than in the Robusta coffees, the highest number of potent odorants (FD 81 to FD 2187) was detected in fermented Robusta coffee. Among the most potent odorants were buttery, vinegar-like, earthy, roasty, caramel-like, woody, popcorn-like, clove-like, cheesy and smoky smelling substances. Some compounds were detected in Robusta coffee at FD > 2187, including 2-ethyl-5-methylpyrazine (roasty), acetic acid (vinegar-like), pyrrole (caramel-like), 2-furanmethanol (cheesy), furaneol (candy floss-like, caramel-like), 2-methoxy-4-vinylphenol (clove-like), 2-ethyl-3,6-dimethylpyrazine (earthy, mouldy), 1-(1-methylpyrrol-2-yl)ethenone (nutty), ethyl 2-phenylethanoate (honey-like), ethyl salicylate (fruity) and benzyl alcohol (marzipan-like). Despite the natural fermentation process improving the overall flavour profile of Robusta coffee, some compounds, such as 2-hydroxy-1-methylcyclopenten-3-one and maltol, which are responsible for the caramel-like notes, were detected only in Arabica coffees. These results suggest that the fermentation process might be used to improve the odours already reported in the Robusta coffees, but further research is needed to examine the formation of more pleasant notes, such as the caramel detected in Arabica coffees.

Keywords: *AEDA, olfactometry, odorants, GC-O/MS*

Acknowledgement: *The São Paulo Research Foundation ((rocess n. 2021/14186-5).*

FOOD CONTAMINANTS (ENVIRONMENTAL)



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F1

SAFE FOOD FOR INFANTS: AN EU-CHINA PROJECT TO ENHANCE THE CONTROL OF SAFETY RISKS RAISED BY MICROBIAL AND CHEMICAL HAZARDS ALL ALONG THE INFANT FOOD CHAINS

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The EU-project SAFFI targets food for EU's 15 million and China's 45 million children under the age of three. It aims at developing an integrated approach to enhance the identification, assessment, detection and mitigation of health risks raised by microbial and chemical hazards along EU and China infant food chains.
SAFFI will benchmark the main risks through an extensive hazard identification system based on multiple data sources and a risk ranking procedure. It will also develop procedures to enhance top-down and bottom-up hazard control by combining management options with a panel of technologies for the detection and mitigation of priority hazards. Furthermore, it will explore unexpected contaminants by predictive toxicology and improve risk-based food safety management of biohazards by omics and predictive microbiology. SAFFI will co-develop with and deliver to stakeholders a decision-support system (DSS) to enhance safety control all along the food chain. This DSS will integrate the databases, procedures and methods described above and will be a framework for a generic DSS dedicated to other food.
This overall methodology will also be implemented in a complementary Chinese side of the project, and exemplified for each side, with four case studies that were selected to cover priority hazards, main ingredients, processes and control steps of the infant food chain. Resulting databases, tools and procedures will be shared, cross-validated, concatenated, benchmarked and finally harmonized for further use in the EU and China.
This EU-China multi-actor consortium of 20 partners involves academia, food safety authorities, infant food companies, a paediatrics association and technological and data-science SMEs.

Keywords: *food safety, baby food, microbial and chemical hazards, risk assessment, decision support system*

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F2

OCCURRENCE OF FLUDIOXONIL IN BLUEBERRIES AND ITS TRANSFERENCE TO ANIMAL PRODUCTS

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Since the emergence of the presence of organochlorine pesticides and polychlorinated biphenyls in the environment and the realization of their toxic effect, their presence has been monitored in food of animal origin. Due to their solubility in fats, they are characterized as the main pollutants. However, sporadic contaminations with other pesticides increasingly indicate contamination of fatty foods with pesticides from other classes of compounds, such as those used to treat fruits, vegetables and animal feed. Modern food production includes more mixed products such as the increasingly popular milk drinks with added fruit. Declared as fermented skimmed milk products, i.e., yogurt with the appropriate percentage of fruit. Analysis of the presence of fludioxonil was carried out in blueberry and blueberry yogurt samples. The aim of the study was to monitor the proportion of fludioxonil pesticide contamination in yogurt by monitoring the amount present in blueberries. Fludioxonil is a phenylpyrrole insecticide that is sprayed on fruit and vegetable crops after harvest to reduce mold loss, both during transit and at the point of sale. Since its introduction in 1994, the production and consumption of fludioxonil has increased dramatically. The absence of a well-confirmed mechanism may be a cause for concern given that the pesticide has long been known to induce stress intermediates in both target and non-target species. Disturbing information indicates that fludioxonil may pose a health risk to users by disrupting the functioning of the liver, endocrine and nervous systems. The analysis involves a solid phase extraction using magnesium sulfate and sodium chloride. Ultrasound-assisted extraction was used for sample extraction. The homogenized was centrifuged for 5 minutes at 5500 rpm. After that, the extracts were cleanup by dispersed solid phase extraction and quantified using a Gas Chromatograph (680 PerkinElmer) equipped with a Clarus SQ8T mass spectrometer (PerkinElmer, Waltham, MA, USA). The maximum residue level (MRL) for fludioxonil in dairy animal products is defined as a value of 0.04 mg/kg (Reg. (EU) 2021/1807), and for blueberries 4 mg/kg. The obtained results of fludioxonil present in blueberry and yogurt ranged from 0.082 to 1.2 mg/kg, and in yogurt from 0.012 to 0.035 mg/kg. The results indicate the correlation and significance of the present share. On the other hand, they indicate the need for monitoring and control of mixed products in accordance with the origin of the raw materials.

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F3

CAPILLARY ELECTROPHORESIS COUPLED TO MASS SPECTROMETRY: A USEFUL ALTERNATIVE FOR THE CONTROL OF MYCOTOXINS AND PESTICIDES IN FOOD AND ENVIRONMENTAL ANALYSIS

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The global concern about food safety and environmental quality has led to the development of a legal framework to control residues and contaminants. In this context, the establishment of analytical methods achieving satisfactory performance in terms of sensitivity, identification, sample throughput and applicability is mandatory. Multiresidue methods mainly based on liquid chromatography coupled to mass spectrometry (LC-MS), have been extensively applied. With the evolution of the instrumentation, capillary electrophoresis (CE) has emerged in the last decades as a very well established separation technique, alternative or complementary to chromatography, for food and environmental analysis. Its versatility, due to the different modes, and the combination with different detection systems, mainly with MS, the applied sample treatments before analysis and the preconcentration strategies have helped to overcome the main limitation attributed to CE that is its low sensitivity.

In this communication, we show the potential of CE-MS for the monitoring of residues and contaminants of recent relevance and great interest, such as neonicotinoids and emerging mycotoxins, in food and environmental samples. We have developed the first proposal for the simultaneous determination of nine neonicotinoid insecticides and the fungicide boscalid, by using micellar electrokinetic chromatography (MEKC) in presence of a volatile surfactant (ammonium perfluorooctanoate), compatible with MS. Furthermore, a scaled-down QuEChERS was developed as sample treatment, involving a lower organic solvent consumption, using Z-Sep+ as dispersive sorbent in the clean-up step. A triple quadrupole mass spectrometer was operating in ESI+. The method has been validated and applied to the determination of these compounds in pollen and honeybee samples.

In addition, CE has shown to be an excellent choice for the analysis of enniatins (ENNs) and beauvericin (BEA), considered as emerging mycotoxins, because their possible toxicological effects, but still not included in legislation. Despite the absence of easily ionizable groups in these compounds, its ionophoric character makes them compatible with CE separation. Due to their apolar nature, a non-aqueous CE (NACE) method coupled to quadrupole time-of-flight–MS has been proposed for the first time to identify and quantify these mycotoxins. A salting-out assisted liquid-liquid extraction was used as sample treatment. Separation of four ENNs (enniatin B, B1, A1 y A) and BEA was achieved in 4 min. "All Ions" acquisition mode was selected as it allows the quantification of the main ENNs (with available standards), and the identification of unusual ENNs (no standards available). The method was validated and applied to wheat samples.

Keywords: *capillary electrophoresis, mass spectrometry, neonicotinoids, emerging mycotoxins*

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F4

A COMBINED APPROACH FOR THE TOXICOLOGICAL EVALUATION OF MINERAL OIL AROMATIC HYDROCARBONS

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The contamination of food with mineral oil residues is still a widely discussed problem. Although there had been significant advancements in the last years, there a remaining knowledge gaps in analysis, exposure assessment, hazard characterisation and risk assessment[1].
Mineral oil hydrocarbons are divided into a saturated and an aromatic fraction. While the mineral oil saturated hydrocarbons (MOSH) are not considered to have a genotoxic relevance, the mineral oil aromatic hydrocarbons (MOAH) may include potential genotoxic and carcinogenic substances, due to the presence of 3-7 ring polycyclic aromatic compounds[2,3]. The European Food Safety Authority recommends to use analytical methods to identify those substances, when MOAH is detected in food[3]. State-of-the-art analysis is done using the online-coupling of LC-GC-FID, but analysis reveals only unresolved humps with unknown origin. Confirmatory techniques using multi-dimensional chromatography, e.g. 2D-comprehensvie GC×GC with various detector types are needed to allow for an adequate substance class identification, recognition of false-positive values and therefore correct quantification of the generated humps. However, there are still knowledge gaps regarding the hazard positive MOAH results have onto human health[1,3].
Therfore, aim of this work is to do a deep characterization of MOAH sub-fractions using a combined approach: deep analytical characterization and evaluation of toxicology using bioassays.
In a first step, mineral oil products, food contact materials and food samples are analyzed for their MOSH and MOAH contamination using the current state-of-art. Afterwards, the MOAH is separated from MOSH and second, is subjected to a HPLC fractionation according to ring numbers[4]. Using those separated fractions, on the one hand, relevant substance classes are identified using 2D-comprehensive GC×GC-ToF. On the other hand, hazard characterization of this sub-fractions is made using the miniaturized AMES-test. The information of both approaches is combined to allow for the needed health risk assessment of the samples tested positive for MOAH.

1) https://doi.org/10.1016/j.tifs.2021.03.021
2) Doi:10.2903/j.efsa.2012.2704
3) doi:10.2903/sp.efsa.2019.EN-1741
4) Doi: 10.1002/jssc.201900833

Keywords: *mineral oil hydrocarbons, 2D-GCxGC, AMES Test, evaluation of genotoxicity*

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F5

ASSESSING THE HUMAN FOOD CHEMICAL EXPOSOME BY NON-TARGETED ANALYSIS: HOW TO CLEAN-UP LIPIDIC EXTRACTS?

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More and more diverse substances are produced by the chemical industries. Some of them may be released into the environment through various ways and may ultimately enter the human food chain. While targeted analyses allow one to investigate known contaminants, non-targeted approaches may help to identify compounds of emerging concern. Sample preparation for target analysis can be highly selective, but to make use of the potential of non-target analysis sample preparation must be less selective.

Most halogenated organic contaminants (HOCs), among them persistent organic pollutants, are lipophilic substances. Thus, the removal of lipids, that will impede the detection of HOCs at trace levels in food matrices, is a key step of the sample preparation for non-targeted analysis. Based on their capacities to remove lipids from extracts, three extraction plus clean-up methods were chosen and compared with regards to recoveries and matrix effects: liquid-liquid extraction followed by gel permeation chromatography (GPC)[1] or sulphuric acid degradation[2] and direct passive sampling using polydimethylsiloxane (PDMS) polymer[3].

To complement the human exposure assessment using food samples, seagulls considered as sentinel species were also studied, based (i) on the assumption that urban seagulls feed on food waste and (ii) that expected concentrations will be higher compared to food. Seen as vermin, their populations are regulated at local levels in France, giving an easier access to the samples compared to human matrices.

A selection of 4 food items of animal origin (cow milk, ham, salmon, mussel) and seagull's egg was considered to perform the comparison. After microwave lipid extraction, 4 replicates were cleaned-up (3 spiked + 1 blank). Two spike mixtures were used with a wide range of hydrophobicity expressed as octanol-water partition constant log P and molecular masses. The first mixture was added to the extract prior the clean-up to assess the recovery of the method (61 compounds). The second mixture was added to the cleaned-up extract prior the analysis, to assess the matrix effect (64 compounds). Samples were then analysed by chromatography (GC and LC) coupled to high resolution mass spectrometry. Data was processed in suspect analysis to semi-quantify the spiked compounds.

Despite initial optimisation of the cut off time, GPC caused clogging into the GC injector and a high matrix effect. Acidic treatment altered acid-sensitive chemicals. Passive sampling using PDMS was suitable for the most concentrated substances.

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[2]Cariou et al., 2016. Analytica Chimica Acta, 936, 130–138. https://doi.org/10.1016/j.aca.2016.06.053.
[3]Baumer et al., 2021. Environ. Sci. Technol., 55 (13), 9097–9108. https://doi.org/10.1021/acs.est.1c01836.

Keywords: *contaminants of emerging concern, non-targeted analysis, sample preparation, lipid removal*

F6

CONTRIBUTION TO THE RISK ASSESSMENT OF POLYCHLORINATED NAPHTHALENES IN FOOD: OCCURRENCE AND CONSUMER EXPOSURE IN FRANCE

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PolyChlorinated Naphthalenes (PCNs) are a group of 75 congeners, of which 73 di- to octaCNs were recognized as POPs in 2015, and are currently in the Annex A and C of the Stockholm Convention. A diversity of emission sources is reported, arising not only from their historical production and use but also from their unintentional presence in PCBs mixtures or their *de novo* formation during combustion. While a number of PCNs congeners have the ability to bind to the aryl hydrocarbon receptor, like the more famous PolyChlorinatedDibenzoDioxins/Furans (PCDD/Fs) and PolyChlorinated Biphenyls (PCBs), the amount of available data is substantially lower than for the latter POPs. Although PCNs are not emerging in the strict sense, the community is expressing renewed interest in them in a context where assessing all the contaminants present is a priority for public authorities, particularly because of their contribution to the overall toxicity associated with POPs.
Analytical challenges still exist, in line with the absence of certified reference materials, and commercial standards for many PCNs implying that in most studies only a limited number of congeners is measured. In this context, taking advantage of the structural similarity between these substances, the present work consisted in integrating the analysis of PCNs into an extraction/purification method routinely applied for PCDD/Fs and PCBs, involving a pressurized liquid extraction followed by an automated purification and an analysis by gas chromatography coupled to high-resolution mass spectrometry.
On the 75 congeners initially targeted, good performances were observed for 69 di- to octaCNs. These included good instrumental linearity (R^2 > 0.99), recoveries (83 % on average), method precision and interlaboratory reproducibility (averaged relative standard deviations respectively equal to 25 and 34 %), and limits of detection (0.1 – 0.3 pg g^{-1} wet weight (ww)).
The application of the method enabled characterizing 60 food items acquired on the French market, including fish, meat, milk and dairy products, eggs, baby foods and vegetable oils. PCNs were found to be ubiquitous, with summed levels falling in the pg g^{-1} ww range, and being up to more than a hundred of pg g^{-1} ww. The measurement of almost all the existing congeners allowed us to demonstrate the predominance of lower chlorinated homologs (2-4 Cl, contributing to ~70 % of total PCNs concentrations), being not commonly measured in other works. In addition, the simultaneous measurement of PCNs with PCDD/Fs and PCBs showed a substantial contribution of PCNs to total POPs concentrations (0.9 – 50 %, 9% on average), although slightly less when considering toxic equivalents (0.4 – 24 %, 5 % on average) The dietary exposure assessment does not suggest a risk to the consumer, based on the food products characterized in this study. This study provides the first data on the occurrence of a large number of PCNs in France in a variety of food items.

Keywords: *polychlorinated naphthalenes, persistent organic pollutants, dietary exposure, toxic equivalents, food analysis*

F7

DIOXINS AND DL-PCBS IN THE AMBIENT AIR OF THE VALENCIAN REGION (SPAIN): LEVELS, HUMAN EXPOSURE AND RISK ASSESSMENT

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Dioxins (polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), as well as dioxin-like PCBs (dl-PCBs), are listed as persistent organic pollutants in the Stockholm Convention. In this study, we measured their concentrations in the particulate phase (PM10) of the ambient air in seven monitoring stations of the Valencian Region (Spain). A total of 82 samples were collected from different sampling sites: four industrial, two urban, and one remote, from February to December 2019. The total concentrations of the sum of PCDD, PCDF, and dl-PCBs ranged from 2.90 fg TEQ/m^3 to 317.98 fg TEQ/m^3. Risk assessment for adults and children was performed using both daily and chronic exposure. Each station showed its specific dioxin profile, related to the main productive activities in each area. The daily inhalation dose (DID) in adults and children was lower than the tolerable daily intake (TDI) of 1-4 pg WHO TEQ kg-1 b.w. d^{-1} for dioxins. In the case of chronic exposure, the cancer risk for dioxins and dl-PCBs was estimated at values ranging from 5.27 E-07 to 5.52 E-05. The cancer risk for dioxins and dl-PCBs estimated at the 95th percentile was higher than 1.0 E-06 in all of the industrial and urban areas.

Keywords: *dioxins, ambient air, risk assessment, gaseous-particle model, cancer risk*

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F8

DERMAL EXPOSURE TO BISPHENOLS – POTENTIALLY TOXIC CHEMICALS IN CLOTHES

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Many of organic substances used in the textile industry can cause not only environmental, but also health problems. A lot of studies suggest that dermal exposure may be a non-negligible way of human exposure to these compounds generally represented by antioxidants, plasticizers, dyes, flame retardants and surfactants. Bisphenol A (BPA) and its derivates are also employed in textile processing, e.g. BPA is used as an intermediate chemical in the manufacture of antioxidants and dyes. Bisphenols (BPs) potentially act as endocrine disrupting chemical and display estrogenic properties. However, the occurrence of bisphenols in clothes is still unknown and the human exposure via skin contact with clothes has not been thoroughly assessed.
The main aim of our study was to investigate the BPA, BPS, BPF and BPB occurrence in adult clothes (14 T-shirts and 11 socks) made of both natural fibers (cotton) and natural / synthetic polymers (viscose, lyocell/elastane, polyester, polyamide) bought in the Czech fashion stores. Then to estimate the dermal exposure to BPs for adults and to perform a simulated sweat leaching experiment for the evaluation of the exposure under sweating conditions.
Sample extraction procedure was based on the ultrasound assisted extraction of 1 g of textile with methanol for 2 hours followed by the ultra-high performance chromatography coupled to tandem mass spectrometry. Limits of quantification for four target BPs were in the range of 0.05-0.5 ng/g and the method repeatability was <20%. The design of the simulated sweat leaching experiment was based on study Wang et al. (2019).
The only BPs found in samples were BPS (100% positives, 0.56-2 470 ng/g, median 3.45 ng/g) and BPA (92% positives, <0.05-54 ng/g, median 7.69 ng/g). Higher median levels of both BPS (5×) and BPA (2×) were detected in textiles made of polymers with cotton content (from 0% to 76%) compared to samples from 100% cotton. The highest amounts of BPS (>100 ng/g) were present in socks (in 3 out of 11 sock samples), it is possible that BPS was introduced to improve material performance and durability.
Median estimated daily exposure from textiles for adults was 0.5 pg/kg bw/day to BPA and 0.2 pg/kg bw/day to BPS. Additionally, dermal exposure dose from highly polluted sweaty T-shirt (with BPA concentration of 54 ng/g) was established as 8 300 pg/kg bw/day. Compared to the estimated intake 17.5 pg/kg bw/day from this sample (non-sweat), dermal contact with sweaty clothes may become a relatively high exposure risk in humans. Additionally, to other exposure routes (dietary and inhalation), the contribution of dermal exposure dose of BPs from daily clothes should not be neglected, especially for most vulnerable infants and children, who are particularly susceptible to endocrine disrupting compounds. *Wang, Lei, et al. "Widespread occurrence of bisphenol A in daily clothes and its high exposure risk in humans." Environmental Science & Technology 53.12 (2019): 7095-7102.*

Keywords: *bisphenols, dermal exposure, UHPLC-MS/MS, clothes*

Acknowledgement: *This work was supported from the grant of Specific university research – grant No A1_FPBT_2022_005.*

F9

APPLYING HIGH RESOLUTION GC-ORBITRAP MASS SPECTROMETRY FOR THE QUANTITATIVE ANALYSIS OF ENVIRONMENTAL CONTAMINANTS IN FOOD

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In a high-throughput testing environment, robust streamlined analytical and data processing workflows are key requirements for the accurate and reliable determination of trace level contaminants in food or environmental samples. These workflows must overcome the challenges of an ever-growing list of compounds, a diversity of sample matrices, and ever-more demanding sensitivity and identification requirements. To date, gas chromatography coupled to a low-resolution GC-MS/MS has been the system of choice for the sensitive and selective detection of a wide range of target compounds. However, a limitation of GC-M/MS systems is the capability to only measure compounds in a targeted list at the time of acquisition. In this study, the analytical performance and suitability of a benchtop high resolution accurate mass (HRAM) Orbitrap GC-MS was assessed. In high resolution MS the default acquisition mode is full scan accurate mass allowing all the ions to be acquired at the same time across a specified mass range, simplifying instrument operation and method setup and giving the analyst the flexibility to decide post-acquisition which compounds and ions to measure. The analysis of trace level contaminants, including pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and brominated flame retardants (BFRs) in olive oil and fish oil is reported. System setup simplicity as well as typical method performance parameters including sensitivity, linearity, and quantitation were evaluated. Analysis of a proficiency test sample provided good agreement with assigned values and to results obtained using GC-MS/MS. Details of linearity, detection limits will be presented in the poster.

Keywords: *PCB, PAH, BFR, OCP, Orbitrap MS, gas chromatography, fish oil*

F10

QUANTITATIVE DETERMINATION OF ACRYLAMIDE IN FOOD ON THE EXAMPLE OF COFFEE USING 2D-LC-ESI-MS/MS

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Acrylamide (2-propenamide) was first detected in 2002 by a Swedish working group in various starch-containing, thermally processed foods like French fries or potato crisps. Legal limits for the content of acrylamide in foodstuffs have not yet been set at both national and European levels. However, there is a recommendation from the European Commission of 08.11.2013 defining signal values for ten food groups. In 2018, benchmark values for the content of acrylamide in various foods were then set in accordance with Regulation (EU) 2017/2158. A comprehensive opinion on acrylamide in foodstuffs, which discusses in particular the mutagenic and carcinogenic effects of acrylamide, was published by the Federal Institute for Risk Assessment (BfR) on 29.06.2011. The procedure presented here describes a method approach for the extraction as well as the measurement by 2D-LC-ESI-MS/MS of acrylamide in different food groups, here on the example of coffee. One goal was the development of a simple and uniform sample preparation procedure, which could be applied to all food matrices. Furthermore, a powerful 2D-LC-ESI-MS/MS method was established in order to achieve a maximum of chromatographic resolution as well as a minimum of ionic suppression and thus a lower limit of quantification.

Keywords: *acrylamid, HPLC, MS, 2D-LC, coffee*

F11

BISPHENOL RESIDUES IN CONVENTIONAL AND UNCONVENTIONAL PROVOLAS CHEESE

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Food of animal origin play an important role in human dietary exposure to bisphenol residues (BPs), due to the high contribution of this exposure pathway [1]. However, migration of BPs from food contact materials is not the only way by which bioaccumulation occurs. In fact, in milk and dairy products, the presence of BPs is also thought to result from dairy cows feeding practices [2]. In this study, the presence of nine BPs in samples of *Provola Ragusana* cheese was investigated to assess the level of contamination and ensure a healthy and safe product for the consumer. The cheese was produced from milk of Friesian cows under two different feeding systems: a conventional diet (CTR) and an unconventional diet (BIO) enriched with dried olive cake (OC) source of bioactive compounds. The sampling was conducted monthly, from March to July 2021. BPs determination was performed by liquid chromatography and tandem mass spectrometry. Bisphenol AF (BPAF) and bisphenol S (BPS) were detected in all samples analyzed, whereas all the others (bisphenol A, bisphenol F, bisphenol E, bisphenol B, bisphenol AP, bisphenol Z, bisphenol P) were below their limit of quantification (LOQ). In the CTR and BIO samples, the mean concentrations of BPAF were 2.53±0.36 µg/Kg and 2.50±0.14 µg/Kg, respectively, whereas those of BPS were 2.21±0.33 µg/Kg and 2.14±0.18 µg/Kg, respectively. Statistical analysis (ANOVA and Tukey's test) of the data showed that the contamination levels in the two diets are similar. Only seasonal variations are observed, with significant increases of BPs concentration in the month of July for BIO and from March to May for CTR. These differences are probably due to different lipid content, seasonal variations in temperature or other forage compositions [3]. The results show that the integration of OC into the animal's` diet does not affect the levels of Provola contamination. Thus, in this case, the source of BPs is not attributable to dairy cow feeding, but likely depends on the production process and environmental contamination. It can be concluded that unconventional Provolas enriched with OC are as safe for the consumer as conventional Provolas.

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Keywords: *provola, cheese, olive cake, cow diets, bisphenols*

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F12

QUECHERS EXTRACTION OF PER-AND POLYFLUOROALKYL SUBSTANCES (PFAS) FROM EDIBLE PRODUCE WITH SENSITIVE ANALYSIS ON XEVO™ TQ-XS MASS SPECTROMETER

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The same sources of environmental per- and polyfluorinated alkyl substances (PFAS) exposure can also lead to contamination in food sources. Cultivating produce using PFAS contaminated water and soils can lead to the uptake of these compounds into the edible fruits and vegetables portions of plants. Thus, it is beneficial to have a straightforward method to monitor the occurrence of PFAS in produce. For this work, the FDA C-010.01 method based on the QuEChERS extraction method was implemented for extraction of PFAS using DisQuE dispersive solid phase extraction (dSPE) products followed by highly sensitive LC-MS/MS analysis on ACQUITY™ UPLC™ I-Class PLUS system coupled to Xevo TQ-XS mass spectrometer. The method was evaluated in five different commodity types including lettuce, strawberry, cranberry, carrot, and potato. Some minor adjustments to the FDA procedure were included in this application to improve the chromatography for better quantitation and identification, and to improve extraction efficiency of target PFAS. These include a dilution prior to LC-MS/MS analysis to improve peak shape of early eluting analytes, removal of GCB to improve overall recovery, and use of buffered salts following AOAC protocol. A PFAS Kit was utilized to modify the LC to isolate possible system and solvent contaminants. This application for PFAS analysis in produce proved to be a simple, time efficient extraction, followed by an accurate, sensitive, and robust analysis for a range of 30 PFAS compounds of varying chemistry classes in the sub ng/g range.

Keywords: *PFAS, QuEChERS, PFOS, PFOA*

F13

TOTAL WORKFLOW FOR THE SENSITIVE ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN FISH, MEAT, EDIBLE OFFAL AND EGGS

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Rising concerns about the long-term impacts of human exposure to PFAS have propelled the scope of PFAS analysis from just environmental matrices into the field of food analysis as well. Over the last decade, cases of PFAS contamination being found in foods have become more prominent in the media. In order to protect the public and understand dietary exposure, analytical methods for the analysis of a large variety of food products are required. This study focused on methods for PFAS extraction and analysis in complex food samples of animal origin, which add complexity to sample preparation needs due to the presence of proteins and fats that can bind PFAS. For this study an alkaline extraction was performed using sodium hydroxide in methanol, followed by solid phase extraction (SPE) clean-up using mixed mode Weak Anion Exchange (WAX) chemistry with analysis performed using UHPLC-MS/MS. This extraction method was evaluated using a suite of 30 PFAS in six different food matrices: salmon, tilapia, ground beef, beef liver, beef kidney, and egg. Detection and quantitation limits were determined to be in the sub-ng/g range. Recoveries were within FDA criteria with utilization of isotope dilution for accurate correction of recovery during calculation of PFAS concentration in samples. Among the samples tested, five PFAS were detected in two different food samples purchased from local grocery stores. This comprehensive method allows for high confidence in results of PFAS in complex food matrices to allow for better monitoring and understanding of the environmental impact of PFAS on our food sources.

Keywords: *PFOA, PFOS, PFAS, animal products*

F14

DETERMINATION OF VOLATILE ORGANIC COMPOUNDS (VOCS) IN POLLEN FROM INDUSTRIALIZED AREA IN NORTHEASTERN ITALY BY USING HS-SPME AND GC/MS

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Volatile organic compounds (VOCs) are a large family of carbon-containing compounds including aliphatic, aromatic and chlorinated hydrocarbon compounds, aldehydes, ketones, esters, ethers, acids, and alcohols. VOCs are ubiquitous in the environment and come from a variety of natural and anthropogenic sources, such as biological processes or industrial and commercial processes with adverse health effects and environmental impacts. Honey bees and bee products, such as pollen, are potential bioindicators of the presence of contaminants in the environment, allowing the monitoring of large areas due to the long distances traveled by honey bees. Indeed, during foraging, the hairs covering the body collect suspended substances, including pollutants.
In this study, a HS-SPME-GC/MS method was developed for the identification and quantification of VOCs: benzene, toluene, ethylbenzene, xylenes (BTEX), ethyl acetate, butyl acetate, isobutanol, methylethylketone (MEK) and 1-methoxy-2-propanol, from pollen collected by honey bees in an area renowned for the tanning industry, located in the north-east of Italy. Solid-phase microextraction was applied as a selective technique to concentrate and isolate analytes. During the development of the method, several parameters were tested to determine the optimum conditions to reach the partition equilibrium of the analytes between the sample and the fiber in the HS mode, which results in increased VOC recovery after chromatographic analysis. The sampling system used for this analysis was the HTA - HT280T autosampler with solid phase microextraction system (SPME). A Shimadzu GCMS-QP2010 SE was used for separation and analysis. The quantitative determination was carried out using calibrated additions with internal standards on distinct portions of the same sample to balance the matrix effect, allowing achieving a LOQ of 0.010 mg/kg. The method was applied to 45 samples of pollen collected between April and September 2021, in sentinel apiaries positioned near the industrial areas and hilly "control" areas. VOCs have been detected in 36% of samples in both study areas, with concentrations varying between 0,010 and 0,107 mg/kg. Industrialized areas showed the presence mainly of BTEX, while the hilly area of alcohols, esters and ketones. To the best of our knowledge, this study is the first one in which HS SPME-GC/MS has been applied to determine VOCs in pollen. Our results support the use of honey bee products as biondicators for the determination of VOCs. Future studies will be directed to increase the number of VOCs determined by this method and to analyze additional pollen samples coming from industrialized areas.

Keywords: *VOCs, SPME, GC-MS, pollen*

F15

COMPARISON OF GAS, ULTRA-HIGH PERFORMANCE LIQUID AND SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED WITH HIGH RESOLUTION MASS SPECTROMETRY IN THE ANALYSIS OF CHLORINATED PARAFFINS IN FATS, OILS AND DIETARY SUPPLEMENTS

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Chlorinated paraffins (CPs) are an emerging and ubiquitous group of environmental pollutants associated with adverse effects on human health (such as endocrine disruption and possible carcinogenicity). They are divided into three main groups by their carbon chain lengths: (i) short- (SCCPs; C_{10}-C_{13}), (ii) medium- (MCCPs; C_{14}-C_{17}) and (iii) long-chain chlorinated paraffins (LCCPs; C_{18}-C_{30}). 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 commercially available standards.
In this study, the CPs were isolated from fish oil based dietary supplements (n=4; selected as type of samples with possible high levels of CPs contamination) by solid phase extraction on a silica gel (deactivated by 2% of water) and then analysed by three instrumental techniques, which were compared. The already validated and established gas chromatography method using 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) was compared to ultra-high performance liquid chromatography (Dionex UltiMate 3000 U-HPLC system, Thermo Fisher Scientific coupled with TripleTOF HRMS system, Sciex; the CPs were separated on Acquity UPLC BEH C18 (Waters) column (100 × 2.1 mm; 1.7 µm) and supercritical fluid chromatography (SFC; Acquity UPC^2 with coupled with Synapt G2 Si high-resolution mass spectrometer, both Waters; the analytes were separated on Viridis HSS C18 SB (Waters) column (100 × 3.0 mm; 1.8 µm) methods, both coupled with HRMS with electrospray ionisation operated in negative mode. The methods were validated, with recoveries in the range of 80 – 129% and repeatabilities (expressed as relative standard deviations) <19%. The limits of detection (LODs) for U-HPLC-HRMS method (from 0.03 to 0.05 µg/g lw) were 5 to 10 times lower than those obtained by SFC-HRMS (from 0.13 to 0.50 µg/lw). The LODs for GC-HRMS were the lowest - 0.005 and 0.013 µg/g lw (SCCPs and MCCPs, respectively). The methods were further compared by analysing coconut fat and lard samples from interlaboratory studies, therefore with assigned values of CP concentrations. Regarding the dietary supplements, the results obtained by SFC, U-HPLC and GC were comparable (SCCPs: 1.2 – 29.6 µg/g lw; 1.0 – 37.2 µg/g lw and 2.5 – 29.8 µg/g lw, respectively and MCCPs: 1.2 – 37.8 µg/g lw, 0.8 – 33.3 µg/g lw and 1.0 – 46.9 µg/g lw, respectively).

Keywords: *chlorinated paraffins, supercritical fluid chromatography, ultra-high performance liquid chromatography, high resolution mass spectrometry, dietary supplements*

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F16

A RAPID SCREENING AND QUANTITATIVE LC-MS/MS METHOD FOR FOOD AND ENVIRONMENTAL CONTAMINANTS USING THE ZENOTOF 7600 SYSTEM

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Pesticides are used before and after harvest to protect crops from infestation by pests and plant diseases. A consequence of their prevalent use in the environment is the appearance of pesticide residues in treated crops and ultimately in human food supplies. Pesticide residues have become a major concern in the area of food safety, and a robust and sensitive screening method for a vast range of pesticides in food matrices is a pressing need. To meet this need, a rapid and robust method was developed for the analysis of food and environmental contaminants such using the ZenoTOF 7600 system from SCIEX. Zeno trap technology significantly improves sensitivity and electron activated dissociation (EA. D) fragmentation technology improves the accuracy of qualitative analysis results. The ZenoTOF 7600 system is equipped with an EAD cell that simultaneously captures precursor ions and free electrons. Precursors then form a free radical that dissociates, often generating more fragment ions and a more informative MS/MS, which ensures precise quantification and qualitative analysis for food and environmental contaminant analysis.

Keywords: *Zeno trap technology, sensitivity, EAD fragmentation technology, accuracy*

F17

ARSENIC, CADMIUM ,CHROMIUM AND LEAD IN INFANT MILK FORMULA AND A PROBABILISTIC DIETARY RISK ASSESSMENT

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Formula milk is the primary nutritional source for infants and toddlers after breast milk. Infant formula is rich with fat, minerals, and protein levels of the human milk with addition of minerals, vitamins and iron. However, infant formulas may contain harmful chemical contaminants due to possible contamination of the raw material in the production chain. Formula may contain heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb) and others like aluminum, copper, zinc and nickel. There are several adverse effects for infants associated with inorganic contaminants, including anemia, nephrotoxicity, developmental and reproductive toxicity, lower IQ, and neurological effects. This project aims to measure the concentration of As, Cd, Cr, and Pb in infant and toddler milk in order to estimate daily dietary exposures and non-cancer hazard indices (HIs). Infant formula samples were collected from 13 brands for ages from newborn to 3 years (108 samples in total) and analyzed for metals using inductively coupled plasma mass spectrometry (ICP-MS). Sample preparation was largely based on simulating the method mentioned on children's cans, and then the extraction of samples was performed by a microwave-assisted digestion with HNO3. We estimated exposure to As, Cd, Cr, and Pb via infant food ingestion using deterministic methods for three different age groups: birth to <1 year; 1 year to <2 years; and 2 years to <3 years. Daily doses of infant milk analysts were compared to recommended intakes and toxicological reference points established by FAO/WHO, and other scientific agencies. The results showed that the concentration ranges were as follows: Cr (24.6±8.9), As (1.01±0.4), Cd (3.95±2.4) and Pb (0.3±0.2). All examined samples of infant formula milk had Pb, Cd, As, and Cr in levels below the reference safety levels. Cr and As were observed in all samples in varying concentrations, while Cd was present in 85 % of samples. The cumulative non-cancer hazard HI (95%) of different ages for all brands was below the permissible limits. Risk assessment indicates that when consumed, infant formula is unlikely to pose risks from heavy metals. However, it is essential to keep monitoring to check the potential toxic elements in infant formulas to protect the health of this sensitive population.

F18

GLYPHOSATE AND ITS MAIN DEGRADATION PRODUCT, AMPA, IN HONEY FROM ARGENTINA

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Argentina is one of the pioneer countries that adopted genetically modified (GM) crops and is currently one of the main producers along with USA and Brazil (ISAAA, 2019). In this scenario, glyphosate (N-(phosphonomethyl)-glycine) is a broad-spectrum, systemic and contact herbicide that is widely used because it attacks all weeds except GM crops such as soybean, corn or cotton. As a large amount of glyphosate is applied in Argentina and worldwide, human exposure and the eco-toxicological environmental risk have drastically increased. For this reason, the concentration levels of this herbicide and its main degradation product (aminomethylphosphonic acid, AMPA) are constantly reported in soil, water resources, rainwater and even in the air. The environmental contribution of glyphosate directly impacts food contamination, and particularly the argentine honey production is strongly affected. This situation motivated us to determine glyphosate and AMPA in honey samples from different regions of Argentina.
We developed the analytical method based on derivatization with FMOC because our goal was to achieve the lowest limit of quantification possible using RPLC-MS/MS. The method involves an extraction with water, then derivatization with FMOC-Cl in an alkaline medium (pH = 9), followed by clean-up by L-L partition. Finally, the extract was analyzed by reversed-phase liquid chromatography coupled to mass spectrometry. The methodology was validated following SANTE guidelines, obtaining satisfactory results in terms of recovery percentages and relative standard deviations (70-120% and ≤20%, respectively). The analysis of commercial honey samples will contribute to better understand the current incidence of glyphosate and its likely ubiquity in the argentine production system and to develop risk assessment strategies.

ISAAA, 2019. ISAAA Brief 55-2019: Executive Summary.Biotech Crops Drive Socio-Economic Development and Sustainable Environment in the New Frontier. https://www.isaaa.org/resources/publications/briefs/55/executivesummary/default.asp

Keywords: *glyphosate, AMPA, honey, LC-MS/MS*

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F19

OCCURRENCE AND RISK ASSESSMENT OF ENDOCRINE-DISRUPTING COMPOUNDS IN FISH MUSCLE: A CASE STUDY FROM DOURO RIVER ESTUARY, PORTUGAL

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The consumption of seafood has more than doubled in the last years; concurrently, the pollution of aquatic ecosystems has become increasingly prevalent at the global level. This study was focused on evaluating the occurrence in fish muscles of persistent organic pollutants (POPs), such as bisphenol analogues, personal care compounds, including some musk fragrances and UV-filters, and selected pesticides, associated with endocrine-disrupting activity. In total, 238 samples of fish muscle were analyzed, including the species flounder (*Platichthys flesus*, *n* = 80 individuals), grey mullet (*Mugil cephalus*; *n* = 79 individuals), and sea bass (*Dicentrarchus labrax*; *n* = 79 individuals). All fishes were captured in the Douro River estuary, Portugal (geographical coordinates between 41°8′37.95″N, 8°40′29.27″W and 41°8′39.11″N, 8°37′49.35″W) over the spring, summer, autumn, and winter, through 2019–2020 years. A sample preparation approach based on the QuEChERS procedure combined with dispersive liquid-liquid microextraction (DLLME), with in situ acetylation of bisphenols, was used followed by gas chromatography-mass spectrometry analysis. Adequate performance characteristics were obtained for the analytical method, including acceptable recoveries for all analytes under study within an accuracy range of 70–120%, and relative standard deviation (RSD) values ≤ 20%. Bisphenol A was detected in only three samples of grey mullet, all caught in spring, with a minimum and maximum content of 0.1 and 52.4 µg/kg ww, respectively. Among the personal care compounds, 2-ethylhexyl salicylate, tonalide, and galaxolide were the analytes most frequently found, amounting to 3.4%, 4.6%, and 15.9% of the samples, respectively. Mullet was the fish species with a greater occurrence of personal care compounds (52%). Interestingly, a higher number of positive samples was observed in the fishes collected during warm seasons, particularly in the spring. About 14% of the sample contained residues of at least one pesticide, with a particular incidence in mullet samples captured in spring. Alachlor, aldrin, *p,p'*-DDT, permethrin, and prochloraz were the main pesticides detected in the samples, with levels ranging from 0.1 µg/kg ww (*p,p'*-DDT) to 37.77 µg/kg ww (prochloraz). Based on a deterministic approach, the estimated daily intake (EDI) was calculated for adults (70 kg) based on the contaminant levels found in the muscle samples and the recommended consumption of fish per day, indicating that there is no health concern at the estimated levels of dietary exposure.

Keywords: *bisphenols, musk fragrances, ultraviolet filters, pesticide residues, GC-MS*

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F20

PESTICIDE RESIDUES AND OTHER ENDOCRINE DISRUPTOR CONTAMINANTS (BISPHENOLS, MUSKS, AND UV-FILTERS) IN BIOTA FROM THE ESTUARIES OF TAGUS AND DOURO RIVERS

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Estuaries are continually threatened as a result of anthropogenic pressures, which causes a large group of contaminants hazardous to human health is affecting the aquatic biota; therefore, it is necessary to monitor their quality.
This study deals with the determination of the levels of four types of endocrine disruptor contaminants (EDC) (21 pesticides, 4 polycyclic musk fragrances, 4 UV-filters, and 7 bisphenols) in several estuarine species (fish, bivalves, crustaceans, earthworms, and macroalgae) collected seasonally along one year in two distinct estuaries: Tagus River and Douro River.
The analysis was achieved by Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) extraction combined with dispersive liquid-liquid microextraction-derivatization, followed by gas chromatography-single quadrupole mass spectrometry (GC-MS). Validation showed recoveries for all target substances near 100% with an average relative standard deviation (RSD) lower than 19%.
In the Tagus river estuary, a total of 14 out of the 36 chemical compounds analyzed could be identified and quantified in the studied biota, including 5 pesticides (alachlor, ethion, *p,p'*-DDT, bifenthrin, and γ-chlordane), 2 musk fragrances (galaxolide and tonalide), 4 UV-filters (EHS, IMC, EHMC, BP3), and 3 bisphenols (BPF, BPA, and BPB).
Biota collected in the Douro River estuary presented lower levels of contamination with pesticide residues and bisphenols (BPs) than those of the Tagus river estuary. Contrariwise, levels and frequency of polycyclic musks and UV-filters were slightly higher in the Douro estuary.
In general, the highest level of analytes in the different species was found in summer in both estuaries.
Due to the contamination levels verified in this study, there is an urgent need to continue the monitoring and enlarge the number of analytes.

Keywords: *seafood, fish muscle, persistent organic pollutants, GC-MS*

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F21

LEVELS OF HEAVY AND TOXIC METALS IN POWER PLANTS OF KOSOVA

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Pollution of the environment by toxic metals is of great concern as the effect of these metals has a negative impact on when they enter the human body through the food chain. These metals often accumulate in the liver, intestines and gallbladder. Heavy toxic metals become dangerous if they start oxidation. They undergo changes and damage all cells. The problem is that they serve as the main food for bad bacteria, viruses, worms, parasites and fungi. This means that these metals can serve as a breeding ground for streptococci A and B, E-coli, H. pylori and viruses. In our study we analyzed the concentration of heavy metals such as As, Cd, Cr, Cu, Fe, Hg, As, Zn and Pb. Determination of the presence of heavy - toxic metals: As, Cd, Cr, Cu, Fe, Hg, Pb and Zn in dairy products such as yoghurt, cheese and cream. Samples of dairy products were collected from three different farmers who have pastures for their livestock near the Kosovo A and B power plants which are located close to each other. These dairy products like yoghurt, cheese and cream are traditionally produced by these farmers. For our research we have preferred exactly these farmers from whom we have taken samples. The sample digestion was performed using the Microwave Oven BS EN13805 method. The analysis is done using ICP-OES (EPA 6010C method). Consumption of dairy products a risk to the health of the local population and further studies are recommended to assess the exposure of pollutants, especially for some vulnerable categories of consumers.Consumption of dairy products in this region near the power plant is a very worrying indicator for the values of toxic heavy metals with highly toxic properties as well as the risk of cancer from As for the age categories.Heavy metals can cause problems which, if left untreated, make it impossible to remove them from food and water and as such enter the body. The best way to remove toxic heavy metals from the intestinal tract is by consuming some vegetables in the form of lettuce, smoothie or tea as they are, celery, parsley, spirulina, sage, garlic, plantain leaves, red clover.

Keywords: *pollution, heavy metals, toxicity, dairy products, Kosovo's power plants*

F22

PESTICIDE RESIDUE ANALYSIS IN VEGETABLES AND FOOD SAFETY CONCERNS OF PESTICIDE USED IN BANGLADESH

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The indiscriminate pesticide uses in vegetables has negative consequences on food safety in Bangladesh but the actual figures for pesticides being used in the country and the persistence of residues are not available. The present study investigated the pesticides used for the production of popular vegetables in Bangladesh; analyzed the pesticide residues on vegetables, soil, and water, and investigated the farmers' perception of the effects of pesticides on human health and the environment. The study was conducted in 5 districts during winter and 6 districts in summer. The study consisted of interviews with randomly selected vegetable farmers in selected 11 districts. The samples comprised 898 farmers (462 for winter and 436 for summer) of focal, proximal, and control farmers. Data were collected on five winter vegetables and five summer vegetables. Pesticide residues were analyzed in 25 winter vegetables, 5 water, 5 soil, and 50 summer vegetable samples by Gas chromatography (GC). Data shows that seven out of 25 winter vegetable samples had residues of organophosphate pesticides: Chlorpyrifos, Dimethoate, and Diazinon at very high concentrations ranging from 14.33 to as high as 323.16 µg/kg. Residues were undetectable in water and soil samples. While out of 50 summer vegetable samples, three Yard Long Bean, two bitter gourds, and one Lady's Finger samples were detected with the presence of Cypermethrin, a synthetic pyrethroid pesticide. Among the farmers, 70-90% agreed that pesticides are harmful to the environment. With few exceptions, farmers (90%) use protection during and after pesticide spray. The contamination levels of winter vegetables were significantly higher than those of summer vegetables. Consumption of such vegetables regularly, even at low levels of contamination, is a matter of important biosafety and environmental concern and can cause chronic illness, which can be fatal.

Keywords: *pesticide residue, vegetables, food safety, pest management, cypermethrin*

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F23

COMPREHENSIVE EVALUATION OF METALS IN SOIL, SACCHARUM OFFICINARUM AND JAGGERY WITH THEIR BIOACCUMULATION AND ASSOCIATED RISK ASSESSMENT: A CASE STUDY FROM LUCKNOW, UTTAR PRADESH, INDIA

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Heavy metal contamination of agricultural soil has emerged as a global issue threatening food security and human health. Toxic metal contamination in food is a major concern for public health and humans who are constantly exposed to toxic metals through the ingestion of crops grown in metal-contaminated soil. In this study, the investigation was carried out to evaluate metals bioaccumulation and health risks of metals contamination in soil, sugarcane, and jaggery in Lucknow region, Uttar Pradesh, India, which is located in the Gomti basin. To characterise the quantitative transfer of a given metal from soil to sugarcane, the Biological accumulation coefficient (BAC) was determined, which is useful for understanding the bioavailability of metals in soils.The process of determining the likelihood of any potentially negative health effects occurring during a given time period is known as Risk assessment.To perform the study, thirty (30) surface soil samples (bulk soil) and corresponding sugarcane samples, as well as jaggery samples, were collected and quantified for metals concentrations. The samples were prepared using microwave digestion method in order to determine the metals of interest.To determine the metal of interest in soil samples, they were digested with an HNO_3-HCl mixture (3:1) using the microwave system in accordance with USEPA 3051A Method. Sugarcane and jaggery samples were digested with a 6:1:1 HNO_3: HCl: H_2O_2 mixtureusing microwave digestion. Metal concentrations were determined using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Further, the method was validated in terms of various analytical parameters. The linear correlation coefficient (R^2) in the range of 0.9827-0.9999, LOD and LOQ was in the range of 0.0018-3.4407 ng/g and 0.00594-11.35431 ng/g respectively. The recovery calculated for soil, sugarcane and jaggery lies in between the range of 80.58-106.94, 67.06-98.47 and 80.63-106.96 respectively. The relative standard deviation was less than 5% for analytical results. The concentration levels of heavy metals in soil, sugarcane and jaggery showed a higher inequality. The BAC is less than 1 for B and Mo suggesting less movement of these two metals whereas, for other metals it was more than one indicating the higher uptake of metals in tested sugarcane from soil. In specific to heavy metals the highest BAC was observed for As (20.96663) and lowest was for Cr (1.30346).The estimated daily intake of heavy metals among adults via sugarcane consumption ranges from 0.015159 (Cd) to 6.99396 (Mn)mg/kg/day and THQ being greater than 1 suggests detrimental effects on local residents after consumption.In case of jaggery consumption the range is from 0.00003 (Cd) to 0.00514 (Zn) mg/kg/day and THQ less than 1 indicates no detrimental effects. The HRI for sugarcane > 1 suggesting not safe for consumption whereas for jaggery HRI < 1 which suggests its consumption to be safe.

Keywords: *saccharum officinarum, bioaccumulation, risk assessment, heavy metals, jaggery*

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F24

DEVELOPMENT OF A NON-SELECTIVE SAMPLE PREPARATION STRATEGY FOR A SEMI- AND NON-TARGETED CHEMICAL PROFILING OF FISH AND MILK

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Fish and milk are matrices of interest in the context of public health and dietary exposure to contaminants. However, their characterisation is difficult due to their high lipid contents, which complicate the extraction of pollutants, particularly the most lipophilic ones. In order to carry out a broad-spectrum global analysis, the challenge of the extraction step lies in its ability to be both non-selective in order to capture a maximum number of markers of interest and sufficiently efficient in terms of eliminating the main interfering endogenous compounds to limit matrix effects and achieve a sufficient level of sensitivity.
The aim of this study was to develop the most exhaustive extraction method possible from fish and milk samples in order to carry out suspect and non-targeted screening (SS/NTS) analyses. This work is conducted within the framework of the European PANORAMIX project (Green Deal H2020) coordinated by the Technical University of Denmark (DTU).
Eleven preparation protocols were applied on conventional whole cow milk and fatty fish from aquaculture (trout and salmon). Solid-liquid (or liquid-liquid) extraction based on one or two solvents (acetonitrile, ethyl acetate, hexane) was considered, with or without QuERChERS and with or without further SPE purification via two compared approaches (i.e. Z-sep or Captiva EMR-lipid). In addition, four sample amounts were tested (0.1 g to 2 g). The extracts were analysed by LC- and GC-HRMS. The performances were assessed by a set of reference exposure markers covering several families of substances and selected to guide this development and evaluate the performances of the tested methods (QA/QC). The results obtained from these different tests (n>100) show significant matrix effects (on average > 40%). Matrix effects were dependent on the exposure markers considered. Similarly, apparent recoveries range from less than 10% to more than 70%. Between half and two thirds of the 100 selected markers were detected depending on the methods applied. The reduction of the sample amount and a delipidation step showed the best results with better apparent recoveries and an average reduction of matrix effects about 15%.
An unselective sample preparation protocol including a delipidation step was developed. This methodology will be applied on a larger scale in order to contribute to documenting the chemical exposure profiles associated with the two food matrices of interest. Because of the constraints and difficulties inherent to these approaches, obtaining equivalent performance to the targeted analyses remains a challenge, especially with these complex lipid matrices.

Keywords: *sample preparation, fish, bovine milk, non-tageted analysis*

F25

SAFE FOOD FOR INFANTS IN THE EU AND CHINA (SAFFI): CHEMICAL HAZARD DETECTION AND DISCOVERY

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Food is a major route of exposure to anthropogenic chemicals in humans. Among the latter, children under the age of three represents a sensitive population group. Safety assessment of chemicals in food generally focuses on single chemical compounds analysis. With this approach, significant health issues caused by chemicals such as persistent organic pollutants (POPs) have successfully been reduced. Nonetheless, other emerging or unknown molecules are still overlooked while potentially causing adverse effects either alone or in mixtures. These effects can occur at low level or *via* repetitive exposure. As such, sensitive and complementary analytical methods are required to capture the presence and the effects of these unknowns and chemical mixtures. Furthermore, the increase in the number of chemical hazards that need to be monitored by the food companies calls for high throughput and cost-effective methods. In this context, the SAfe Food for Infants project (SAFFI, EU H2020 SFS 37,) bringing together both academic and industrial partners from Europe and China was initiated in 2020 (Engel et al., 2022). The SAFFI overall goal is to develop an integrated approach to enhance the identification, assessment, detection and mitigation of safety risks raised by microbial and chemical hazards all along European and Chinese infant food chains. This poster presentation will be centered around the development of novel approaches based on analytical chemistry and bioassays for the detection, monitoring and discovery of chemical hazards. Among these approaches, an innovative method based on sample pooling and data processing has been initiated for several known priority contaminant/food pairs to minimize analytical costs while still having the ability to identify outliers in pooled samples with little additional analysis. Additionally, several innovative targeted methods such as ion mobility are being evaluated in comparison to more conventional methods to improve throughput, sensitivity and selectivity of historic and emerging contaminants. Finally, SAFFI has been studying the complementarity between non-targeted chemical analyses and bioassays in a multi-step approach in order to discover unsuspected and unknown chemical hazards (Van der Burg et al., 2022). The initial identification at a realistic threshold of the presence of toxic compounds in infant food by bioassays and the implementation of high-resolution mass spectrometry with bio and chemo-informatics processing will provide a robust and efficient system to improve infant food safety.

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Keywords: *infant food safety, chemical mixtures, chemical analysis, bioassay, sample pooling*

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A SURVEY OF NITRITE AND NITRATE CONTENT IN "SUPERFOODS" BY IC-UV

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In the fitness communities, food and nutrition plays a very important role in the everyday life. The fitness industry often relies on tightly controlled and restricted diets consisting of a very limited number of food items that are considered extra healthy. These food items are often referred to as "superfoods". Sometimes a specific type of "superfood" will become fashionable and spill over into the diets of the general population. This type of restricted diet will lead to excessive intake of a few similar food types, and while this may contribute with a lot of healthy vitamins and other bioactive components, consumers also risk a larger exposure to contaminants that will come naturally with these types of food. In this study, we report results that are a part of a larger survey of superfoods products available for the Danish consumers, covering products of vegetable origin, as well as animal and marine origin. The poster will focus on nitrite and nitrate, the latter of which, is a contaminant that is known to be present in significant concentrations in green leafy vegetables such as spinach and arugula, in various "superfood" samples bought on the Danish market.
The method used for determining nitrite and nitrate is a modified version of the ISO EN 12014:2017 method described for meat products, using an alkaline extraction with a clean-up step and ion chromatography with UV detection at 225 nm with a secondary detection at 214 nm. The method achieved optimal separation for detecting nitrite in small quantities, even in samples with very high concentration of nitrate and a clean-up step was included for removing possible interferences. Overall the method is very well suited for complex food matrices.

Keywords: *contaminants, ion chromatography, spinach, vegetables*

F27

GC-ECNI-HRMS OPTIMIZATION FOR CHLORINATED PARAFFINS ANALYSIS

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Introduction: During the last decade, GC-ECNI-HRMS has become the most widely used analytical technique in the detection and the semi-quantification of chlorinated paraffins (CPs). However, it has already been suggested that some parameters, for example, the geometry of the ionization source, can impact the quantification of CPs, while the influence of the GC and ECNI parameters on CPs response are not well understood. Indeed, the complexity of the CP mixtures and their wide range of volatility as to be taken into account during the separation process. Therefore, the main objective of this study was to highlight the influence of some key parameters focused on the GC part and the response of CPs.
Materials and Methods: A mixture of six technical standards from Dr. Erhenstorfer including two SCCP mixtures, two MCCP mixtures and two LCCP mixtures, was analyzed using GC-ECNI-HRMS. In order to better understand the influence of the main GC and ionization source conditions, various parameters were tested. The influence of the column chemistry (Optima 1 or Optima 5 HT), the column length (30, 15, 12 or 10 m) and the column film thickness (0.25 or 0.10 µm) were studied on the detection sensitivity of CPs and the homologue group profile. Then, using the selected column, the influences of the injector, ion source and column oven temperatures were also studied.
Results and discussion: Comparing all the parameters listed above, optimal sensitivity conditions in the detection of CPs have been highlighted. These optimal conditions include a GC final oven temperature of 340 °C, an ion source temperature of 200 °C and an injector temperature of 280 °C. Using the OPTIMA 5 HT chromatographic column, a better sensitivity was reached compared to the OPTIMA 1 column. The column length and film thickness of 12.5 m and 0.10 µm, respectively, exhibited the best detection sensitivity on the widest range of CPs. Concerning the homologue group profile response factors, parameters that influence the most the response factor of CPs were the temperature of the ionization source, the film thickness as well as the chemistry of the stationary phase.
Conclusion: This study shows the best conditions to increase the detection sensitivity using GC-ECNI-HRMS and allow to identify the main parameters influencing the response factors of CPs.

Keywords: *GC-ECNI-HRMS, chloroparaffin, optimization*

F28

PFAS ANALYSIS AT LOW PPT-LEVEL IN FRUITS AND VEGETABLES

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Per and polyfluorinated compounds (PFAS) are a group of substances that recently have received a lot of attention. PFAS have versatile applications due to its exceptional chemical properties. PFAS are used, among other things, in grease- and/or water-repellent coatings, in fire-fighting foams, and in thermal and chemically resistant plastics for various applications. Due to their extensive use, PFAS can now be found everywhere in our environment and in varying food products. Because many PFAS are already harmful at low concentrations, it is of increasing importance that detection limits are reduced further and further.
In this current study, we developed a method to reliably detect and quantify a total of 20 PFAS down to the sub-ppt-level in five categories of fruits and vegetables. The food categories that were selected were: leaf crops, fruits, tuber crops, garlic/unions, and 'other' vegetables. In each category, six different fruits/vegetables were included in the validation. Fruits and vegetables were quantified based on matrix fortified calibration lines prepared in fruits or vegetables of the same category. The method is based on a solid-phase extraction (SPE; weak anion exchange), followed by detection by ultra-performance liquid chromatography (SCIEX Triple Quad™ 7500 LC-MS/MS). Separation was performed on a C18 column with methanol and an ammonium acetate buffer (20 mM) as the mobile phase. Low ppt-level detection limits were obtained by (1) increasing the sample intake, (2) lowering the solvent volume in the final extracts, and (3) implementing a new, sensitive LC-MS/MS system.
The method proved to be quantitative for the perfluorinated carboxylic acids, with chain lengths C5 – C14 and C16 (excluding C13, due to the lack of isotopically labeled internal standard), and the perfluorinated sulfonates with chain length C4 – C10, as well as for GenX, NaDONA, 9Cl-PF3ONS, and 11Cl-PF3OUdS. The method proved to be reliable to quantify concentrations of over three orders of magnitude, with the lowest quantification limits being 0.5 pg/g (0.5 ppt). The developed method was applied in a large-scale study of vegetables from kitchen gardens in the vicinity of large fluorochemical production plants (FPP) in the Netherlands. The study was performed on over 800 samples of various local fruits and vegetables. Arcadis provided the samples from these gardens. The samples in the vicinity of the FPPs showed elevated concentrations of PFHxA, PFHpA, PFOA, and GenX; especially concentrations of PFOA and GenX were significantly elevated up to 5 ng/g. Fruits and garlic/unions were, in general, less contaminated than leaf crops and tuber crops. The Dutch National Institute for Public Health and the Environment (RIVM) performed a risk assessment on the published results and has issued general advice regarding the consumption of vegetables from the gardens. The advisory report is published on the website of the RIVM.

Keywords: *PFAS, sub-ppt LOD, LC-MS/MS, SPE*

Acknowledgement: *The local authorities close to the FPP are gratefully acknowledged. Arcadis is gratefully acknowledged for providing the samples.*

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ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN EDIBLE OILS BY A SIMPLE AND FAST CLEANUP METHOD BASED ON MOLECULARLY IMPRINTED POLYMERS

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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 mostly by intake of food. As these compounds are highly soluble in lipophilic matrices, edible oils can be an important source of contamination by PAHs. In 2011, EU Commission Regulation No 835/2011, amending Regulation 1881/2006, 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.
Some matrices remain challenging to analyze. To preserve the integrity of analytical devices and to reach a certain limit of detection, it is often necessary to perform sample preparation. The molecularly Imprinted Polymer (MIP) technology applied to Solid Phase Extraction (SPE) brings higher selectivity in comparison to classic polymeric or silica based SPE. It allows a thorough sample cleaning prior to analysis.
For PAH analysis, both canola and olive oils were tested. To do so, they were spiked with a mixture of 8 PAHs at 2µg/kg which is compliant with the maximum levels fixed by European regulation. Recovery yields ranging from 83% to 95% were obtained, with relative standard deviation inferior to 8%. The analyses were carried out by GC-MS/MS by the national reference laboratory LABERCA. Moreover, the sample cleaning was also demonstrated to be suitable for LC-FLD analysis.
In a second set of experiments, PAHs analysis were performed on a "full spectrum" type CBD oil which is a more complex type of oil to analyze. The cleaning procedure was carried out on the oil using a combination of a "Pass-through" SPE cleanup and a MIP cartridge. The preliminary data are presented in this work. Very good results were obtained with an efficient cleanup and satisfying recovery yields.

Keywords: *polycyclic aromatic hydrocarbons (PAHs), sample preparation, Oils, SPE, mass spectromerty*

F30

PFAS: FROM ENVIRONMENT TO WATER AND FOOD; THE PFAS CONTAMINATION HAS REACHED OUR FORK. HOW DO YOU FEEL?

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Between 2010 and 2020 the PFAS (Per- and polyfluoroalkyl substances) were identified by EFSA as an emerging risk at the food level for the health of consumers[1] and the evaluation of the data obtained from the subsequent monitoring carried out at European level has led to a first regulation[2] and subsequent updates and revisions which are still in progress today.
Since 2009, PFOS, PFOA and their derivatives have been included in the Stockholm Convention to eliminate their use. Similarly, Perfluorohexane sulfonic acid (PFHxS) and derivatives are being evaluated for inclusion in the same list. Starting from July 2020, the production and marketing of PFOS and its derivatives as a substance as such was prohibited. In particular, new legal limits on food for human consumption are expected by mid-end 2022 (update of Regulation (EC) No 1881/2006 on animal origin products) and action limits on baby food (EU COMMISSION RECOMMENDATION on the monitoring of perfluoroalkylated substances).
Mérieux NutriSciences has been working on PFAS since the first emergencies in 2017-2018 which mainly affected the territories of the provinces of Vicenza, Verona and Padua (for an estimated population of 300,000 inhabitants) and has had the opportunity in recent years to develop, validate, and apply chemical-analytical methods for the determination of PFAS based on liquid chromatography with mass spectrometry at both high resolution (HRMS and MS/HRMS Orbitrap technology) and low resolution (MS/MS MRM quadrupole technology).
Today, in line with the provisions of EURL 2022[3], we are able to achieve performances in terms of selectivity, specificity and sensitivity that were unthinkable until a few months ago. We are talking about validated and accredited LOQ values equal to 1 ppt on the main 4 PFAS such as PFOA, PFOS, PFNA and L-PFHxS on the most critical matrices from the toxicological point of view regarding human nutrition: baby food.

[1] Risk to human health related to the presence of perfluoroalkyl substances in food, EFSA Journal 2020;18(9):6223.
[2] Regulation (EC) No 1881/2006 on animal origin products.
[3] EURL for halogenated POPs in feed and food (2022): Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed (version 1.2 of 11 May 2022).

Keywords: *PFAS, contaminants, food, baby food, circular economy, mass spectrometry*

F31

EXPERIENCES OF DEVELOPING AND VALIDATING A ROBUST MULTI-PFAS METHOD FOR THE ANALYSIS OF FOOD AND ENVIRONMENTAL SAMPLES

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Per-and polyfluoroalkyl substances (PFAS) are a complex group of man-made chemicals. The Organization of Economic Co-operation and Development identified 4730 PFAS related CAS numbers[1]. PFASs were first introduced to the global market in the 1930's and have been used in a variety of applications[2]. They prevent food from sticking to cookware, make textiles resistant to water and stains, and create firefighting foam. To date, most of the analytical efforts have focused on PFAS in the environment. The estimation of PFAS exposure from food sources is rapidly increasing in food safety evaluation. Current initiatives are in food contact materials, food from regions directly impacted from environmental contamination, and certain food types having an increased potential of PFAS contamination. In its opinion of 2020, European Food Safety Authority highlighted the need for more sensitive analytical methods for PFAS in food[3].

We present the development of a multi-analyte procedure using targeted LC-MS/MS for the quantitation of 30 legacy and emerging PFAS in a variety of food matrices following the recent EURL guidance document on PFAS analysis[4]. An isotope dilution mass spectrometry method was developed as the "gold standard" for quantitative PFAS analysis. With many food safety laboratories currently developing LC-MS/MS based methods for PFAS testing ahead of forthcoming EU Regulations, we evaluated the performance of different models and generations of technology and characterise the capabilities and limitations. Advances in MS technology contribute to improvements in sensitivity and the performance of analytical methods, here we report our findings from working with "real world" samples.

Extraction techniques including solid phase extraction (SPE); accelerated solvent extraction (ASE); "QuEChERS like" and dispersive SPE (dSPE) were evaluated for a variety of food commodities and considered for automation of the process. A thorough assessment of the commercially available analytical columns was performed. Our findings and recommendations for the ultra-short chain (USC), short to mid chain, long chain sulfonates and carboxylates and the emerging compounds are presented. A comparison of two ionisation mechanisms, electrospray (ESI) and Unispray (US) in negative polarity was also conducted. The performance of the two ionisation mechanisms is presented across the scope of the PFAS in terms of signal intensity and signal to noise (S/N) in food matrices. Preliminary observations relating to background levels of PFAS in UK food supply is reported. Finally, we share our experiences, recommendations, and best practices for setting up a targeted method for the accurate quantitation of PFAS in food commodities.

1] https://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/
2] Buck, R.C., *et al. Integr Environ Asses*. 2011 (7)513–541.
3] EFSA Journal 2020;18(9):6223
4] https://eurl-pops.eu/news/guidance-document-pfas/guidance-document-pfas

Keywords: *PFAS, environmental contaminants, isotope dilution mass spectrometry, food contaminants*

Acknowledgement: *The Food Standards Agency (FSA), U.K. are kindly acknowledged for provision of financial support.*

F32

POLYMERIC SPE SORBENTS FOR PFAS EXTRACTION AND CLEAN-UP IN DIFFERENT MATRICES

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Introduction: Per- and polyfluorinated alkyl substances (PFAS) products have been in use for more than 60 years. They get into the environment during their manufacturing process and also during their use and disposal. The analytical interest in these compounds has rapidly increased in the last few years. Research has revealed the high toxicity of PFAS compounds and thus the resulting need to regulate the substances. Analyzing these PFAS compounds is challenging and dedicated lab equipment helps to avoid blind values and standardizes the processes in sample preparation. The US-EPA and US-DoD have already published several methods for PFAS analysis in different matrices. For the compliance of these methods, a list of acceptance and quality control criteria have to be fulfilled.
Materials and Methods: The full workflow of PFAS sample preparation is presented. Analyte extraction of environmental samples were conducted via a new vertical shaker (MIX-TRACTION). For the enrichment and/or purification of PFAS compounds manual and automated solid phase extraction (SPE) via FREESTYLE with single cartridge solutions of different compositions were used. For the critical evaporation step a vacuum centrifuge with cold trap (D-EVA) is used. Results: The MIX-TRACTION shaker ensures that all samples are shaken under identical conditions, thus showing consistent and reliable extraction results. The newly introduced polymeric sorbents successfully enrich and purify PFAS compounds to meet the requirements of the US-EPA and US-DoD methods. Single SPE cartridge solutions of different compositions show high recoveries, low standard variations and reliable clean-up of PFAS analytes in different matrices. The automated FREESTYLE system is proven to perform without blind values and allows to run predefined methods while leaving space for ongoing method development. The D-EVA avoids the loss of neutral PFAS, has handling advantages, saves time and reduces errors.
Conclusions: The presented workflow shows a streamlined sample preparation process for the PFAS analysis with new polymeric SPE cartridges with a superior matrix reduction performance in specific samples.

Keywords: *PFAS, clean-Up, SPE, solid phase extraction, US EPA*

F33

EVALUATION OF SELECTED PERSISTENT ORGANIC POLLUTANTS IN BIVALVES FROM THE BULGARIAN BLACK SEA COAST

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In last decades the levels of persistent organic pollutants (POPs) in the environment are steadily declining, but residues of polychlorinated biphenyls (PCBs), organochlorine pesticides and polycyclic aromatic hydrocarbons (PAHs) continue to wide spread in marine ecosystems and to bioaccumulate in animal tissues. Bivalves are often used as bioindicators of pollutants in marine environment due to their higher capability of bioaccumulation. The aim of the study was to assess the present contamination status of POPs using black mussel (*Mytilus galloprovincialis*). Wild and cultivated mussels were collected from different sites of the Bulgarian Black Sea coast in the period 2021 – 2022. The concentrations of 13 PAHs, 15 PCBs and organochlorine pesticides (such as DDTs and HCHs) were determined in mussel soft tissues by simultaneous extraction of POPs in accelerated solvent extractor (ASE) and were detected by gas chromatography system with mass spectrometry (GC-MS).
The total levels of DDTs, PAHs and PCBs in mussels from Bulgarian Black Sea coast were found 1.5 ng/g ww (wet weight), 22.1 ng/g ww and 1.2 ng/g ww, respectively. Lindane and its isomers had very low concentrations in all samples investigated. The metabolite p,p'-DDE was the abundant organochlorine contaminant in mussel samples (from 0.73 to 1.66 ng/g ww). DDT was present mainly in the form of its metabolites p,p'- DDE and p,p'- DDD, suggesting contamination in the past. The most of dioxin-like PCB congeners were found below the analytical detection limits in all mussel samples. Regarding PAHs contamination, the phenanthrene and fluorene were the most abundant compounds in all samples investigated. Although benzo(a)pyrene was detected in 25% of analyzed samples, the concentrations did not exceed the limit set in EC Regulation. The results showed that low molecular weight (LMW) PAHs (3 and 4 aromatic rings) were predominant accounting 97% of total PAH levels. The ratio LMW/HMW PAHs was higher than one, suggesting petrogenic origin of pollution. The levels of PCBs, DDTs and PAHs in mussels were found lower or comparable to levels measured in the similar species from other aquatic ecosystems.These results confirm that the persistent organic pollutants continue to be present in marine environment in the Black Sea.

Keywords: *polychlorinated biphenyls, organochlorine pesticides, polycyclic aromatic hydrocarbons, mussels, Black Sea*

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F34

CONSTRUCTING A ROBUST HIGH THROUGHPUT SCREENING WORKFLOW FOR ANIMAL FEED UTILISING TARGET, SUSPECT AND NON-TARGET DATA

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In a typical target or suspect screening workflow authentic standards are used where available to ensure an analytical data processing method has confidence in annotating the correct identification of a given compound, in addition as per the associated guidelines fragmentation information in the form of qualifier ions are also used to confirm the identification. The approach runs into problems when there is a requirement to measure hundreds of compounds in a high throughput manner due to time constraints in the chromatographic space and also due to related compounds sharing very similar fragments.
When screening for large numbers of compounds a triple quadrupole mass spectrometer is limited by dwell time especially when speed is of most importance. In this work we describe a workflow utilising UHPLC-QToF for robust high throughput screening of feed. Building a database with >600 targets, hundreds of suspect compounds including predicted retention time and non-target information from random screening constantly updates the targets/suspects using structure elucidation.
More importantly we have developed a robust software screening workflow which uses isotope pattern, retention time/predicted retention time with appropriate windows and qualifiers. Method parameters have been setup individually at a compound level and the associated databases have been created and curated extensively. Robustness testing of the analytical and data processing method is currently underway and preliminary results will be shown.

Keywords: *screening, QTOF, high throughput, UHPLC*

F35

OCCURENCE OF PERFLUOROALKYL SUSTANCES (PFAS) IN POTENTIALLY CONTAMINATED DRINKING WATER SOURCES IN THECZECH REPUBLIC

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Per- and polyfluoroalkyl substances (PFAS) are a complex group of manmade environmental pollutants. Owing to their stability, they are persistent in the environment and may bioaccumulate in organisms and food chains. Widespread surface and groundwater contamination with PFAS as become of great concern in the last few years. In 2020, the European Food Safety Authority (EFSA) CONTAM Panel, reevaluated and reduced tolerable weekly intake (TWI) of 4.4 ng/kg bw per week for Σ4 PFAS (perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorohexane sulfonic (PFHxS) and perfluorooctane sulfonic acid (PFOS)), as the knowledge on their harmful effects on human health is broadening. The new European Union Directive 2020/2184 on the quality of water intended for human consumption from December 2020 defines two parameters for monitoring: 'sum of PFAS' (sum of ten perfluorocarboxylic acids, PFCAs, and ten perfluorosulfonic acids, PFSAs, C4-13) limited up to 0.1 µg/L and 'PFAS total' with limit up to 0.5 µg/L. The main aim of this study is investigation of sources of drinking water with a potentially higher level of PFAS contamination which could represent a higher health risk for the consumer. Based on obtained results, the estimated weekly intakes (EWIs) from drinking water will be calculated and evaluated with respect to the latest EFSA´s TWI. For the analysis a total of 26 PFAS, the method based on solid phase extraction catridge (weak anion exchange) followed by ultra-performance liquid chromatography coupled to tandem mass spectrometry with low limits of quantification (0.02–0.25 ng/L), recoveries 70–120% and repeatabilities ≤ 20%.
The most abundant contaminants (%positives, median, min-max in ng/L) were perfluoropentanoic (PFPeA, 92, 0.42, <0.02-21.75), perfluorohexanoic (PFHxA, 83, 0.34, <0.02-28.08) and perfluoroheptanoic acid (PFHpA, 79, 0.31, <0.02-13.13). PFOS isomers were found in 52% of samples in concentration ranging from <0.02-5.81 ng/L. Median and maximum concentration of Σ4 PFAS in water samples can be filled 4.1% and 240%, respectively, of the TWI intake for average consumer (70 kg bw, 2 liters). Obtained results shows that the drinking of commonly available tap water can contribute to dietary exposure to PFAS. But, the occurrence of PFAS in drinking water in the Czech Republic is relatively low, is up to 90× lower than the current limit for 'sum of PFAS' (< 0.1 µg/L), established in the EU Drinking Water Directive (EU 2020/2184).

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Keywords: *PFAS, drinking water, LC-MS/MS*

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F36

DETERMINATION OF MINERAL ELEMENTS IN ETHNIC FOOD PURCHASED IN THE MARKETS OF SOUTHERN ITALY

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The consumption of Oriental ethnic products, in particular seaweed and dried fish [1], is now strongly present in European cuisine. These products, however, may have negative characteristics terms of food safety, especially due to possible contamination by toxic elements [2].
In this regard, in this study, the content of six toxic and potentially toxic mineral elements (As, Cd, Cr, Hg, Pb, Tl) in seaweed and dried fish purchased from markets located in southern Italy was evaluated. In addition, iodine in seaweed was quantified. The most abundant element in algae was arsenic (mean = 8.19 ± 6.62 mg/kg), followed by cadmium (0.38 ± 0.25 mg/kg), and lead (0.12 ± 0.10 mg/kg).
In fish, however, As was always the most abundant element (mean = 0.47 ± 0.37 mg/kg), this time followed by Hg (0.12 ± 0.07 mg/kg). Only 19.2%, or 5 out of a total of 26 seaweed samples exceeded the Cd limit set by the *Center d'Etude et de Valorization des Algues (CEVA, France)* [3], while 1 out of 8 fish samples, or 12.5%, exceeded the maximum Pb limit imposed by *Regulation (EC) No. 1881/2006*. Correlations were observed between Hg-As, Cr-Tl, Pb-Tl (seaweed), and As-Cd, As-Pb, Cd-Pb, As-Cr (fish). Chemometric analysis allowed optimal grouping of samples by product type, while that by geographic origin was less accurate and effective.
In conclusion, although the content of non-essential minerals in the fish and seaweed analyzed, as well as the iodine levels in algae, did not show results of concern, nevertheless continuous monitoring is essential to constantly assess their possible contamination and their potential risk to the consumer.

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3. CEVA, Centre d'Etude et de Valorisation des Algues. (2014). *Edible seaweed and Frenche regulation-synthesis made by CEVA (31/03/2014)*. Pleubian, France.

Keywords: *toxic elements, ethnic foods, food safety, fish, seaweed*

F37

MINERAL CONTENT IN WELLNESS HERBAL TEAS

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The consumption of wellness herbal teas has now reached very high levels. Their use can bring several beneficial effects, but negative ones also. In fact, environmental chemical residues, such as toxic and potentially toxic mineral elements in plants used to formulate herbal teas can be accumulated [1,2].
In this research samples of seven tea types were analyzed: mint, fennel, moringa, mate, chamomile, rooibos, and licorice. The teas were packed in pods and filter. The analyses were first carried out on the leaf samples and then on the corresponding herbal drinks obtained by infusion.
In all 28 samples the content of Mg, Ca, Na, K, Zn, Mn, Co, Cr, B, Fe, Cu, Ni, Mo, Se, Ba, Al, Cd, As and Pb was determined by ICP-MS and of Hg by DMA-80; the percentage of transfer from the dry material to the drink obtained from the infusion of the same was calculated; and finally, was evaluated if the intake of these herbal teas can positively or negatively affect the consumer health.
Among the pod teas, mint showed the highest amount of potassium and calcium; while the highest contribution of magnesium and sodium was found in moringa and rooibos, respectively. Among filter herbal tea samples, the same trend was observed, except for potassium which was present at higher concentration in fennel. Iron, zinc, and copper were present in abundant amounts in both pods and filters. Again, all samples showed a concentration of lead and cadmium below the legal limits.
The percentage of transfer from the dry material to the infusion was higher for filter than pod herbal teas. Among the latter, moringa and chamomile samples had the highest transfer rates; while among those in filter, the highest transfer rates were observed in mint, rooibos, chamomile, and mate samples.
The consumption of one cup (250 mL for pods, 150 mL for filters) of herbal teas resulted in a full amount of magnesium and manganese intake (the value exceeded the Recommended Dietary Allowance in all analyzed samples), and of Ni and B (in almost all analyzed samples). The trends of the other minerals were very variable but did not exceed the daily intakes.

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Keywords: *wellness herbal tea, mineral content, ICP-MS, DMA-80, mineral intake*

F38

SIMULTANEOUS DETERMINATION OF MELAMINE AND PRIMARY AROMATIC AMINES IN AÇAÍ-BASED (EUTERPE OLERACEA MART.) PRODUCTS BY UPLC-MS-MS

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Overall, the *açaí*-based products are stored in colorful packaging. For this reason, it may be a source for migration of primary aromatic amines into food. In addition, melamine contamination of food can occur through leaching from the packaging to the food. Furthermore, this compound may come from fertilizers or classified as an environmental contaminant. In this study, an ultra-high performance liquid chromatography coupled to triple-quadrupole mass spectrometry (UPLC-QqQ-MS) method is described for the simultaneous identification and quantification of melamine (MEL / CAS 108-78-1) and primary aromatic amines (benzidine (BNZ / CAS 92-87-5); *o*-toluidine (o-T / CAS 95-53-4); 4,4'-methylenedianiline (4,4-MDA - CAS 101-77-9)) in *açaí*-based products. Firstly, the compounds were extracted using 3% HAc, followed by clean-up process in the SPE cartridges with a retention mechanism of cation exchange. All targeted analytes investigated in this work were successfully validated. The fitness-of-purpose of the method was verified by analytical selectivity, limits of detection (LOD – 0.20 up to 1.5 μg kg^{-1}) and quantification (LOQ – 0.67 – 2.7 μg kg^{-1}), linearity in solvent and matrix-matched calibration curves ($R^2 \geq 0.0995$) and adequate recoveries (81–111 %) and precision (RSDs ≤ 19.21 %), under repeatability and within-laboratory reproducibility conditions. The validated method was applied to commercial *açaí*-based products samples, and the investigated compounds were not detected in any sample. In summary, the application of the developed method to *açaí*-based products marketed in Brazil contributes to the first set of data on melamine and PAAs.

Keywords: *UPLC-QqQ-MS, açaí, validation, primary aromatic amines*

Acknowledgement: *The authors are grateful to the São Paulo Research Foundation (FAPESP) for financial support (grant #2020/10990-1 and 2020/02728-5) and Project RTI2018-097805-B-I00 from the Spanish Ministry of Science and Innovation.*

F39

IDENTIFICATION OF MICROPLASTICS IN WATER AND FOOD USING PYROLYSIS GC WITH HIGH RESOLUTION ORBITRAP MASS SPECTROMETRY

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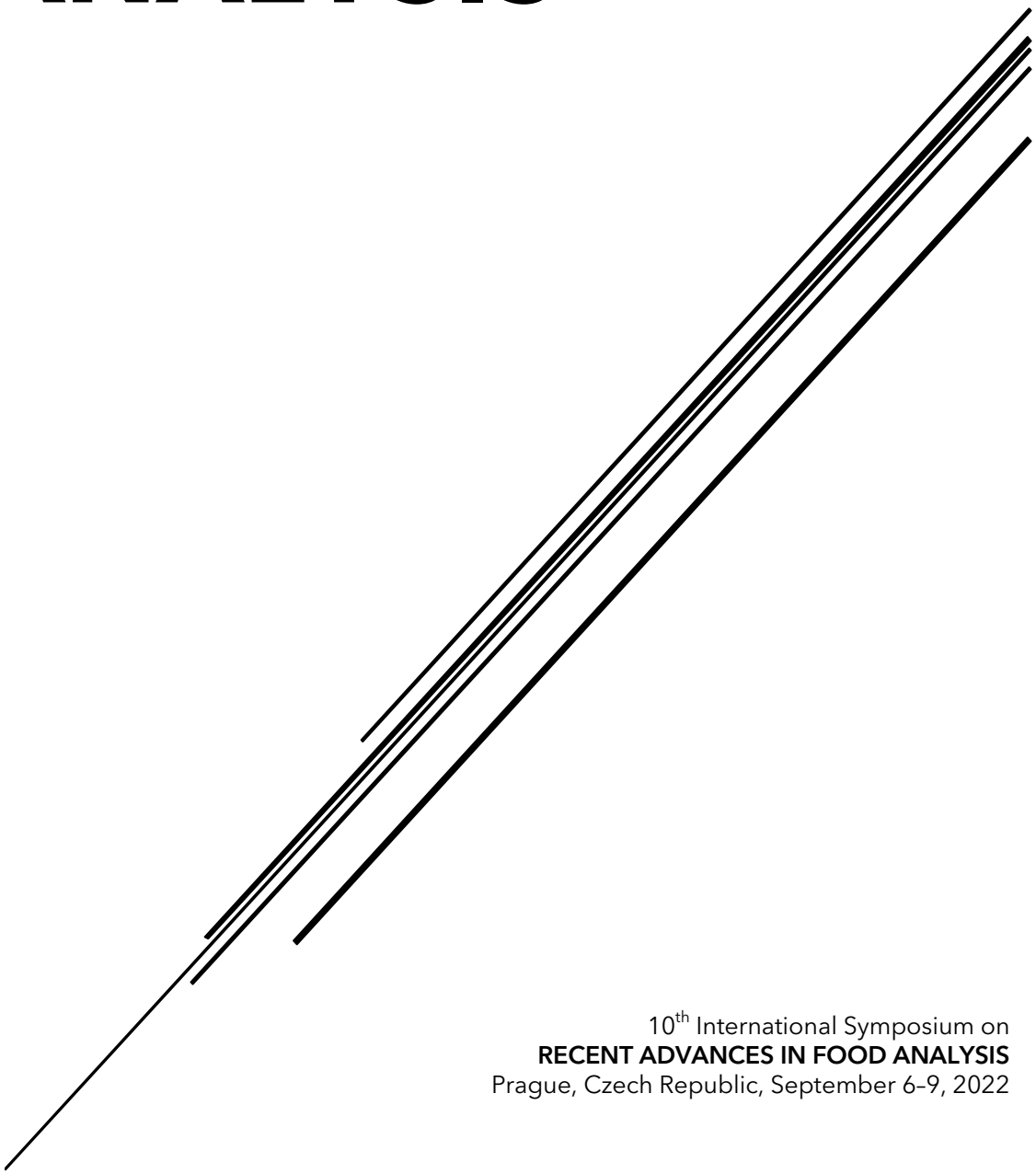
Microplastics are small particles made from synthetic polymers with a diameter typically ranging between 5 mm and 1 µm; nanoparticles cover particles sizes of sub 1 µm, because of their small size they can migrate very quickly and can be easily incorporated into the food chain. Microplastics may consist of not only the pure synthetic polymer, but also include residuals of the monomer, plasticizers, flame retardants, and many other toxic additives that can have a negative impact on human health. Among the analytical techniques used for the analysis of microplastics, Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy and microscopy-based techniques. However, especially for microscopy-based analysis, the number of samples that can be screened is limited. Pyrolysis gas chromatography-mass spectrometry (py-GC-MS) presents a promising alternative for surveillance and identification of microplastics where throughput is critical. Furthermore, this analytical approach enables time-saving detection of bulk amounts of micro- and nanoplastics below the lower size limit of the microscopy techniques.
A pyrolizer (Frontier Laboratories) was mounted on a Thermo Scientific™ Orbitrap Exploris™ GC 240 mass spectrometer. The mass spectrometer was operated in full-scan mode using 60,000 mass resolving power.A double-shot method was used for the analysis of the food and environmental samples.
A series of polymer standards were subjected to pyrolysis to find characteristic fragmentation products that can be used for polymer identification in unknown samples. The resulting pyrograms were screened to find the pyrolysis products known from the literature. To simplify further data treatment during the analysis of samples, a targeted processing method was created. The processing method included all compounds previously identified, with each compound´s presence confirmed using a minimum of three representative ions extracted from the TIC using a mass extraction window of ±5 ppm. During the data processing, benzene, naphthalene, and fluorene were found in the stormwater sample. Styrene, allylbenzene, α-methylstyrene, and toluene were revealed in two remaining samples (milk and steak). The study demonstrates that Py-GC-Orbitrap is a robust tool for the confirmation of the presence and identity of microplastics in different sample types. High selectivity and sensitivity were achieved in combination with a targeted screening approach using both Compound Discoverer software and Chromeleon software. The presented method allows for target microplastics analysis in different sample types, including complex food and environmental matrices.

Keywords: *microplastics, py-GC-MS, gas chromatography, Orbitrap MS, milk, meat, water*

Acknowledgement: *Cassie Rauert, University of Queensland for method consultation and supplying samples.*

GENERAL FOOD ANALYSIS



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G1

DEVELOPMENT OF INNOVATIVE ACTIVE ANTIOXIDANT FOOD PACKAGING SYSTEMS BASED ON NATURAL EXTRACT FROM FOOD INDUSTRY WASTE

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The protection of food from contamination or degradation is an important aspect in the field of food safety and in the "EU green deal" economy purpose of reducing food waste. Antimicrobial and antioxidant packaging represent a future solution with a strong impact on the packaging industry and the citizens health. Developing stable and safe materials from food waste on an industrial scale is a challenge and requires standard procedures and methods for evaluating the packaging performance.
This work concerns the development of innovative active packaging materials, starting from wine and olive oil industrial waste products (seeds, skin, pomace) and from *Moringa oleifera* leaves, obtained by different extractive procedures.
The active ingredients obtained from maceration were analyzed and the antioxidant efficacy of many fractions of the plant extracts was measured by multiple standard assays and the results were correlated with their polyphenols content and implemented in different ways on a biodegradable cellulose-based product.
The antioxidant properties of the obtained new active films were measured by both indirect and direct analytical methods demonstrating good free radical scavenging properties for all the three kind of active agents and a good correlation of the results. The highest radical reduction capacity, related to a higher content of polyphenols, was obtained by the films with 5% w/w of moringa and grape extracts added to cellulose by coating (direct food-contact) and in the adhesive between two layer of cellulose (indirect action mechanism), respectively. Therefore, they were tested for their ability of delaying oxidation of ground beef meat, as an application on a real complex food matrix.
Both packaging systems revealed to prevent meat from oxidation by at least 50% after 16 days compared to simple cellulose, result which was confirmed by both canonical assays (like thiobarbituric acid reactive substances) and *in situ* analysis of the meat performed by vibrational spectroscopies.
The aim of this work is to develop new prototype laboratory-scale materials in a green chemistry context that can be easily transferred to the industrial level. Furthermore, this work, proposing the use of sustainable and degradable materials and an innovative way to recover food industry waste to prolong the food shelf-life, perfectly fits in the actual compelling need to reduce pollution and global waste production in accordance with the recently signed European Green Deal purposes of more sustainable "from farm to fork" food production systems.

Keywords: *active packaging, natural antioxidants, food shelf-life prolongation, food industry waste recovery, sustainable packaging*

Acknowledgement: *We kindly acknowledge the Department of Analytical Chemistry of the Aragon Institute of Engineering Research I3A, EINA-University of Zaragoza, Spain, and especially Prof. Francisco Jesùs Salafranca Lazaro for the materials, the extractions and the support on packaging realization.*

G2

ASSESSMENT OF ANTIMICROBIAL RESISTANCE OF PSEUDOMONAS AEROGINUSA IN BOTTLED DRINKING WATER

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Pseudomonas is a type of bacteria (germ) that is found commonly in the environment, like in soil and in water. Of the many different types of *Pseudomonas*, the one that most often causes infections in humans is called *Pseudomonas aeruginosa* is an opportunistic gram-negative bacilli, aerobic, and motile, which can cause infections in the blood, lungs (pneumonia), or other parts of the body after surgery.
These bacteria are constantly finding new ways to avoid the effects of the antibiotics used to treat the infections they cause. Antibiotic resistances occurs when the germs no longer respond to the antibiotics designed to kill them.
The study was conducted in order to assess antimicrobial resistance of *Pseudomonas aeruginosa* in bottled drinking water.
In SFDA labs, *Pseudomonas aeruginosa* are one of the routinely tested organism in bottled water according to GSO 1016/2015. *Pseudomonas aeruginosa* was Detected by method of ISO 16266 Water quality – Detection and enumeration of Pseudomonas aeruginosa –by membrane filtration. Forty four isolates from Riyadh,10 Isolates from Jeddah, and 6 Isolates from Dammam of *Pseudomonas aeruginosa* were collected during the period between April 2020 to November 2020 and send to Riyadh lab for antimicrobial resistance testing by Vitek 2 according to the manufacturer instruction.
Samples were classified according to the geographic territories. the results of resistance in central region was 50% , While in western region was 33%, in the eastern region was 10% ,5% in northern region and Finally southern region showed 2%. Three different patterns of antibiotic resistance were detected. These three patterns are consistent in the following antibiotics: Ampicillin/sulbactam, Minocycline, and Tigecycline. The results showed that all isolates (60 isolates) had the same inhibitory concentration. While these three types differ in one antibiotic, which is Trimethoprim/Sulfamethoxazole, where the resistance of bacteria was in the first type at a concentration of (80) in (46 isolates), and in the second type at a concentration (160) in (11 isolates), and for the third type at a concentration of (40) in (3 isolates).
From these results, it becomes clear to us that all the bacteria that were isolated were resistant to more than one type of antibiotic, and this makes them classified as multi drug resistant bacteria - MDR, which makes them of high risk to consumers' health

Keywords: *assessment*

Acknowledgement: *Dammam Lab and Jeddah Lab*

G3

MONITORING OF PRESENCE OF HISTAMIN IN FISH AND FISH PRODUCTS IMPORTED IN KOSOVO

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Histamine is one of the most important and toxic biogenic amine in food. It is produced during bacterial decarboxylation of the histidine present in fish muscles. The key actions to prevent production of histamine are adequate refrigeration. It is also important to respect the time and low temperature during unloading, transportation, storage, and processing of fish. Determination of histamine is important not only because of its toxicity for humans, but also as an indicator of the freshness of fish and fish products.
According to national legislation which is harmonized from EU legislation (Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs), Food Safety Competent authority based on annual official control plan, collect the samples of fish and fish products from import, in order to monitor presence of histamin.
Histamine concentration in samples collected from fish and fish products did not exceed the allowable limit, indicating that they are safe for consumers.

Keywords: *smoked fish, EU Scientific Committee on Food (SCF), carcinogenic, fish products, histamin*

G4

BLOCKCHAIN AND IOT BASED FOOD SAFETY MONITORING FRAMEWORK FOR FOOD SUPPLY CHAINS

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Blockchain and IoT are recognized as key technologies enabling process automation and boosting performance and trustworthiness in modern food supply chains. The blockchain technology is being applied to automate order matching procedures and provide immutable traceability records. The IoT solutions are used to manage environmental conditions under which food is handled and processed. The agrifood domain also requires IoT systems for on-the-spot analysis to ensure food quality standards and requirements are fulfilled.
Our approach applies blockchain and IoT technologies to trace food quality and safety parameters throughout complex food supply chains. The goal is to reduce food waste and improve risk management capabilities by providing predictions about possible quality degradation and contamination that are subject to measured food quality parameters and environmental conditions. The solution proposes instantiation of interoperable measurement checkpoints which write measurements directly to a shared blockchain ledger. Interoperability comes from the deployment of the IoT environmental sensors, IoT gateways and on-the-spot food analyzers (e.g. IoT spectrometer solution developed during the PhasmaFOOD H2020 project) which support the same communication interfaces, protocols, and data models and have the same knowledge representation. The shared ledger is based on a private permissioned blockchain - the Hyperledger Fabric. This allows us to configure access permissions hierarchically, accommodating different relationships within supply chains. All stakeholders can conduct a complete audit of overall measurement checkpoints to ensure data and process integrity. Data analysis models have access to trusted measurement data on which to run prediction processes. These models also write analysis results into the shared ledger for multi-layer decision making.
We demonstrate our approach through experiments conducted on a testbed capable of emulating food supply chain conditions. The testbed includes 10 measurement checkpoints comprising IoT sensors, IoT gateways with edge processing capabilities, and IoT-enabled spectrometers for measuring food quality and safety parameters. All checkpoints write measurements into Hyperledger Fabric blockchain and predictive machine learning models run on the collected data to identify risks for food safety. The obtained results of the proof of concept implementation show that the data flow performance between measurement checkpoints and the machine learning models is comparable to the centralized cloud based approach without distributed trusted leger. The interoperability of the checkpoints allows for comprehensive analysis of the supply chain processes and their interdependencies and provides strong predictive capabilities that can drastically reduce food waste in practical use.

Keywords: *internet of things, blockchain, supply chain, food safety, trustworthiness*

Acknowledgement: *This work is based on the "Federated and Trusted Food Supply Chains" project (FT-Chain) financed by the German Federal Ministry of Education and Research (BMBF) under grant reference number 01DS21011.*

G5

SUPERCRITICAL FLUID CHROMATOGRAPHY SEPARATION OF FLAVANONES´ ENANTIOMERS. APPLICATION TO BEE POLLEN

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Flavanones are a sub-class of flavonoids produced by plants as compounds of secondary metabolism. They are widely distributed in nature and have attracted the attention of researchers due to their health benefits and important properties; in particular, they have been reported to have antioxidant, anticarcinogenic, cardioprotective, or anti-inflammatory activities. Flavanones have one chiral centre at the C2 position and 3-hydroxyflavanones possess two chiral centres at the C2 and C3 positions. Since it is well known that a pair of enantiomers can display different bioactivities or metabolism, the chiral separation of flavanones is an important challenge and there is a need for enantiomeric methods of analysis to advance the knowledge of their stereospecific disposition or pharmacokinetics.
Traditionally, liquid chromatography (LC) has been the technique applied in the chiral analysis of these compounds, but in most cases, the studies are limited to one or three compounds. The simultaneous enantiomeric separation of a high number of flavanones has not been reported yet. Chiral separations are one of the fields where supercritical fluid chromatography (SFC) has been employed with great success. The physicochemical properties of the supercritical fluids allow to obtain separations with high efficiencies, short analysis times, and reduced waste generation if compared with LC. Nevertheless, SFC has been scarcely applied in the enantiomeric analysis of flavanones.
The main goal of this work was to study, for the first time, the chiral separation of ten flavanones (flavanone, 2'-hydroxyflavanone, 4'-hydroxyflavanone, 6-hydroxyflavanone, 7-hydroxyflavanone, naringenin, naringin, hesperetin, pinostrobin, and taxifolin) using SFC. For this purpose, seven different chiral stationary phases and modifiers were checked in order to obtain the most favorable separation in terms of resolution and analysis time. The best results were obtained with the Chiralpak AD column and a mixture of ethanol/methanol (80:20, v/v) containing 0.1% of trifluoroacetic acid as an organic modifier. The proposed method was applied to the analysis of bee pollen samples obtained from local markets and experimental apiaries. The 2S enantiomer of pinostrobin was detected in two of them in variable concentrations (8 and 80 mg/kg).

Keywords: *flavanones, bee pollen, SFC, enantiomeric separation*

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G6

VALORIZATION OF FOOD WASTE: COMPARISON OF THE POLYPHENOL PROFILE, EXTRACTED BY ULTRASOUND USING NATURAL DEEP EUTECTIC SOLVENTS

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The valorization of waste, sustainability, and green chemistry are the goals for future research. Therefore, the use of green solvents such as Natural Deep Eutectic Solvents (NADES), with a wide range of physicochemical properties, such as polarity, conductivity, or pH, have become more widespread and are also good candidates for the extraction of polyphenols. This study aims to compare the UAE (ultrasound-assisted extraction) of polyphenols from the orange peel with four NADES. Four different combinations of NADES were used, where in all cases choline chloride was used as hydrogen-bond acceptor (HBA), and fructose, glycerol, proline, malic acid, and lactic acid were used as the hydrogen-bond donor (HBD). In parallel, ethanol (EtOH) (50% v/v) was used as a control. The extraction was done by UAE, 400W of power, and 10 min at < 40°C. The characterization and quantification of fifteen polyphenols were done by HPLC/UV-VIS (Agilent Technologies 1120 Compact LC, USA), equipped with a UV detector, and a C18 column (Phenomenex, USA) following the method described by Anticona, et al (2022). Standard calibration curves were done under the same conditions as samples. Choline chloride:Lactic acid (ChChl:LA) turns out to be the worst NADES for the extraction, but it was better than ethanol for hesperitin (70.5 ± 1.0 µg/100ml, 62.3 ± 0.1 µg/100ml respectively). For vanillic acid (11.0 ± 0.8µg/100ml), hesperidin (489 ± 71 mg/100ml), quercetin (83.3 ± 4.9mg/100ml), *trans*-cinnamic acid (0.013 ± 0.00 µg/100ml), and apigenin (2.33 ± 0.48) the best NADES was choline chloride:Fructose (ChChl:Fruc). The NADES made by three components (Choline chloride:proline:malic acid (ChChl:LP:MA)) was better for the extraction of ferulic acid (0.48 ± 0.12µg/100ml), *p*-coumaric acid (45.4 ± 1.6µg/100ml), narirutin (47.0 ± 4.0µg/100ml) , and naringenin (102 ± 1.8µg/100ml). Choline chloride:glycerol (ChChl:Gly), a polyalcohol-based NADES, resulted to be the best solvent for the extraction of catechin (4.73 ± 0.88µg/100ml), caffeic acid (4.83 ± 0.54µg/100ml), chlorogenic acid (8.91 ± 0.13µg/100ml) and naringin (361 ± 16.7 mg/100ml). In conclusion, more of one polyphenol could be extracted based on the type of NADES utilized to extract bioactive compounds from the orange peel. The optimum NADES for hesperidin extraction came out to be ChChl:Fruc. Hesperidin is the primary flavonoid in orange peel.

Keywords: *natural deep eutectic solvents, orange by-products, polyphenols, green extraction, ultrasound-assisted extraction*

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G7

HEADSPACE-SPME AS A VERSATILE MONITORING METHOD FOR THE DETECTION OF EARLY INSECT INFESTATION IN RICE

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Stored rice can be infested by a variety of insect pests that cause grain damage and reduce grain quality leading to grain storage losses of 9% in developed countries and even larger worldwide.[1] Modern stored-product integrated pest management (IPM) programs represent a more environmental and safe approach versus chemical insecticides for pest control. IPM makes decisions using knowledge of population dynamics and threshold insect density, where grain probe traps that target adult insects are used as a conventional method for monitoring insect densities.[2-4] However, an adult female insect can produce hundreds of eggs before being detected which could delay pest control actions and increase the risks of grain losses.[1] Specific volatile organic compounds (VOCs) have been detected and identified as insect biomarkers for detecting the presence of a distinct insect.[1, 5, 6] Thus, the use of new monitoring methods for early insect detection would be highly beneficial for fine-tuning and improving IPM programs. In this regard, headspace monitoring technologies that allow the detection of specific VOCs in the store grain resulting from the activity of the larvae in the early stages of insect infestations are needed in IPM programs.
Several studies had identified VOCs such as benzoquinones, hydrocarbons, alcohols, furans, and aldehydes as insect biomarkers.[5-8] Specifically, isopentenols and polysulfides have been reported as potential early biomarkers for the presence of moths and beetles in rice. The aim of this study was to develop a headspace solid phase microextraction (HS-SPME-GC-MS) method for high-throughput analysis and detection of early volatile biomarkers (prenol, prenal, isopentenol, hexanal, dimethyl disulfide, dimethyl trisulfide, 2-methylfuran, and 2-pentylfuran) in rice. After examination of four commercially available SPME coatings, Carboxen-PDMS coating was found to be most effective in the extraction and desorption of the volatile components compared to the other fibers. We demonstrated that HS-SPME can be used as a fast and versatile insect monitoring technology in IPM programs.

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Keywords: *volatile organic compounds (VOC), headspace SPME GC-MS, rice, food analysis, detection of insect infestation*

G8

ELUCIDATION OF THE VOLATILE COMPOSITION OF HONEY SAMPLES BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY – MASS SPECTROMETRY COMBINED WITH SOLID-PHASE MICROEXTRACTION ARROW

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The elucidation of the volatile profile of honey samples is critical both for the evaluation of their quality and for their classification according to their botanical and geographical origin [1-2]. In this work, solid-phase microextraction Arrow (SPME Arrow) combined with comprehensive two-dimensional gas chromatography – mass spectrometry (GC×GC-MS) was used for the first time for the exploration of the volatile profile of honey samples. Due to the significantly higher sorption phase of SPME Arrow in comparison with conventional SPME fibers, the utilization of this technique results in enhanced sensitivity [3]. At the same time, GC×GC-MS serves as a powerful analytical tool for untargeted analysis due to its high sensitivity, selectivity, and peak capacity [4]. The main parameters that could potentially affect the extraction efficiency were optimized. Under optimum conditions, extraction was performed using divinylbenzene/polydimethylsiloxane (DVB/PDMS) SPME Arrow fibers and adsorption of the target analytes was carried out at 50 °C for 50 min under constant stirring. As a proof-of-concept, the proposed method was successfully employed for the analysis of multifloral and unifloral secretion and flower honey samples. The combination of GC×GC-MS and Arrow SPME enabled the determination of more than 200 compounds of different chemical classes including aldehydes, ketones, furanic compounds, and terpenes.

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Keywords: *comprehensive two-dimensional gas chromatography, honey, volatile organic compounds, headspace solid phase microextraction*

G9

MULTIVARIATE DATA ANALYSIS OF LOW AND HIGH FIELD NMR DATA OF BREWED COFFEE FROM FERMENTED COFFEE BEANS

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Coffee is the second largest global commodity, and its characteristic aroma is essential for its appeal. Green coffee bean fermentation is carried out to modify and enhance the sensory profile of roasted coffee beans. In the literature, various bacteria and yeast strains are described for controlled coffee fermentations. By controlled fermentation of the coffee beans, flavour and sensory profiles of the coffee beans may be optimized to achieve higher specialty coffee association (SCA) gradings on specialty coffees. This is of high interest for the industry due to lower production costs of such coffees.For the comparison of the sensory properties of fermented coffee beans, SCA trained experts conduct cup tastings or cuppings, grading the coffees by specific sensory attributes.
For this study, roasted and brewed coffee samples were analysed by means of high (300 MHz) and low field (60 MHz) NMR, as well as headspace GC-MS/IMS to identify volatile and non-volatile compounds responsible for the enhanced sensory profiles of fermented coffee beans.The samples were of single origin of the variety *Canephora* var. Old Paradenia, which were fermented with either one of five different yeasts or three different bacterial strains before the whole cherries were dried accordingly to conventional natural coffee processing. As the cultures were isolated from the farm dried cherries, one aim of the study was the characterization of those strains that lead to similarly good taste results. Consequently, these strains could also be used as inoculants to suppress the growth of microbes leading to off-flavours. The sensory profile of coffee beans is highly dependent on various factors including variety, processing, origin, profile of roasting and fermentation inoculum. The analysed coffee beans share the same variety, processing and profile of roasting and differ only in their origin and fermentation inoculum.
This poster presents our multivariate data analysis strategy of low and high field NMR data of freshly brewed coffee from fermented beans. The applicability of low field NMR data, as well as high field NMR data to discriminate fermented coffee beans according to the inoculum will be assessed. Furthermore, the potential of data fusion of complementary NMR and GC-IMS data will be discussed to increase discriminative power.

Aditiawati et al. 2020; Bressani et al. 2020; da Silva et al. 2021; Elhalis et al. 2020; Evangelista et al. 2014; Martinez et al. 2017; Wang et al. 2020.

Keywords: *low-field NMR, high-field NMR, multivariate analysis, fermented coffee*

G10

FREE AMINO ACID ANALYSIS IN BEVERAGES USING THE ACCQ•TAG™ ULTRA DERIVATIZATION KIT WITH UPLC UV DETECTION

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Amino acid analysis can be used to ensure the quality and consistency of beverage products. This work demonstrates the application of the Waters Amino Acid Solution for the analysis of free amino acids in beverage samples. Retail samples for fermented beverages such as white wine, cider and kombucha alongside soft drinks like coconut water, energy drinks, apple juice, vitamin water and sports nutrition beverage powder have been derivatized with the AccQ•Tag™ Ultra Derivatization Kit. The separation and analysis were performed with an established set of methods on an ACQUITY UPLC H-Class System with PDA detection at 260 nm. Quantitation was performed against the Amino acid food and feed standard kit with norvaline used as internal standard. Dilution factors for different beverages were optimized and quantitation within the calibration range could be achieved by analysing pure, 1:10, 1:20 and 1:100 dilutions of the samples respectively. Baseline separation for all components was achieved. A seven-point bracketed calibration line was run with all sample sets. R^2 ranged from 0.9933 (Cys) to 0.9999 (Ala). Repeated derivatisation (n=3) showed low standard deviations for technical replicates with an average of 4% when calculated for area count.

Keywords: *amino acids, beverage, analysis, wine, cider*

G11

ANALYSIS OF ORGANIC ACIDS USING A MIXED-MODE LC COLUMN AND A QDA MASS DETECTOR

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Organic acids are an important group of compounds for many industries including food and beverages, animal feeds, and human health. The separation of fifteen organic acids on a mixed-mode LC column, the Atlantis™ Premier BEH™ C_{18} AX Column, has been studied using an ACQUITY™ H Class System coupled with an ACQUITY QDa™ Mass Detector. The effects of the chromatographic conditions, such as organic solvent content, ionic strength and pH of the mobile phase, on the retention and selectivity of organic acids were studied. An analytical method for organic acids has been developed and applied to fruit juices. The performance characteristics of the analytical method, including the limit of quantitation (LOQ), the relationship between the chromatographic peak area and concentration, the precision and the accuracy have been evaluated. This analytical approach has good retention and resolution of OA, the run time is short, and the detection is sensitive and selective. This solution is suitable for the determination of organic acids in fruit juices and beverages as well as other application areas.

Keywords: *organic acids, mixed-mode, beverages, food, QC*

G12

NON-TARGETED ANALYSIS STRATEGY: DEVELOPMENT AND APPLICATIONS

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While globalisation of the food supply chains has allowed for wider variety of foods to meet evolving consumer demands, coupled with the effects of climate change, this has also resulted in an increasing number of unexpected and emerging food safety risks. It is thus imperative to develop strategies that will enable rapid identification of unknown chemical hazards at the earliest opportunity, so that food safety risks may be addressed before they pose a threat to public health. Most targeted methodologies have limited scopes of detection in specific types of food and are incapable of detecting compounds beyond the validated scope. On the other hand, non-targeted analysis (NTA) leveraging on ultra-high performance liquid chromatography high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) is a powerful methodology for the detection and identification of unexpected and unknown hazards. While incredibly useful, NTA can be especially challenging for food analysis due to sample diversity and complexity. Data processing is usually the bottleneck of NTA as a single sample can generate millions of data to be analysed, making the entire manual workflow tedious and labour intensive. In response to the increasing needs for food safety assurance and risk assessment, this presentation will share on the development of an AI-promoted smart NTA strategy with proven transferability and efficiency to identify potential chemical hazards in food.

Keywords: *non-targeted analysis (NTA), food safety, high resolution MS (HRMS), chemical contaminants*

G13

HILIC-LC-MS METHOD FOR DETERMINATION OF CARBOHYDRATES IN VARIOUS FOOD MATRICES

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Carbohydrates are fundamental and integral part of almost all food products. Being major nutrient not only for humans, but also for bacteria, it allows to produce a variety of fermented products such those in basis of this work: yoghurts, fermentation broth, fermented grains, but also many others. Production of fermented products requires precise control of the process and is essential to obtain final products with desired flavour and taste, while also pursuing lesser turnaround time to achieve desirable parameters for a final product. Optimizing conditions of a controlled fermentation process require precise analysis of consumed medium, including different carbohydrates. Analysis of carbohydrates with classical methods based on HPLC-RI/ELSD or derivatization-based GC-MS suffer from high limits of quantification, complex sample preparation techniques, relatively long running times per sample as well as selectivity problems. The use of LC-MS or UPLC-MS based methods, as in this work, allows to reduce the time spent on sample preparation while achieving lower limits of detections and better selectivity in complex food matrices. Here, we present the method for determining six carbohydrates: fructose, galactose, glucose, sucrose, maltose, lactose and C_{13}-isotopically labelled analogues respectively in previously mentioned food matrices using the HILIC-LC-MS approach. Sample preparation steps have been kept as simple as possible, which includes serial dilution of the starting material, employing as low as 20 µl of a sample with addition of internal standards. The developed method was assessed for linearity (R^2 coefficient of calibration curve), detection (LoD) and quantification (LoQ) limits and recovery. The method has been found to have an acceptable linearity (R^2 values were higher than 0.99 for all analytes), LoQ were in the range of 0.2 to 0.7 mg/L and recoveries between 103 and 113% based on isotopically labelled internal standards. The method was applied to several food matrices, such as plant-based yoghurts, fermentation broth, sprouted oat beans and fava bean spread, to measure selected carbohydrates. The results have showed a wide concentration range of the measured carbohydrates (from <0.1 g/L up to 20 g/L with suitable dilutions) in the analysed matrices with relatively small RSD% (<10%).

Keywords: *HILIC-LC-MS, fermentation, carbohydrates, fermented food*

G14

BAKING PROPERTIES OF DIFFERENT SORGHUM VARIETIES GROWN IN AUSTRIA

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Globally, sorghum is the fifth most cultivated crop [1]. Properties such as drought tolerance, the abundance of micro- and macronutrients, the low allergy potential due to the absence of gluten [2] and low water requirement are increasingly placing sorghum at the center of baking research, helping the grain to gain a forward-looking image and a wide range of applications. Furthermore, sorghum is also increasingly being grown in areas too dry for maize [3]. Sorghum flour has a neutral flavor and thus can partially replace wheat flours in common bread and bakery recipes, adding a nutritional and technological value. Traditionally, sorghum is consumed mainly as porridge in Africa (e.g. Sudan, Ethiopia), whereas in the Middle East, sorghum flour is used to make flatbread [4].
The prevailing climate change is resulting in wheat with increased gluten contents in Austria, which are reflected in hard doughs. The addition of sorghum to standard wheat recipes could improve the handling of bulky wheat-based doughs and furthermore, give an additional nutritional value to the baked goods.
Three different sorghum varieties (*Armorik, Ggolden, Ggivry*), grown and harvested in Austria in 2019, were assessed for their feasibility in standard bread and sponge cake recipes as a gradual addition as whole meal flour to wheat from 0-20% in 5% steps.The baked goods were subsequently evaluated based on defined quality parameters by physical and software-based methods. Both breads and sponge cakes enriched with sorghum resulted in an improved handling of the doughs and the baking and physical properties of the baked goods were comparable with the reference formulation of 100% wheat flour, especially when 10-20% of sorghum was added.
In addition, the sorghum varieties *Janus, Annggy, Alligator, Kalatur, Armorik, Huggo, Icebergg, Arabesk, Ggolden, Arsky* and *PR88Y92*, grown and harvested in Austria in 2020, were analyzed chemically, to identify their composition and their potential in baking applications. Overall, the chemical analysis of the 8 sorghum varieties yielded results in line with the literature, which confirms the suitability of Austria for the cultivation of sorghum and their competitiveness with other sorghum varieties in the baking field.

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Keywords: *sorghum, baking, chemical composition, drought tolerance*

G15

HIGH QUALITY CURATED HRAM MSN SPECTRAL LIBRARIES AND REAL TIME LIBRARY SEARCH FOR THE CONFIDENT ANNOTATION OF FLAVONOIDS IN TEA

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Purpose: Compound class specific deep scan for confident annotation of relevant analytes using a high quality curated High Resolution Accurate Mass (HRAM) MSn spectral library in conjunction with Real Time Library Search (RTLS).
Methods: A Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer is used for collecting all MS and MSn data. For data acquisition, MS/MS is always collected with precursor ions detected in the survey MS scan within 1.2 second cycle time. High order MSn (3-5) is collected using the built-in spectral library creation templates using LC-MS. The generated MSn tree data are processed using Thermo Scientific™ Mass Frontier™ 8.0 and Thermo Scientific™ Compound Discoverer™ 3.3 software. Flavonoid standards were used for the library creation and putative flavonoids were annotated in tea extract. Thermo Scientific™ mzVault™ 2.3 software was used for creating the spectral library to be used for RTLS.
Results: Use of standard spectral library and RTLS allows more unknown flavonoid compounds being identified from the natural products. Nearly 100 more putative flavonoids were annotated using this workflow from tea extracts.

Keywords: *flavonoids, spectral library matching, high resolution MS, real time library search, MSn annotation*

G16

INTELLIGENT DATA ACQUISITION FOR UNTARGETED METABOLOMICS OF MILK SAMPLES COUPLED WITH QUANTITATIVE HIGH-RESOLUTION ACCURATE MASS DATA COLLECTION

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Purpose: Development of an intelligent data acquisition workflow for untargeted LC-MS metabolomics with deep metabolome coverage and confident compound annotation to identify components for a high-throughput and robust quality screening study in milk.
Methods: Reversed-phase LC-MS methods using Hypersil GOLD™ separation were developed utilizing a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled to a Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer to assess and quantitate metabolic variation among different milk samples; bovine milk with various fat content, almond, oat, coconut, and soy milk.
Results: Higher levels of amino acids were shown to classify plant-based milk from bovine milk. Hippuric acid and orotic acid were verified as markers for bovine milk compared to plant-based milk. Gluconic acid, however, was verified as a marker for soymilk.

Keywords: *milk, untargeted metabolomics, plant-based milk, high-resolution accurate mass, AcquireX*

G17

QUANTITATIVE ELEMENTAL ANALYSIS IN THE FOOD CYCLE SUPPORTED BY AN AUTOMATED ELEMENTAL ANALYZER

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The food cycle includes a variety of processes and materials that are carefully regulated by federal authorities and various international organizations to ensure product quality and consumer and environment safety. One of the main tools for quality control of materials in the food cycle is elemental characterization. Elemental analysis utilizes carbon, hydrogen, nitrogen, sulfur and oxygen, which help determine the structure of an unknown compound, as well as to evaluate the structure and purity of a synthesized compound. The Thermo Scientific™ Flash*Smart*™ Elemental Analyzer (EA) enables quantitative elemental determinations at high and low levels of concentrations for solid and liquid samples in one single system. Here we present data of different samples in the food cycle to demonstrate how:
Elemental characterization of soils, leaves, plants and crops allows optimization of agronomy plans and fertilization practices.
Determination of protein content in food and feed enables consumers to perform price and quality comparisons based on % protein declarations.
Elemental analysis of recycled waste is critical for quality control of fertilizers and the subsequent effect that agricultural practices have on the environment.
The Flash*Smar*t EA copes effortlessly with modern laboratory requirements such as high sample throughput, day to day reproducibility and accuracy.

Keywords: *elemental analysis, protein, characterization, fertilizers*

G18

APTASENSOR DEVELOPMENT FOR GEOBACILLUS STEAROTHEMOPHILUS SPORES DETECTION IN CANNED FOOD – SPORES-QUANTUM

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Over the past ten years, consumer concerns on health, nutritional and organoleptic qualities of heat-treated [BL1] foods have been constantly increasing. In response to this demand, canning manufacturers are continually improving the packaging and the process of their products. These modifications may favour the emergence of certain biological contaminants because of their adaptation to their environment, including thermophilic spore-forming bacteria. Ended, the presence of highly resistant spores of the non-pathogenic bacteria *Geobacillus stearothermophilus* or *Moorella thermoacetica* is responsible of around 70% of the cases of economic losses in the canning industries.
To limit economic losses and food waste, risk assessment of *G. stearothermophilus* can be carried out before thermal treatment using classical microbiological methods. Unfortunately, these cultivation methods are long, tedious and do not allow to obtain a result in less than 3 to 5 days. Therefore, it is important to explore some new routes to develop alternative tools to survey and anticipate canned food spoilage.
That's why the SPORES-QUANTUM project, supported by the Qualiment® Carnot Institute, proposes to develop specific biosensors dedicated to the detection in real time of *G. stearothermophilus* spores. This project aims to use a DNA aptamer as the biorecognition element of the proposed biosensor. To this purpose, a SELEX protocol was performed, including numerous counter selection rounds, performing PCR amplifications in emulsion, and using Next Generation Sequencing in order to obtain specific aptamers, to allow a deeper candidate analysis and to monitor aptamers evolution over cycles.
To date, the first step of selection of specific aptamers from *G. stearothermophilus* spores has been completed. The bioinformatic analysis has highlighted some over-represented sequences that are potential candidates for target recognition. The affinity of these aptamers for *G. stearothermophilus* spores was confirmed by binding assays based on PCR analysis performed directly on the spore surfaces. Other strategies are currently being investigated to characterise more precisely aptamer-target recognition: i) visualisation of labelled oligonucleotides on the surface of spores by confocal epifluorescence microscopy and ii) precise study of spore attachment to immobilised aptamers by Quartz Crystal Microbalance. Once sorted, the resulting aptamers will be integrated into optical and electrochemical biosensors.

Keywords: *aptasensor, canned food, bacterial spores*

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G19

EFFECTS OF DIFFERENT PROCESSING METHODS ON THE QUALITATIVE PARAMETERS OF RAPESEED OIL

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Rapeseed (*Brassica napus*) is the most important oil seed crop of moderate climates and is the third most important produced edible oil in the world (after palm and soybean oils). Rapeseed has been cultivated in the territory of the Czech Republic since the beginning of the 19th century, and the production has taken up around 15 % of agricultural land in recent years. Rapeseed oil has significant health benefits compared to other vegetable oils, due to the unique fatty acid profile; moreover, cold-pressed rapeseed oil also contains antioxidants such as tocopherols, phytosterols, carotenoids, etc. In this study the profile of fatty acids and the content of minor components of rapeseed oils from different plant production and different oil extraction methods were studied.
The rapeseed oil samples of 5 producers in the Czech Republic were divided into 3 groups: (1) cold-pressed oils, (2) hot-pressed and solvent extracted oils, (3) refined, bleached and deodorized (RBD) oils. The obtained results were compared with literature data and parameters found for novel rapeseed varieties.
All samples tested showed typical fatty acid composition for rapeseed oil. There were no differences between the groups of samples ($p>0.5$). The average content of monounsaturated fatty acids was 64.3 %; polyunsaturated fatty acids 27.0 % and saturated fatty acids 8.6 %. The most abundant fatty acid was oleic acid (62.1 %), followed by linoleic acid (18.4 %) and linolenic acid (8.3 %). The content of erucic acid was <0.2 %. Carotenoids and chlorophylls are known to be natural antioxidants that scavenge reactive free radicals and singlet oxygen physically. Their content was dependent on the manufacturing process. The group of hot-pressed and solvent extracted rapeseed oils has the highest content of carotenoids and chlorophylls (measured as pheophytin, average 38.3, resp. 14.1 mg/kg), the group of cold-pressed oils content in average 18.3 mg carotenoids/ kg oil and 5.9 mg pheophytin/ kg oil. The group of the most processed RBD oils has the lowest content of carotenoids and chlorophylls (2.6 mg carotenoids/ kg oil and <0.1 mg pheophytin/ kg oil).
The important customer choice criterion is the odour quality of edible oil products. Gas chromatography with mass spectrometry using SPME fibre was used for the identification and quantification of volatile compounds in the tested samples. The 59 volatile compounds were detected, most of them in cold- and hot-pressed oils. The identified compounds were in agreement with literature and were typical of rapeseed oils.

Keywords: *rapeseed oil, fatty acids, volatiles, cold-pressed, rafined*

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G20

CREATION OF A EUROPEAN METROLOGY NETWORK FOR SAFE AND SUSTAINABLE FOOD

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Safe food is a prerequisite for good human health, however currently almost one out of ten people in the world fall ill after eating contaminated food. National food safety authorities need to be able to manage food safety risks along the entire food chain. The Official Controls Regulation (EU) 2017/625 was put in force to ensure that the food and feed law stipulates the need for validated analytical methods. However, whilst food safety method development and proficiency testing are well established by European Union Reference Laboratories and National Reference Laboratories, validation of the measurements involved, certified reference materials and internationally recognized calibration and measurement capabilities are currently lacking. This erodes trust in the accuracy of the measurements.
This paper describes the European Metrology Network for Safe and Sustainable Food, which was recently approved by the European Association of National Metrology Institutes (EURAMET). The network aims to establish a long-term dialogue between the metrology community, reference laboratories and regulatory bodies, in order to (i) identify stakeholders' needs, (ii) develop a sustainable knowledge-sharing programme and web-based platform for stakeholders and (iii) develop roadmaps and a strategic research agenda. Improved access to more reliable and accurate food safety measurements will enable reference laboratories to more confidently and effectively compare their measurement results and support accreditation. This is particularly important when national food safety authorities need to assess potential new contaminants, novel food ingredients and newly emerging food risks, that requires new measurements methods as well as input into new documentary standards. The activities for sustainable food intend to boost the activity within the "EU green deal framework" to reduce food waste and losses, and the development of standards and methods that allow the reuse of food waste.

Keywords: *food safety, food sustainability, European network, reference laboratories*

G21

ALKALPO : SOLANINE AND CHACONINE IN BELGIUM POTATOES AND POTATO PRODUCTS

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Glycoalkaloids (GAs) are natural compounds found in the Solanaceae family, which includes potatoes, tomatoes and eggplants. The main GAs in potatoes (Solanum *tuberosum*) are α-solanine and α-chaconine. which together account for 95% of the total content. These GAs are found in all parts of the plant with the highest levels in the flowers, sprouts, eyes and skin, while the lowest are found in potato tubers.
These substances are naturally toxic and are one of natural self-defense of the plant. Low concentrations of GAs in potatoes cause a bitter taste and higher consumption of these compounds may provoke acute fever, headache, neurological or gastrointestinal disturbances.
Severe glycoalkaloids poisoning, can cause paralysis, respiratory or heart failure and coma and is considered as potentially lethal in exceptional circumstances.
Based on the most recent knowledge, EFSA has determined the lowest observed adverse effect level (LOAEL) of 1 mg total potato GAs/kg body weight per day. A MOE greater than 10 indicates that there is no health concern.
The research project ALKALPO aimed to determine the concentrations of glycoalkaloids in potatoes and their processed products in Belgium, to identify factors that influence their presence and to assess the exposure of the Belgian consumer.
A sampling plan comprising 500 samples was drawn up on the basis of the Belgian 2014 Food Consumption Survey and previously identified risk factors. The samples were collected taking into account certain criteria: state of the potato, potato category, distribution of place of sampling to regions according to the Belgian population density, distribution channels and intermediate results. A series of information relating to each sample was also noted (light, humidity, greening, wounds and germination).
An UHPLC-MS/ MS method was developed, validated and accredited. The solanine and chaconine concentrations available were determined in potatoes and potato products.
Potatoes with high GAs values both in the flesh and in the whole tuber are potatoes with either greening, injury or sprouting or a combination of these parameters. These parameters clearly have an impact on the GAs content.
For heavily contaminated potato products, an estimate of exposure by the Belgian consumer was determined. The MOEs estimated on the basis of the minimum or average concentration of GAs indicate a health problem for consumption of these products.

Acknowledgement: *Project funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 16/5 ALKALPO.*

G22

COMPARISON OF DAIRY AND DAIRY-FREE CHEESE FLAVOR PROFILES USING THE NEWLY DEVELOPED GC-ECTOF

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An increased awareness to health, environmental and animal welfare issues and a growing acceptance of vegan products, especially amongst the younger population, has led to an increase in non-dairy product sales. To attract new customers, vegan foods often try to imitate more established non-vegan products. Hence, characterization of flavor and aroma profiles is of particular interest.
Flavor is one of the most important criteria determining consumer choice and acceptance, especially with non-dairy cheese. The development of cheese flavor is the result of a complex combination of microbial and biochemical activities throughout the storage period, leading to the formation of a heterogeneous mixture of volatile and nonvolatile compounds. A newly developed time of flight (TOF) mass spectrometer operating an electron ionization (EI) and a chemical ionization (CI) source in parallel is used to characterize and compare the cheese flavor profiles of vegan-cheeses to those of non-vegan equivalents. By directly coupling a single gas chromatograph (GC) to both ionization sources, target and suspect screening analysis is improved as well as effective non-target analysis rendered possible. Concurrent structural as well as accurate mass molecular ion information is generated, which helps in the identification of compounds of interest, either flavor dominant compounds such as ketones, esters, aldehydes and organic acids, or possible off-flavor compounds. With this, the potential for the GC-ecTOF during product development and quality control, and as a tool for authenticity concerns, is highlighted.

Keywords: *flavor comparison, non-target analysis, quality control, GC-ecTOF, authenticity*

G23

USE OF TD-NMR FOR INSTANT TEA POWDER CHARACTERIZATION

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Significant amount (25 tones/year) tea leaves remain during tea production. The goal of this study is to valorise this waste to produce various instant tea products. Functional black tea powders have been produced by using cistus, ginger and sage. Tea waste was first brewed at different temperatures (25°C and 100°C) followed by spray and freeze drying of the extracts. Dried extracts were mixed using a ball mill with the plants that were already grounded and instant tea powders were produced. Due to the super grinding process, the particle size of instant tea powders decreased to less than 100 μm. For characterization of instant tea powders, water hydration behaviour and crystallinity were measured using Time Domain Nuclear Magnetic Resonance (TD-NMR) techniques. Spin-lattice (T_1) and spin-spin (T_2) relaxation times are measured. CPMG, saturation recovery and solid echo sequences were used for hydration and crystallinity experiments. X-ray Diffraction analysis was also conducted on the samples to confirm the results obtained from TD-NMR. Results showed that tea produced at different brewing temperatures and mixed with different plants showed differences on hydration and crystallinity. Thus, TD-NMR has been shown to be used as analytical technique for powder tea characterization.

Keywords: *food powder, tea, plants, TD-NMR, hydration and crystallinity*

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G24

FERMENTED CUCUMBERS WITH REDUCED BIOGENIC AMINES CONTENT – APPLICATION OF SELECTED LACTIC ACID BACTERIA STRAINS

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Enriching diet with fermented products can positively affect human health. However, except plenty of benefits, consumption of fermented food can trigger adverse effects due to ingestion of considerable amount of biogenic amines (BAs). In order to prevent accumulation of high doses of those microbial metabolites during fermentation process it is necessary to apply well-characterised starter cultures that are not able to produce BAs.
Two non-aminobiogenic lactic acid bacteria strains - *Lacticaseibacillus casei* KKP 3272 and *Pediococcus pentosaceus* KKP 3273 - were individually applied in three different concentrations – 10^4, 10^6 or 10^7 CFU×mL^{-1} - with an aim to examine BAs content reduction in a model cucumber fermentation system. Spontaneous fermentation was carried out as a control. Next generation sequencing and liquid chromatography – mass spectrometry were applied to assess microbial profile and biogenic amines concentration. After 6 months of storage, in spontaneously fermented samples Enterobacteriaceae and Lactobacillaceae families predominated and constituted 46.52 and 42.94 % of bacteria community, respectively. Amongst genera, *Enterobacter* (35.21 %), *Lactobacillus* (22.41 %), and *Pediococcus* (20.40 %) prevailed. In the samples fermented with starter cultures, the higher was the number of bacteria cells added, the greater share represented members of genus inoculated. Bacteria community in the samples inoculated with 10^7 CFU×mL^{-1} of *P. pentosaceus* KKP 3273 was dominated by members of Lactobacillaceae family (96.71 %), *Pediococcus* genus (93,09 %). Inoculation with 10^7 CFU×mL^{-1} of *L. casei* KKP 3272 resulted in predominance of Lactobacillaceae family (78.95 %), and *Lactobacillus* genus (78.62 %). BAs content reduction was mainly affected by the number of bacteria cells added as starter cultures. Generally, in comparison to control, both strains tested effectively prevented histamine, tyramine, putrescine, and cadaverine formation, when applied at concentration of 10^7 CFU×mL^{-1}. At the end of the experiment, percentage reduction of those amines reached 91, 64, 96, 17 % for *L. casei* KKP 3272, and 99, 96, 95, 51 % for *P. pentosaceus* KKP 3273, respectively. Samples inoculated with 10^6 or 10^7 CFU×mL^{-1} of starter cultures were classified as posing a low risk of adverse health effects, while control samples were classified as posing a medium risk according to Biogenic Amine Index.
Obtained reduction of BAs content resulted from the ability of the non-aminobiogenic starter cultures used in proper amount to predominate among other bacteria and thus inhibit the growth of autochthonous BAs-producing microorganisms. Acquired data indicate that number of inoculated cells significantly affects safety and quality of the final fermented product and no less than 10^6 CFU×mL^{-1} of cells should be added to initiate fermentation process.

Keywords: *liquid chromatography - mass spectrometry, next generation sequencing, Biogenic Amine Index, food safety*

G25

SUITABILITY OF SPECIFIC VOLATILE COMPOUNDS AS INDICATORS OF LIPID OXIDATION IN FISH FILLET

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Lipid oxidation is one of the main causes of food degradation, especially in sources rich in highly unsaturated fatty acids such as fish. The development of lipid oxidation can jeopardize the nutritional value of fish, the sensory acceptance due to the formation of volatile compounds and may produce some molecules with negative biological effects. Thus, it is mandatory to have adequate analytical methods to evaluate lipid oxidation to assure the quality of fish for human consumption. The TBA test is one of the most widely used methods, as it easily allows the determination of mainly malondialdehyde, a main non-volatile product of lipid oxidation. One alternative to the TBA test can be the study of some volatile compounds, where the attention is usually focused on volatile aldehydes, especially on hexanal. However, the production of specific volatile compounds depends on the composition of the sample.
The aim of this work was to evaluate the suitability of different volatile compounds found in fresh and refrigerated fish fillets as lipid oxidation indicators, using the TBA value test as a reference method.
Specimens of European sea bass (*Dicentrarchus labrax*) were fed 5 diets with 16 % of added fat that was fish oil (F, control), or fish oil plus another fat (at 25:75, w/w): olive pomace oil (OP), olive pomace acid oil (OPA), crude soybean oil (S) or soybean-sunflower acid oil (SA). After 3 months, fillets were sampled (5 analytical replicates per diet). At 24h post-slaughter, fillets were stored under commercial refrigeration conditions ($CO_2/N_2/O_2$; 40/30/30; 2°C) for 0 (fresh fillets) or 6 days (refrigerated fillets). The TBA values were determined through a third derivative spectrophotometry at 521.5 nm after the reaction of TBA with the acid aqueous extract of 1.5 g of homogenized sample. The volatile compound analysis was performed with SPME-HS-GC-MS on the homogenized mixture of 1g of sample with 0.2 µg of internal standard (4-metil-2-pentanol), 20 µg of EDTA, 2 µg of propyl gallate and 2 mL of a 20% NaCl water solution. For the SPME a fibre of divinylbenzene/carboxen/polydimethylsiloxane was used and for the GC separation a Supelcowax-10 capillary column.
The TBA values in fillets increased after the refrigeration, while fillets from F diet showed the highest TBA values in fresh and refrigerated fillets. Twelve volatile compounds were identified in fresh and refrigerated fillets, being the main ones hexanal, 1-penten-3-ol and 1-octen-3-ol. All the identified compounds were present in higher amounts after the refrigeration. In fresh fillets, the higher lipid oxidation development of F fillets was only noticeable in the case of 1-octen-3-ol, while in refrigerated fillets the effect of the diet was significant for 1-octen-3-ol, 1-penten-3-ol, propanal and (Z)- and/or (E)-2-pentenol. The 1-octen-3-ol was the volatile compound with the greatest correlation with TBA values and, therefore, seemed to be the best one as lipid oxidation indicator.

Keywords: *fish fillets, lipid oxidation, TBA values, volatile compounds, fillet quality*

Acknowledgement: *This study has been supported by the grant AGL2015-64431-C2-2-R funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe"; the grant RYC-2017-23601 funded by MCIN/AEI/10.13039/501100011033 and by "ESF Investing in your future"; and by Spanish Ministry of Universities through the pre doctoral contract within the FPU program (FPU18/01010).*

G26

PRESENCE OF FREE GLUTAMATE IN OUR MEALS: WHAT ARE THE CHALLENGES?

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Glutamic acid is a non-essential amino acid, naturally occurring in food as a component of proteins but also in free form. This free molecule is responsible for the savoury "umami" taste. For this reason, glutamate is added to food products as a flavour enhancer. Indeed, glutamic acid and five of its salts are authorised as a food additive by the European Commission (Regulation (EC) No 1333/2008) in most food products, with a maximum permitted level of 10 g kg^{-1} or at *quantum satis*.
In 2017, the European Food Safety Authority (EFSA) re-evaluated glutamic acid and set an Acceptable Daily Intake (ADI) of 30 mg kg^{-1} bw day^{-1}. They concluded that the newly set ADI was exceeded in different European countries, causing potential adverse effects in humans. However, their assessment pointed to different uncertainties, leading to an overestimation of the free glutamate exposure. Therefore, the exposure of the Belgian population should be evaluated. First, an accurate and high throughput analytical method was developed. Free glutamate is extracted using acidified water and analysed using UHPLC-MS/MS after a precolumn derivatisation step using propylchloroformate. The method was validated for all types of food matrices.
Next, 600 food samples were carefully selected. This was very challenging since free glutamate can originate from different sources: monosodium glutamate can be used as a food additive, but free glutamate is also naturally present in food ingredients at various levels, and is naturally produced through several processes (e.g. ripening of cheese, fermentation of soy sauce). Furthermore, glutamate food additives tend to disappear from ingredients lists. Instead, yeast extracts can be added to foods to enhance flavours. This ingredient is known to contain glutamates. Protein hydrolysates are also suspected of containing high levels of free glutamate. As making a distinction between naturally present and added free glutamate is impossible from an analytical point of view, the selection of the samples is crucial. Similar products with and without added glutamate were compared when possible. Furthermore, ingredients like yeast extract and protein hydrolysates were also considered. In this manner, it will be possible to evaluate the contribution of the food additive and alternatives to the total exposure to free glutamate.
The results will show how to deal with the various sources of free glutamate, the validation performance of the method, and the expected and surprising results obtained when analysing the selected samples.

Keywords: *free glutamate, food additive, UHPLC-MS/MS, source identification*

Acknowledgement: *The research that yielded these results was funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RT 20/3 FREEGLUTAMATE.*

G27

CLASSIFICATION OF FOOD MATRICES FOR EFFICIENT VALIDATION PROCESSES: APPLICATION OF THE AOAC TRIANGLE

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The simultaneous analysis of different food additives entails several challenges. Firstly, food additives comprise many substances with varying physical and chemical properties. Secondly, they can be present in a wide range of concentrations, and food matrices are very diverse in composition and can be complex mixtures. Thirdly, the analysis of food additives cannot rely on a European Reference Laboratory nor specific guidelines regarding quality criteria and validation processes related to their analysis. Finally, certified reference materials are scarce, which also hampers method development and validation.
Developing and validating a method applicable to all food matrices is challenging and expensive since validating the method in all matrices like meats, cheeses, yoghurts, beverages, prepared meals, etc., would lead to unmanageable numbers of validations. For example, the colour carminic acid can be added to different drinks, fine bakery wares, cheese and meat products, sauces, breakfast cereals, fruit and vegetable products, and snacks, among others. By clustering food matrices, the number of validations can be reduced. One of the strategies that can be applied is the Food Matrix Organization System, developed by AOAC, which classifies foods based on the contents of fat, carbohydrates, and proteins. This classification results in 9 triangles. As the water content of the food product is not considered in the AOAC system, a distinct group for foods containing more than 85 % water was created. Foods classified in the same triangle are considered to have the same analytical behaviour, so they were grouped in the same validation process. To still encompass the variety within the triangle, the different days of validation were performed on different matrices from the concerned triangle. For instance, a lasagna, a yoghurt and a pudding sample were selected to validate the triangle containing 33 to 67 % carbohydrates, less than 33 % proteins and less than 33% fat.
This approach has been applied successfully in several research projects. For example, a multi-method was developed for the analysis of 27 food additives in more than eleven types of matrices. By applying the AOAC classification system, the number of validations was reduced to six.Regrouping food matrices using the AOAC model enabled efficient validation of the methods as one validation covered several types of foods, still taking into account the variation within the AOAC food group.

Keywords: *food additives, AOAC classification system*

G28

ANALYSIS OF SMALL ORGANIC ACIDS IN FOOD AND BEVERAGES FROM THE BELGIAN MARKET USING ION CHROMATOGRAPHY WITH CONDUCTIVITY DETECTION

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Organic acids (OAs) are ubiquitous in many foods and beverages, and they can play a significant role in the definition of flavour, texture and freshness. Moreover, they can be added (as such or as salts) to foodstuff to fulfil multiple technological functions and act as preservatives, acids, acidity regulators, sequestrants, antioxidants or flour-treatment agents. Since OAs can be naturally present or used as a food additive (FA), with a high co-occurrence rate, there is a need for fast and wide-applicable *multi*-methods. Many extractions and quantification methods have been reported in the past. However, they often focus on an individual OA or a specific food matrix, and they suffer from many challenges such as the need for derivatisation steps, interference of UV-active compounds or poor retention/selectivity.
In the present study, a method was developed to analyse 7 OA (i.e. citric acid, malic acid, propionic acid, fumaric acid, lactic acid, sorbic acid and benzoic acid) in food and beverages using ion chromatography coupled to a conductivity detector. The analysis of these 7 OAs corresponds to a total of 24 FAs since these FAs are very often salts of OAs. Next, the method was validated in-house and applied to >300 food and beverages belonging to an extensive range of food categories, from dairy products to fine bakery wares, including sauces, salads and savoury-based sandwich spreads, flavoured and alcoholic drinks, and composite foods. The concentrations found during the analysis of the samples varied extensively due to the nature of the food/beverage and the specific Maximum Permitted Levels (MPLs) defined by Regulation (EC) No 1333/2008 for some FA/Food Category combinations. However, an interesting result was the highest co-occurrence rate of the OAs detected compared with what was declared on the ingredient list. Moreover, seventeen samples were non-compliant based on the specific MPLs related to sorbate and benzoate content. Finally, remarkable results were obtained for composite foods. In this food category, FAs are allowed according to the *carry-over principle*. Nevertheless, the average concentrations were higher than previously reported, and the compliance evaluation was challenging due to the complex assessment of the potential source of the FA. The method proved to be an easy, cost-effective method for OAs monitoring scopes, while the results raise the need for a further investigation of OAs use in composite foods.

Keywords: *food additives, organic acids, ion chromatography, market survey, composite foods*

G29

TARGETED AND NON-TARGETED ANALYSIS OF PUMPERNICKEL BREAD AROMA COMPOUNDS BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED TO TIME-OF-FLIGHT MASS SPECTROMETRY (GC×GC-TOFMS)

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Pumpernickel bread is a special type of sour rye dough bread that is steam-cooked under moderate conditions for relatively long time. It originates from Westphalia and has a very dark and dense crumb. Consumers appreciate it for its sweet taste and intensive aroma, typically described as brown, caramel, and malty. Gas chromatography−olfactometry (GC−O) analysis was considered as the technique of election for the recognition of key aroma compounds with specific odor notes in pumpernickel bread and its intermediate products obtained during the fermentative process,
In this research, three pumpernickel bread extracts obtained from products with varying ingredients were investigated by comprehensive two-dimensional gas chromatography (GC×GC) coupled with time-of-flight mass spectrometry (TOFMS). At first, previously defined target compounds with a significant odor activity value (OAV) were determined[1]. Furthermore, relative differences between the investigated extracts were explored taking advantage of a supervised statistical analysis based on the Fisher-ratio approach elsewhere described[2]. The differences in the aroma composition of the three pumpernickel bread extracts can be directly linked to the use of the different ingredients and provide valuable clues for the recipe and/or manufacturing steps. The careful identification of key aroma compounds formed during the preparation of the food item with the recognition of technology parameters influencing its formation can provide producers with the knowledge about possibilities to positively influence the overall aroma of the final product.

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Keywords: *GCxGC, TOFMS, pumpernickel bread, two-dimensional*

G30

VACUUM IN-TUBE EXTRACTION FOR EFFICIENT EXTRACTION OF FLAVORS, FRAGRANCES, OFF-FLAVORS AND FOOD-BIOMARKERS DIRECTLY FROM COMPLEX FOOD MATRIX

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Extraction of volatile compounds is a widely used approach for analysis of foods and beverages. The applications include flavours, fragrances, off-flavours, and contaminants in food stuffs. Typical sampling techniques are Headspace, Dynamic Headspace (DHS), Solid Phase Micro Extraction (SPME), and In-Tube Extraction (ITEX). These techniques are typically carried out with heated samples at increased pressure levels. At these conditions, the limiting factors of extraction performance are vapor pressure of the analytes with higher molecular weight and polarity.
These limitations can be overcome by sampling at decreased pressure levels using vacuum conditions. This has been shown in several publications using vacuum sampling techniques, but so far these require manual steps of sample preparation.
In this study, a fully automated approach of Vacuum In-Tube Extraction (V-ITEX) is presented. The sample preparation is fully integrated in a PAL System autosampler. It is using a dedicated modules for vacuum and inert gas control, which is fully integrated in the ITEX Method. The presented data shows the simplicity and advantages in throughput and minimizing artefacts during the extraction thanks to reduced temperature and extraction time. The application examples include flavors, fragrances, off-flavors and biomarkers by extractions directly from the food sample matrix.

Keywords: *volatile compounds, vacuum, headspace, flavours, analysis*

G31

COMPREHENSIVE CHARACTERIZATION OF THE BEER METABOLOME

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Beer has accompanied mankind and the emergence of civilization for thousands of years. Brewing research and the fascination with fermented beverages have produced numerous inventions and findings that have shaped our society today (Pasteur, Enzinger, Linde, Hansen). Rising from and building on this pioneering work, modern analytics allows us to paint a comprehensive picture of beer at the molecular level.
In our work, we examined over 500 beer samples from around the world to reveal the complexity and diversity of molecules in beer. The instrumental range covered the entire analytical space from separation sciences (UPLC) over spectroscopy (2D-NMR) to spectrometry (LC-ToF-MS, DI-FT-ICR-MS). Tens of thousands of molecular signals were detected and characterized. Ultra-high resolution mass spectrometry coming with highest mass accuracy allowed the molecular compositions of each molecule to be determined. Utilizing exact mass signals, the compounds were related by molecular networks (mass difference networks) and specific chemical signatures were found. The brewing process contributes to the complexity of the beer just as decisively as the raw materials themselves. The hopping technology, classic or dry hopping, is reflected in hundreds of bitter acid derivatives and polyphenolic compounds. The basic principles of the Maillard reaction seem to be largely understood; the immense molecular diversity that develops in the brewing process still is almost undescribed ("dark metabolome"). Multivariate chemometric analysis based on metabolomics data made it possible to construct a reaction network comprising more than 2,800 Maillard reaction products. This can be read as an image of the reaction sequences in the malting and brewing process and opens up a comprehensive view of this complex reaction network. Against the background of the German Purity Law, beers were differentiated on the basis of the starch sources used. Molecular networks of the metabolites of barley, wheat, corn, and rice could be detected both at the compositional level (DI-FT-ICR-MS mass difference networks) and at the structural level (LC-ToF-MS^2 similarity networks) in the finished beer. From these specific molecular signatures, individual marker molecules were identified that could serve as evidence of the corresponding grain.
Ultimately, the comprehensive non-targeted metabolomics approach succeeded in interpreting the molecular imprint of a historical beer from the German Imperial period (1885) and reconstructing brewing technology in the late 19th century, when pioneer works by Pasteur, Enzinger, Linde, and Hansen paved the way for modern science and industrial food production.

Keywords: *metabolomics, beer, FI-ICR-MS, foodomics, molecular networks*

G32

ACCURATE AND RELIABLE ANALYSIS OF FOOD SAMPLES USING ICP-MS

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The food supply is regularly monitored for potentially harmful residues and contaminants, including the analysis for pesticides, persistent organic pollutants and also toxic metals. This task is commonly fulfilled by analytical testing laboratories, which analyse large numbers of different sample types to provide results to manufacturers, food retailers and regulatory authorities.
For elemental analysis, the use of inductively coupled plasma mass spectrometry (ICP-MS) is widespread due to the outstanding detection limits that can be achieved. ICP-MS is also recognised as a robust analytical technology which suffers lesser sample matrix effects compared to other MS technologies, primarily because of the greater destruction of the sample matrix during sample preparation. Nonetheless, matrix effects can still be observed when samples contain differing amounts of major elements, or when, the digestion procedure results in different acid concentations. Although internal standardization provides a means to account for changing responses, the stringent QC requirements applicable in regulated laboratories may require a particular sample to be diluted and re-run, even in the case of small differences in response. This causes additional labor time and cost for the laboratory.
This presentation will highlight how ICP-MS can be used for the analysis of food samples daily using automated dilution of the sample extracts with argon gas, directly in the instrument, saving time and costs. The method was fully characterized and initially validated using a certified reference material and spiking experiments, and has been demonstrated to provide accurate and reliable results.

Keywords: *food analysis, ICP-MS, toxic elements, nutritional elements, robustness*

G33

APPLICATION OF ICP-OES FOR THE SIMULTANEOUS ANALYSIS OF NUTRITIONAL AND TOXIC METALS IN VEGETABLE OILS

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Vegetable oils are widely used for cooking, but despite their utility and nutritional properties, they may not always be free from toxic components and thus, potential health hazards. Regular and rigorous monitoring of the trace metal content of vegetable oils is required to ensure that they are fit for human consumption. Regulations such as the EU EC No. 1881/2006, for example, limit the highest acceptable level of lead in such products at 0.1 $mg \cdot kg^{-1}$. In this study we present a method for mutli-elemental analysis of vegetable oil samples using an inductively coupled plasma – optical emission spectroscopy (ICP-OES) technique. The developed analytical method targets both nutritional and toxic elements and helps to deliver the accurate, precise and fast results required by analytical science laboratories.
The Thermo Scientific™ iCAP™ PRO XP ICP-OES Duo, offering high sensitivity and stability across the full suite of analytes, was used for analysis in this study. The instrument provides flexibility to choose suitable analytical wavelengths and offers effective interference correction for target elements in a vegtable oil sample matrix. An optimized sample introduction system and simple sample preparation with minimal dilution helps achieve excellent detection limits even for those anlytes in the challenging ultraviolet wavelength region, such as phosphorus, lead etc. These results along with outcomes of uninterrupted measurement over several hours will be highlighted.

Keywords: *vegetable oils, ICP-OES, food regulations, toxic metals, nutritional elements*

G34

EVALUATION OF THE DETERMINATION OF DIOXIN IN FOOD AND FEED BY GC-MS/MS AND THE DIOXIN WORKFLOW KIT

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The Dioxin Workflow Kit was used for the monitoring of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in food of animal origin and feed using the Agilent 8890B GC and the Agilent 7010B Series Triple Quadrupole GC/MS system. The method setup and startup was done using the Dioxin Workflow Kit giving the chromatographic and MS settings, facilitating a quick and easy start.
The reporting was performed using the supplied dedicated Dioxin Reporting Solution. Overall, the method was shown to give linear response over the required concentration range, good repeatability of response and quantitation down to low pg /TEQ/g levels. The comparison of analytical results for the measured real matrix samples (egg, and animal feed) by GC-HRMS and GC-MS/MS indicates the suitability of the used Workflow Kit for the routine screening of dl-PCB congeners, that meets the requirements of current European Union legislation.

Keywords: *dioxins, furans, GC-MS/MS, food, feed*

G35

INFLUENCE OF CONVECTIVE AIR DRYING ON THE CHEMICAL COMPOSITION OF PUMPKIN PULP (CUCURBITA MAXIMA D.)

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Pumpkin is a fruit vegetable that is commonly cooked, baked and processed into various pumpkin products in the food industry, such as pumpkin pie, bread, cookies, cheesecake, desserts, donuts, cereals, ice cream, lasagna dishes, etc. Due to the large global production and use, it is necessary to use preservation methods that allow to preserve the properties of pumpkin for a long period of time. One of the most commonly used methods is convective hot air drying, which reduces water activity and thus minimises microbiological changes. However, during the drying process, structural and chemical changes can occur in the fresh material, ultimately affecting product quality. Therefore, with the aim of extending the shelf life of fresh pumpkin pulp (Cucurbita maxima D), hot air drying was also used in this work. Experiments were conducted at three different temperatures (50, 60, and 70 °C) and velocities (1.5, 1.0, and 0.5 m/s) using the following combined parameters: 50 °C (1.5, 1.0, and 0.5 m/s), 60 °C (1.0 and 0.5 m/s), and 70 °C (0.5 m/s). The chemical properties of the dried samples were evaluated based on moisture content, crude ash, crude fat, and crude fibre content. To evaluate the chemical composition of the dried samples and the efficiency of drying, analyses were also performed on fresh, undried pulp. In addition to the dried and undried pumpkin samples, the peel and seeds were also analysed as potential sources of nutrients generated during the preparation of fresh pulp for drying. As the result showed, pumpkin pulp and peel contained significantly higher moisture content (93.97 and 87.78%, respectively) than dried pulp (11.78-19.55%, depending on temperature and drying speed) and seeds (9.81%). The highest ash content is found in dried pumpkin peel (5.55 - 8.23 %), followed by pulp (0.55 %), peel (0.97 %) and seeds (2.75 %). The fat and total dietary fibre content is particularly high in pumpkin seeds, 21.44% and 56.55%, respectively. The high fat (3.24%) and fibre (18.52%) content is also found in the shell. Therefore, both by-products can be considered as valuable sources of fat and fibre, compared to dried samples with slightly lower amounts of these nutrients (1.03 - 3.13 % for fat) and (8.52 - 13.99 % fibre). In conclusion, the drying process resulted in a decrease in moisture content, which increased the shelf life of the squash. The dried samples are likely an excellent source of minerals due to their high ash content compared to the other samples. Although the fat and fibre content decreased due to the drying process, the results indicate that the functional properties of the pumpkin can be maintained after drying.

Keywords: *pumpkin pulp dried pumpkin pumpkin by-products hot air dying chemical composition*

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G36

NUTRITIONAL PROFILE OF BUTTERNUT SQUASH PULP, DRIED PULP AND BY PRODUCTS

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Global production of pumpkin (family *Cucurbitaceae*) has increased in the past year due to various consumption alternatives. Pumpkin can be consumed raw or cooked, baked, frozen, dried, crystallised, and freeze-dried. One of the most commonly used pumpkin processed products is flour, as it can be stored for a long time. As a natural colour additive, it can be added to baked goods, soups, sauces, and instant noodles to not only increase the content of various nutrients, but also improve their flavour. The simultaneous separation of pumpkin pulp for different uses results in large amounts of waste fractions, i.e. peel and seeds. From a nutritional point of view, the pumpkin pulp, pumpkin flour, peel and seeds are valuable sources of proteins, fibres, sugars, minerals, etc. Considering their functional properties and benefits for human health, the main objective of this work was to study the quality parameters of pumpkin pulp (*Cucurbita moschata*, "butternut" pumpkin), dried pulp and by-products in order to compare which of them represent nutrient-rich sources. In this context, dry matter, crude ash, crude fat and crude fibre were analysed in all pumpkin fractions. The drying process was carried out with air at 50 and 60 °C and a velocity of 1.0 m/s. The results showed that pumpkin peels and pulp had significantly higher moisture content (90.43 and 91.56 %) than the dried pulp samples (11.9 - 18.62 %) and seeds (5.44 %). On the other hand, dried fruit pulp samples contained high ash content (7.91 - 8.23 %), followed by pulp (0.78 %), skin (1.22 %) and seeds (4.83 %). The fat and total fibre contents are particularly high for pumpkin seeds, 35.57% and 24.86%, respectively. The high fat (1.90 %) and fibre (16.68 %) contents are also found in the shell compared to the pulp and dried pulp. The pulp contains 1.30 and 12.79 % fat and fibre, respectively. The dried pulp contains between 0.39 and 0.43% fat and 10.37 to 12.23% fibre, depending on the drying temperature.
In conclusion, all the pumpkin components studied in this work can be considered a valuable source of nutrients. For example, the dried samples with low moisture content extended the shelf life of pumpkin and due to their high ash content are an excellent source of minerals. Considering that seeds and peels are usually discarded, both by-products are interesting from a nutritional point of view due to their high fat and fibre content. Finally, the pulp, with its high moisture content, could be considered a useful source of water intake as part of a balanced diet.

Keywords: *pumpkin pulp, dried pumpkin, pumpkin by-products, chemical composition*

Acknowledgement: *This work was supported by means of the Croatian Science Foundation project IP-2019-04-9750.*

G37

ANALYSIS OF ORGANIC ACIDS IN BEER BY ION-EXCLUSION CHROMATOGRAPHY AND POST-COLUMN PH-BUFFERING CONDUCTIVITY DETECTION

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Yeast generates during fermentation numerous chemical compounds including ethanol, carbon dioxide, aldehydes, alcohols, fatty acids and organic acids, among others. The last compounds (mainly acetic, citric, formic, lactic, malic, succinic, and pyruvic acids) can influence besides the flavor (sour, bitter or salty) also the pH of beer. The presence of the acids can also contribute to inhibition the growth of some bacteria helping to improve shelf-life of beer. Therefore, the control of content of organic acids in beer is important.
Ion-exchange chromatography with gradient elution or ion-exclusion chromatography used in isocratic mode are established liquid chromatography methods for analysis of organic acids. The acidic eluent usually applied for ion-exclusion chromatography improves separation of organic acids, but the sensitive conductivity detection is affected by low-ionization grade of the analytes at low pH.
This poster presents a method for analysis of organic acids in beer based on ion-exclusion chromatography and pH-buffered conductivity. The method involves the successive addition of a pH-buffering reagent after column separation, to adjust the pH level to close to neutral. This not only reduces background noise, but also dissociates organic acids from the substance being analyzed. Consequently, electrical conductivity detection can then detect these organic acids with high sensitivity and selectivity.

Keywords: *organic acids in beer, ion-exclusion chromatography, pH-buffered conductivity detection*

G38

PHYSICOCHEMICAL AND VOLATILE PROFILE CHARACTERISATION OF BIDENS, MINT, COFFEE AND BLONG SONG HONEY ORIGINATED FROM VIETNAM

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Bidens honey (*Bidens pilosa*), mint honey (*Mentha Longifolia*), coffee honey (*Coffea robusta*) and blong song honey (*Schefflera heptaphylla*) are among the specific unifloral honeys in Southeast Asia and significantly differ from multifloral honey in their sensory patterns and availability. The task was to characterize the basic physicochemical properties and the specific volatile compounds present in 24 honey samples collected in seasons 2021 and 2022.
The pollen analyses confirmed the presence of specific botanical species in most of the tested honey. The four groups of honey did not differ from each other in the moisture content, which varied from 18.0 to 20.0 %; the rest of the following parameters (acidity, diastase activity, electrical conductivity, hydroxymethylfurfural, colour, monosaccharides and disaccharides) was found to be floral origin-specific. Coffee honey, which had the highest diastase activity (8.8 to 12.6 DN), the highest electrical conductivity (28.1 to 37.7 mS/m) and the darkest colour (41 to 48 mm in Pfund scale), was the most different honey from the others. And, unlike the others, it contained only a negligible content (less than 6 g/kg in total) of disaccharides sucrose, turanose, maltose, trehalose and melibiose. Subsequently, the volatile profile was analysed by SPME-GC/MS. A total of 30 volatile compounds were identified and quantified in the samples and classified into 5 groups according to their chemical structure, of which esters of organic acids represented the largest group. PCA applied to visualize data distribution revealed sufficient clustering of the groups of honey according to the floral source.

Keywords: *Vietnam, honey, chemical composition, quality parameters*

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G39

URBAN CONTROLLED ENVIRONMENT AGRICULTURE: SHORTENING SUPPLY CHAINS AND INCREASING SAFETY

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Recently food security has been impacted by two major global crises. First, the COVID-19 pandemic exposed food safety issues combined with the lockdowns affecting the food production and processing industries. Now we see the war in Europe challenging access to fertilizers and blocking grains from reaching the global market. These long food chains between countries and regions, for example, in the USA, where most of the food production concentrates in a few states, expose a vulnerability in moments of crisis and contribute to an increase in food insecurity. Over the years, urban areas have become exclusive food consumers, almost completely disconnecting food production and consumption. This situation contributes to unhealthy dietetic habits, massive food waste, increased food footprint, and cultural disconnection of the urban population with their meals. Urban agriculture (UA) helps reduce these impacts and increase benefits from social and environmental perspectives using innovative ideas and community-based solutions. Controlled Environment Agriculture (CEA) grows crops recirculating a balanced water nutritious solution to optimize the plant's growth. CEA production benefits by saving scarce nutrients, reducing between 70% to 90% of water use, and increasing yield using less space, helping reduce the lack of arable land, another problem that agriculture faces nowadays. These greenhouses facilities have complete control of the physical environment, such as temperature, humidity, CO2 level, and light. These enclosed spaces are also less likely to face contamination outbreaks. When food production becomes an urban activity, people are most likely to frequently connect with the local producers or become enthusiastic about urban farming, supporting access to some fresh vegetables in their diet. It contributes to the social, physical, and cultural reconnection with food. We are analyzing the social, economic, and environmental benefits and trade-offs of increasing the CEA food production in Washington DC using the well-established input-output methodology. We are creating a model to compare a soil-based and soil-less food supply, questioning that only cost and benefit analyses do not adequately capture social and environmental elements. In standard economics, money overtakes the value of listed environmental and social benefits. By reducing the distance between "farm and fork," we create a wide range of opportunities that contribute to urban sustainability and reduce the urban footprint. Using this framework, we can implement public policies to increase food safety and security, reducing health problems related to foodborne diseases created by the unsustainable food supply chain that is in place.

Keywords: *urban sustainability, controlled environment agriculture, food safety, food security, urban agriculture*

Acknowledgement: *This research was supported by the College of Agriculture, Urban Sustainability and Environmental Sciences of the University of the District of Columbia.*

G40

LOCAL CHIKEN BREEDS VALORIZATION BY IMAGE ANALYSIS APPLICATION ON EGGS PRODUCED IN ORGANIC SYSTEM

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The evaluation of food color is a psychological problem of sensitivity to attractiveness or to irritation. In the case of egg yolk color there exists an "optimal-yellow", which must be "appetizingly pretty," as determined by the "corruptible human eye". The yolk color is affected by genetics, housing and feeding system. Actually, for yolk measurement, the scale consists of 15 different color stripes ranging from light yellow to dark orange and red according to the Yolk Color Fan(Roche)scale. Consumer acceptability thus depends on visual impression, but the human eye is not very sensitive to the darker shades of yellow. To solve this problem, the aim of this study was to assess the pattern of color pigmentation of the egg yolk of Siciliana, Livornese and Lohmann White hens reared in organic system, using red green blue (RGB) image, for a future standardized technique with lowering human error by individual visual perception. For the trial, 63 eggs were sampled from 3 groups (21 eggs/group) of chicken breeds reared in organic system: Siciliana (S), Livorno (L) and Lohman White (LW). The individual egg yolks were placed on Petri dishes (50mm diameter) and photographed in a measurement chamber, with a camera for high-resolution data acquisition (16 million colors) by using an E-eye (Iris Visual Analyzer 400-Alpha MOS). The application of the software available in the instrument (Alphasoft, version 14.0) allowed to group color spectra in range of 16 bit for each coordinates RGB obtaining 4096 variables shown as histograms. To evaluate the ability of the E-eye in discriminating the different egg-producing breed, data collected on the samples of each group were processed by PCA. A selection of the most discriminant variables has been performed in order to improve the separation between samples. Results showed that, for S group, greater color homogeneity described by the predominance of a lower number of bars (colors) was seen (5 codes color); on the contrary, the number of bars increased passing from L (7 codes color) to LW group (11 codes color). The PCA analysis explained 99.53% of the total variance (98.61 for PC1 and 0.93% for PC2). Considering the locations of products on the surface (PCA score) was possible to note that S and L samples were quite grouped in a cluster, whereas LW samples were clearly differentiated from S and L, but divided in two groups mainly as a function of PC1. Different direction of vectors (PCA loadings), shows which variables (colors) were involved in the appearance variations among samples. Variables "colors 2144 and 2400" which describe the strongest yellow intensity affected mainly the position of S samples, on the contrary, the darkness variable "color 2128", was opposite and characterized L samples. Our results, which described yolk eggs colors and identified the 3 breeds, suggest that image processing can be applied to extract RGB image of yolk color, which could be used to develop the model of color recognition.

Keywords: *organic, eggs, yolk, color, local breeds, E-Eye*

Acknowledgement: *Study funded by PSR SICILIA 2014/2020 mis 10.2. project BIOSAVE, CUP G49J2100676009*

G41

TRUSTED ASYNCHRONOUS FEDERATED LEARNING FOR FOOD SUPPLY CHAINS

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Food supply chains are complex systems with many participating stakeholders. When spanning borders, regulatory domains, and heterogeneous business goals, there is an inherent challenge of trustworthiness even when technology like artificial intelligence, or IoT is applied to automate processes. Private permissioned blockchains and smart contracts emerged as a practical tool to establish trust among the cooperating partners and ensure the proper flow of goods along the supply chain, without having to rely on a central authority that manages the whole chain.
To meet the standards for food safety and track product quality, new devices for fast, on-the- spot, non-destructive sensing are being developed. These sensing devices can be easily operated by anyone and enable rapid collection of food quality measurements, but they often fail to meet the certification standards of regulatory bodies. Hence, their success crucially depends on using sophisticated machine learning models, trained on high-quality data, labeled with certified reference methods.
Instead of centrally collecting expensive training data for a specific use case and then deploying the sensors across the supply chain, we propose a novel trusted federated learning approach to learn prediction models for these kinds of sensors in a decentralized fashion wherein the data collection task is distributed along the whole supply chain. This collaborative learning empowers learning from a wide variety of quality datasets from various stakeholders in the supply chain and also reduces the operation cost burden from each stakeholder to procure reliable data. This results in smart decision-making to timely detect food contamination/spoilage risks in turn avoiding food wastage completely. Our approach tightly integrates the distributed model training with the blockchain, thus enabling secure and trusted model training and accurate predictions. The proposed framework is implemented to work asynchronously and is capable of respecting varying compute capabilities and availability of the participating clients. Based on Hyperledger Fabric and off-chain distributed storage, the framework is compatible with state-of-the-art machine learning tools, highly scalable, and allows full tracking of the learning process.

Keywords: *federated machine learning, food supply chains, trustworthiness, blockchain, sensing*

Acknowledgement: *This work is based on the "Federated and Trusted Food Supply Chains" project (FT-Chain) financed by the German Federal Ministry of Education and Research (BMBF) under grant reference number 01DS21011.*

G42

NOVEL APPLICATION OF ARTIFICIAL SENSES (E-NOSE AND E-TONGUE) AND CHEMOMETRICS APPROACH FOR RAPID ORGANOLEPTIC EVALUATION OF MILK

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Flavour is composed principally of the sensations of smell and taste. To separate and identify sapid substances, traditional methods of chemistry have been employed. By now, methods such as these have been applied and whole books have been devoted to the sensory assessment of milk. The origin of the significant flavorants in milk probably originate from the cow's metabolism; however, there is potential for manipulating flavour by means of feed. The aim of this study was to evaluate the efficiency of artificial senses combined with chemometric technique used almost like a human panel for to evaluate the effect of olive cake supplementation on the organoleptic/aromatic profile of cow's milk, in a process of valorization of by-products in the circular economy. In fact, the use of olive cake as animal feed represents a sustainable alternative reducing the costs associated with animal nutrition. For the trial, 24 multiparous dairy cows, breeding in the same condition, were divided into two homogeneous groups: Control (C), received conventional diet; Treated (T) received a conventional diet integrated with 7% of dried olive cake. Raw milk collection took place in the spring period (April-May 2022), in 3 sampling dates: after 15 days of dietary adaptation, then at 20 and 40 days of the trial. In the laboratory, the individual samples of milk from each group were mixed and analyzed 10 times using an E-nose (FOX 4000, Alpha M.O.S., Toulouse, France) with 18 MOS sensors and a potentiometric E-tongue (αAstree, Alpha M.O.S., Toulouse, France) with 7 chemical sensors. The results were subjected to exploratory PCA and expressed based upon the discrimination index (DI). Then, for increase the probability of correct classification, a low-level data fusion was investigated and the single-matrix with data from all sensors was subjected to a new PCA. On the basis of Mahalanobis distance, the pattern discrimination index (PDI) among the 4 groups was calculated.PCA analysis of raw data was able to discriminate all sampling groups, highlighting the effect of both time and diet. The DI was 83% and the first two components (PC1 and PC2), represented 95% of the total variance between the sample measurement. Observing the PCA plot, the groups are distributed along the first component (PC1 = 78.68%) as a function of the diet, while in upwards direction, along the second component (PC2 = 19.79%) as a function of time. The PDI results confirmed that the greatest differences in the taste and smell of the milk from the two groups are due to time. The organoleptic distances between the diet-related samples are very small, confirming the capacity of E-nose and E-tongue to find even minimal differences. The results highlighted by the artificial senses, shows that is possible to suggest the use of E-nose and E-tongue as effective tools to recognize a product made with alternative eco-friendly resources, addressed to minimize the environmental impact of the olive oil industry

Keywords: *milk, olive-cake, E-nose, E-tongue, chemiometry*

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G43

QUANTITATIVE ANALYSIS OF HAZARDOUS VOLATILE ORGANIC COMPOUNDS IN BABY FOOD USING HEADSPACE SPME-ARROW-GC/MS

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The quantification of dangerous volatile contaminants in baby food products is a relevant topic in food analyses. In this regard, we developed a HS-SPME-Arrow-GC/MS for the simultaneous analysis of furfural, furans, several fumigants, BTEX, THF and acrylonitrile residues.
For the validation was used 1g puree consisting of instant oatmeal and milk. The sample was weighed into a 20 ml HS vial with water, sodium chloride and a mix of suitable internal standards [1]. The vial was extracted at 40 °C with a DVB/carbonWR/PDMS SPME ARROW, that is even more sensitive than the common SPME phases [2]. The extracted sample was measured with a GC/MS Agilent 7010B GC/TQ, using a single ion monitoring acquisition.
The method was specific for all analytes and a good precision was reached. Linearity was verified in a range of at least two orders of magnitude and the LOQs varied from 0.1 to 5 µg/kg, confirming that the method complies with the current LOQ criteria for the analysis of furfural, furan, alkyl furans [3] and fumigants [4] in jarred baby food and of acrylonitrile [5] and BTEX in food. The recovery values revealed a low matrix effect for all analytes (82%-110%).
Under these premises we started to investigate other kind of products as powder milk, vegetable-, fruit-, meat- and cereal-jarred baby food of the German market. The main analytes found in high concentration in the samples were furans and furfural, especially in vegetable- and fruit-based products (Furans sum: max. 320 µg/kg; Furfural: max. 3500 µg/kg). This may be related to the degradation of ascorbic acid [6],[7] and to the reactions of pentoses [8], both naturally occurred in fruits and vegetables. Remarkable for the meat samples was the precence of 2-pentyl furan (max. 110 µg/kg), that may be generated from the protein and lipid fractions, during the cooking process through Maillard reactions [9].
Given the good validation and analytic results, we decided to continue to assess the risk associated with VOC contaminants in baby food and to validate new analytes of interest.

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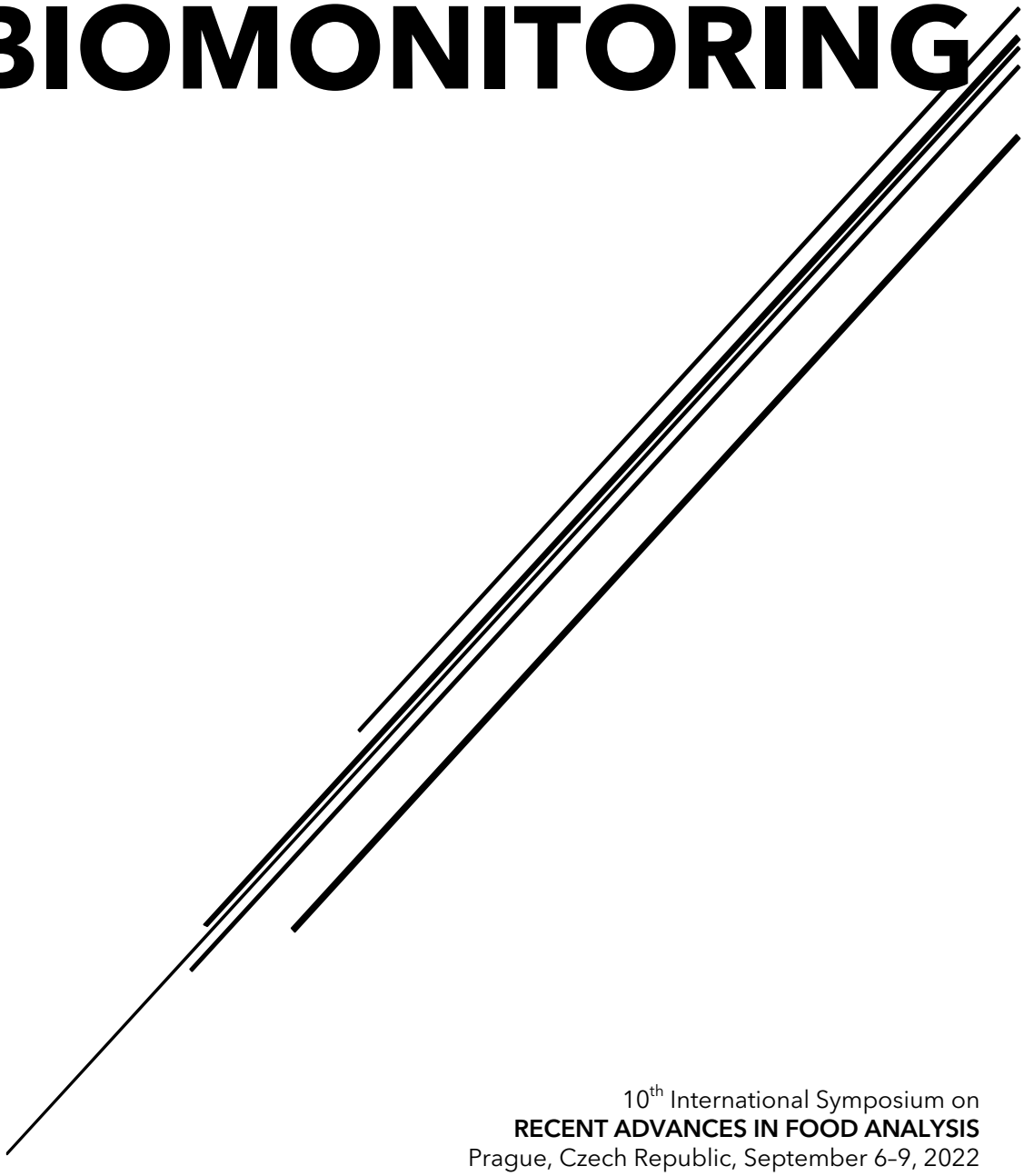
Keywords: *baby food, SPME-Arrow, VOC*

HUMAN BIOMONITORING



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H1

HUMAN BIOMONITORING OF URINARY ACRYLAMIDE BIOMARKERS IN THE EASTERN SPANISH ADULT POPULATION

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Acrylamide (AA) is a chemical compound classified as "Probably carcinogenic to humans" by International Agency for Research on Cancer (IARC) [1]. It is formed in processed carbohydrate-rich foods and in tobacco smoke, so the general population mainly exposed to AA by dietary intake and inhalation. Thus, exposure to AA might represent a potential public health threat. After dietary exposure, humans eliminate about 50% of AA in 24h in the urine as mercapturic acid conjugates, primarily as N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA), N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA3), and N-acetyl-3-[(3-amino-3-oxopropyl)sulfinyl]-L-alanine (AAMA-Sul), which can be used as short-term biomarkers of AA exposure.
A representative sample of the adult population (aged 18-65 years) living in the Valencian Region (Spain) were recruited in 2020-2021 as part of the BIOMOVAL project. The main aims of the present study were: i) to determine the urinary levels of AA metabolites, and ii) to estimate the risk associated to AA exposure in this population group.
First-morning urine samples were collected and analysed using a "*dilute-and-shoot*" method. The major urinary AA metabolites, AAMA, GAMA3 and AAMA-Sul, were determined by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS (QqQ)). The limit of quantification (LoQ) was 0.25 $\mu g \cdot L^{-1}$ for all metabolites.
To evaluate the risk associated to AA exposure, the estimated daily intake (EDI) was calculated based on the obtained urinary levels and compared with the Benchmark Dose Lower Confidence Limit ($BMDL_{10}$) by calculating the margin of exposure (MOE). The forward dosimetry approach was also used, where urinary AAMA levels were compared with its Biomonitoring Equivalent (BE) [2], by the calculation of the hazard quotient (HQ) [3].
AAMA, GAMA-3 and AAMA-Sul, were detected and quantified in 100% of the analysed samples, with geometric means (GM) of 84, 11 and 26 $ng \cdot mL^{-1}$, respectively. The calculated EDIs of AA ranged between 1.29 and 2.06 µg/kg-bw/day in the GM.
In a risk context, only a MOE lower than 125 was found in the assessed population group at the P95 distribution. By contrast, AAMA levels at GM were significantly higher than the BE, leading to HQ values between 4.6 and 8.2 in the GM.
Due to the high detection frequencies of AA metabolites on the analysed urine and that urinary levels of AAMA are between 5 and 8 times higher than the corresponding BE, the risk of exposure to AA in the target population could not be discarded. Thus, AA exposure in the Eastern Spanish population should be closely monitored and continuously evaluated in a risk context.

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Keywords: *acrylamide metabolites, human biomonitoring, urine, LC-MS/MS, risk assessment*

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H2

QUANTIFICATION OF FOURTEEN METABOLITES OF PHTHALATES IN HUMAN URINE USING DILUTE AND SHOOT AND LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY

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A quick, sensitive and high throughput method for determination of fourteen phthalate metabolites in human urine was developed. The proposed method includes an incubation with β-glucuronidase followed by dilution in 96-well plates. The diluted samples were directly injected in a liquid chromatograph coupled to triple quadrupole mass spectrometer for their quantification. The ionization mode selected was electrospray in negative mode. During the method validation the recoveries ranged between 80 and 120% with RSDs lower than 20% for all analytes using spiked levels from 1 to 500 ng mL^{-1}. The limit of quantification was 1 ng mL^{-1} for most of the metabolites and the linearity showed a R^2>0.99 in all cases. The proposed method was successfully applied for the determination of those compounds in 10 urine samples of volunteer adults from the Valencian Region (Spain).

Keywords: *phthalates, biomonitoring, urine, liquid chromatography, mass spectrometry*

Acknowledgement: *This work was developed in the framework of the BIOMOVAL project with the support of the Public Health Directorate of Valencia and FISABIO. The study was co-funded by the European Union through the European Regional Development Fund Operational Programme (ERDF) of the Valencia Region (2014- 2020). Pablo Dualde acknowledges his "Ayudas para la contratación PTA" (PTA2018–016320-I) from "Ministerio de Ciencia e Innovación" (Spain). Sandra Fernandez acknowledges her predoctoral contract ACIF/2019/083 (Generalitat Valenciana and the European Social Fund). Borja Peris would also like to thank the Valencian Government for his "ACIF" (Subsidies for the hiring of predoctoral research staff) predoctoral fellowship which also support this study".*

H3

RECENT ADVANCES IN DATA MINING FOR NON-TARGETED SCREENING WITH HIGH-RESOLUTION MASS SPECTROMETRY USING SCIEX OS SOFTWARE 2.0

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Non-targeted screening (NTS) workflows enable simultaneous detection, characterization, identification (if reference was reported previously) as well as relative quantitation of multiple environmental chemicals, metabolites and other small molecules. Non-targeted screening with high resolution mass spectrometry (HRMS) helps overcome challenges of characterization of thousands of compounds present in samples of interest (e.g. environmental exposure samples as well as human samples).
NTS data analysis is challenging in many ways: in order to obtain higher level of confidence in identification, often, advanced data-mining techniques including suspect screening by *in-silico* prediction, case-control strategy, stable isotope labeling, mass defect filtering, and product ion filtering are needed. SCIEX OS software enables NTS and suspect screening workflows in one workflow, as well as sample-control comparison and grouping by adducts. In the latter approach, a group of ions including adducts and isotopes of the same chemical that form a chromatographic peak at a specific retention time was considered to comprise the same feature. For each datapoint (within a feature) SCIEX OS software NTS workflow returns several ion characteristics such as retention time, molecular mass, fragment spectra and signal intensities as well as elemental composition and elemental formula derived from the accurately detected mass. In addition, advanced data querying and filtering functionality of the software enables application of logical statements including Boolean function e.g. IF, AND, OR as well as custom calculations on the results of analysis further optimizes advanced data-mining when performing NTS utilizing HRMS. Here we present the SCIEX OS software data-mining workflows applied to NTS analysis of several example data including indoor environmental and food and beverage samples.

Keywords: *non-targeted screening, library searching, suspect screening*

H4

MONITORING OF BENZOPHENONE AND CAMPHOR UV-FILTERS IN HUMAN URINE

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By monitoring biomarkers in urine, a person's exposure to selected chemical substances can be assessed. In this study, 9 organic UV-filters were monitored in 494 urine samples of mothers and their newborns from an industrial (Karvina) and a reference area (Ceske Budejovice) during the winter and summer season. The analytical method consisted of enzymatic hydrolysis, liquid-liquid extraction (ethyl acetate), purification by dispersive solid-phase extraction (Z-Sep sorbent), and ultra-high performance liquid chromatography coupled with tandem mass spectrometry [1]. Limits of quantification of the method ranged from 0.001 – 0.100 ng/mL, with recovery 92 – 115 % and repeatability 2 – 7 %.
Benzophenone-1 (BP-1) was found in all analysed urine samples. On the contrary, 3-(4-methylbenzylidene)camphor (4-MBC) and 3-benzylidenecamphor (3-BC) were not determined in any urine sample. The analyte with the highest concentration was benzophenone-3 (BP-3) with a median concentration 5.95 µg/g creatinine. Comparable concentrations of UV-filters were found in the urine of mothers (20.9 µg/g creatinine) compared to their newborns (19.2 µg/g creatinine) in both locations in both periods. The presented results indicate that the exposure to UV-filters is approximately 1.6× higher in the industrial area (24.3 µg/g creatinine) than in the reference area (15.2 µg/g creatinine) for mothers and their newborns in both seasons. A comparison of the urine sampling season showed comparable concentrations of UV-filters in measured urine samples except for the urine samples collected in Karvina from mothers in winter (13.7 µg/g creatinine) and summer (30.5 µg/g creatinine) where approximately 2.2× times higher concentrations were found in urine samples collected in summer. The concentrations of UV-filters from the presented research are comparable with results from Brazil [2], the USA [3], and Denmark [4] and 10× lower than concentrations of these compounds measure in urine in China [5].

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Keywords: *biomonitoring, biomarkers, Czech mothers and newborns, UV-filters*

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H5

EXPANSION AN LC-MS/MS EXPOSOME BIOMONITORING METHOD FOR VETERINARY DRUGS AND PESTICIDES

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Humans are constantly exposed to a cocktail of xenobiotics through diet, environment and consumer products. Only a limited number of exposed xenobiotics and their metabolites are monitored through targeted approach in human biomonitoring programs in the EU. Measuring the chemical exposome, i.e., the totality of chemical exposures is a challenging task owing to its complex and dynamic nature. LC-MS/MS has been used as a method to determine targeted xenobiotics in environmental and biological samples. Recently the LC-MS/MS exposome biomonitoring method of Jamnik et al. [1] have shown promising results for simultaneous determination of >80 xenobiotics in human bio-fluids at the pg-ng/mL level. However, veterinary drugs and pesticides, an important class of xenobiotics, have not yet been systematically investigated within the exposome framework. Therefore, the current research focused on the expansion of Jamnik's targeted exposome method to determine veterinary drugs and pesticides, which may fill existing research gaps for better understanding of the chemical exposome. Tuning and multiple reaction monitoring (MRM) parameters were successfully optimized for veterinary drugs (n=28) and pesticides (n=22) including selected internal standards (n=11). Tuning was performed on a QTrap 6500+ mass spectrometer with an ESI source that was operated in positive and negative ionization mode. The best precursor ion (Q1), product ions (Q3), declustering potential (DP), collision energy (CE) and cell exit potential (CXP) were established. MRMs were used to select the two intense ions as quantifier and qualifier respectively. First real-life samples will be screened for veterinary drugs and pesticides. In conclusion, the expanded LC-MS/MS method could be beneficial for routine biomonitoring of multiple co-exposures incorporating veterinary drugs and pesticides in human biofluids.

1. Jamnik T, Flasch M, Braun D, Fareed Y, Wasinger D, Seki D, Berry D, Berger A, Wisgrill L, Warth B (2022). Next-generation biomonitoring of the early-life chemical exposome in neonatal and infant development. Nature Communications, 13, 2653

Keywords: *LC-MS/MS, biomonitoring, veterinary drugs, pesticides, exposome*

Acknowledgement: *The research was funded by the FWF-Austrian Science Fund through a Lise-Meitner postdoctoral fellowship program (Project No. M3217-N).*

H6

SPECIFIC PERSONAL EXPOSURE TO PARTICULATE POLYCYCLIC AROMATIC HYDROCARBONS IN THE CZECH REPUBLIC

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Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants produced during incomplete combustion of organic matter. Humans can be exposed to them via several pathways (inhalation, digestion, dermal exposure).[1] PAHs introduced into the atmosphere are distributed in both gaseous and particulate matter (PM). Different size of particles found in PM can bound various PAHs depending on volatility and molecular structure. Long-term exposure to PAHs is correlated with mortality from cancer, cardiovascular and respiratory diseases.[2] Limited studies published until now have explored the association between PM bound PAHs within personal air monitoring. The aim of our study was to assess the relationship between the total personal exposure to PAHs bound to PM in different size (<0.25, 0.25 – 2.5, and >2.5 μm). A total set of 129 participants from two localities of the Czech Republic (České Budějovice and Ostrava) took part in 24 h personal air sampling, at various seasons. Extraction of target 23 PAHs was carried out by organic solvent extraction (hexane: dichloromethane, 3:1 *(v/v)*) in the ultrasonic bath followed by gas chromatography coupled to tandem mass spectrometry in electron ionization (GC-EI-MS/MS). The recoveries of all target analytes were within the range from 70 % to 75 % and the repeatabilities were below 21 %. The limits of quantification (LOQs) were in the range from 0.001 to 0.02 ng/m^3 . The total amount of sum 23 PAHs ranged from 0.02 to 26.13 ng/m^3, with median 0.3 ng/m^3. Concentration of Benzo(a)pyrene, as an air quality standard issued in Europe (daily averaged concentration of 1.0 ng/m^3), ranged from 0.01 to 3.27 ng/m^3, with median 0.02 ng/m^3. The particle size distribution of PAHs during personal exposure showed that up to 87% of the total amount of PAHs is bound to the fraction < 0.25 μm, 12% of PM bound PAHs were in the fraction 0.25-2.5 μm and only 1% in the fraction larger than 2.5 μm. Based on a comparison of the median concentrations of the analyzed PAHs and the daily inhalation exposure at the monitored locations, it was found the air in Ostrava is twice higher compared to in the Czech Budejovice, which is related to the increased industry in this area.

[1] Kim, K; Jahan, A; Kabir, E; Brown, RJ. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. *Environment International,* 2013, *60*, 71-80
[2] Pelucchi, C; Negri, E.; Gallus, P.; Boffetta, P.; Tramacere, I.; La Vecchia, C. Long-term particulate matter exposure and mortality: a review of European epidemiological studies. *BMC Public health* 2009, *453*.

Keywords: *polycyclic aromatic hydrocarbons, air pollution, risk assessment*

Acknowledgement: *This work was financial supported from a specific university research (A1_FPBT_2022_05) and the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798), which we are gratefully acknowledged.*

H7

BIOMONITORING OF MULTICLASS PERSISTENT ORGANIC POLLUTANTS IN PAIRED BLOOD SERUM OF CZECH MOTHERS AND THEIR NEWBORNS

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Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and poly- and perfluoroalkyl substances (PFAS), represent a wide group of chemicals with high stability, persistency, mobility over long distances and toxicity for organisms and the environment. For that reason, many of them are listed under the Stockholm Convention on POPs and are monitored within biomonitoring, which represents a scientific approach used for the assessment of the human internal exposure to various chemicals via analysis of proper biological fluids and tissues.
The main aim of the study was complex assessment of the body burden of Czech mothers and their newborns to both classic and new groups of POPs. Altogether 8 PCBs, 12 OCPs, 9 BFRs, and 29 PFAS were analyzed in 415 samples of maternal blood serum (MB) paired with 415 samples of the umbilical cord serum (UC). A method consisted from multiple extraction of PCBs, OCPs and BFRs into *n*-hexane:diethylether mixture with purification on a silica column, after removing of organic layer PFAS and some BFRs were isolated by acetonitrile. Both gas and liquid chromatography coupled to (tandem) mass spectrometry were used.[1]
PCBs (# 138, 153, 180) and OCPs (*p,p'*-DDE, HCB) were the major chlorinated POPs found in >86% of all samples, while higher levels were found in MB compared to UC serum. Their median amounts (in ng/g l.w.) were as follows: PCB 138 (MB 17.0; UC 10.0), PCB 153 (MB 27.8; UC 14.8), PCB 180 (MB 27.7; UC 13.4), *p,p'*-DDE (MB 64.2; UC 48.0), and HCB (MB 7.9; UC 6.5). The concentrations of BFRs as well as their detection frequencies were generally lower compared to PCBs and OCPs. Only BDE 47 was found in >62% of samples at 0.42 in MB and 0.51 ng/g l.w. in UC. The other BFRs were found in <25% of samples. The trend in BFR concentrations is opposite to PCBs and OCPs, i.e. slightly higher amounts were observed in UC. From PFAS group, PFOA, PFNA, PFDA, PFHxS, and PFOS were found in all samples. PFOA and PFOS were the major PFAS at median concentrations (in ng/mL) 0.4 and 0.9 in MB and 0.3 and 0.9 in UC, respectively. For 8 POPs strong linear dependence among levels in paired samples was observed. Correlation coefficients of PCB 138, *p,p'*-DDE, and PFOA were 0.87, 0.81, and 0.91, respectively. Our data were lower compared to our previous study[2] and with long-term data from National Institute of Public Health. We should conclude that the amounts of POPs in Czech population gradually decrease over years. Generally, the present levels of PCBs, OCPs, and PFAS are lower compared to worldwide studies.

1. Svarcova, A. *et al.* Integration of five groups of POPs into one multi-analyte method for human blood serum analysis: An innovative approach within biomonitoring studies. *SCI TOTAL ENVIRON* 667, 701–709 (2019).
2. Polachova, A. *et al.* Biomonitoring of 89 POPs in blood serum samples of Czech city policemen. *Environ. Pollut.* 291, 118140 (2021).

Keywords: *human biomonitoring, persistent organic pollutants, internal exposure, maternal blood serum, umbilical cord serum*

Acknowledgement: *This work was supported by the European Regional Development Fund under Grant Healthy Aging in Industrial Environment HAIE (CZ.02.1.01/0.0/0.0/16_019/0000798) and also received funding from the grant of Specific university research – grant No A1_FPBT_2022_005.*

H8

A QUECHERS-BASED PROTOCOL FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN HUMAN MILK

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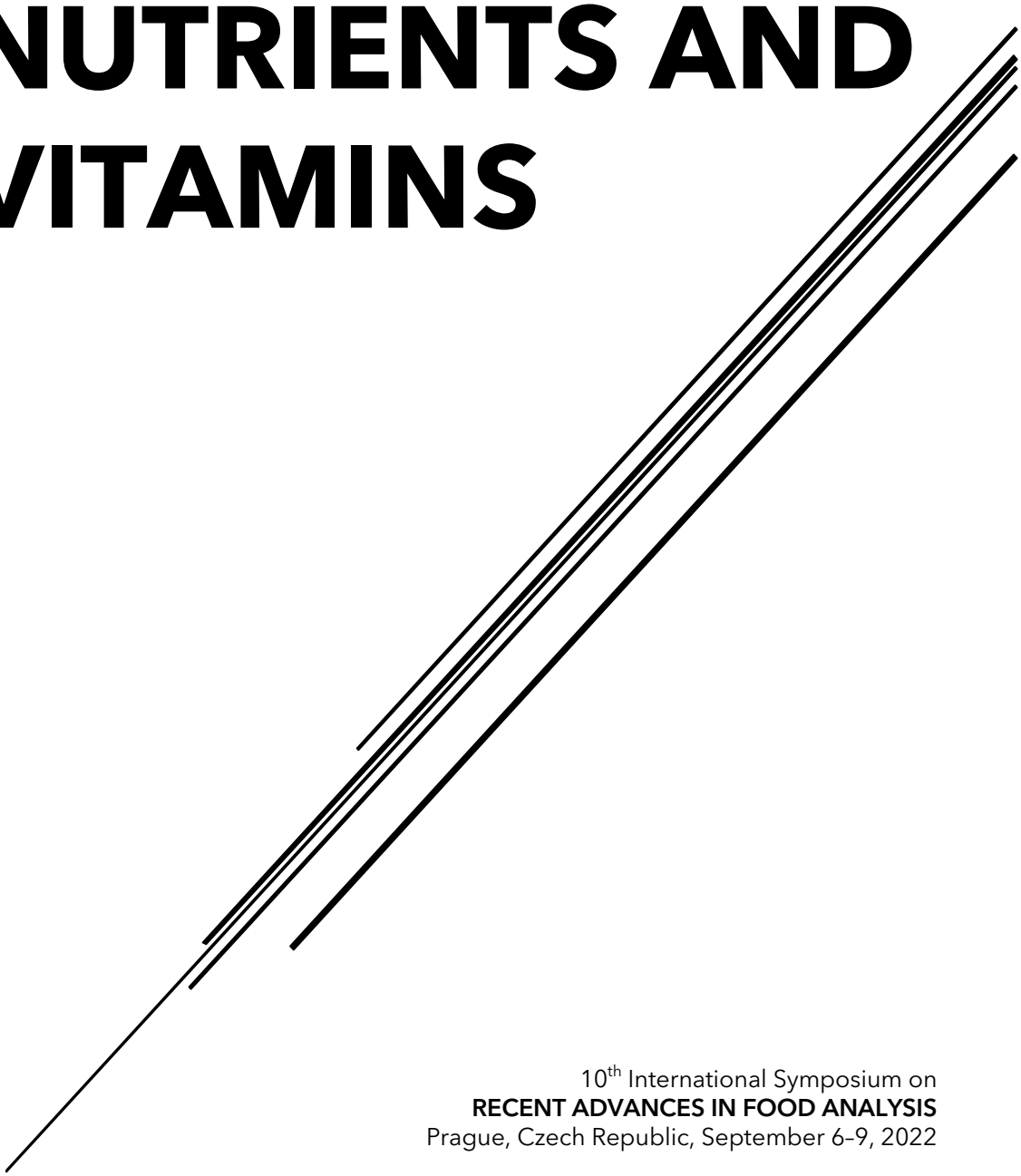
Human milk is the gold standard for infant nutrition due to supplies all nutrients necessary for the healthy growth. In view of this, the World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of life. On the other hand, The composition of human milk might be affected by mother's diet and lifestyle. Due to the lipophilic characteristics of human milk, some contaminants ingested by mothers, such as polycyclic aromatic hydrocarbons (PAHs), might be detected in human milk. PAHs are carcinogenic organic compounds resulting from incomplete combustion of carbon-based materials, such as fuel or being formed in foods during cooking at high temperatures. Up to date, there is no information on the incidence of PAHs in human milk from Brazilian mothers. Because of this, the objective of this study was to evaluate the incidence of 18 PAHs in human milk donated by mothers from Campinas-SP by gas chromatography-mass spectrometry. The mothers were questioned about their place of residence, food and smoking habits, as well the use of consumer goods to identify any correlation between the incidence of PAHs in human milk. Next, the samples were prepared using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach. For this purpose, it was evaluated the effect of PSA, NaCl, C18, $MgSO_4$, and acetonitrile through Plackett-Burman design. The variables were evaluated using 10% of significance, to avoid the exclusion of any important variables. The method was validated in term of linearity, recovery, inter and intra-day precision and showed satisfactory results according to SANTE/2020/12830 guidelines. Afterwards, the method was applied to 78 samples of human milk. This is the first set of data on PAHs in human milk from Brazilian mothers.

Keywords: *human milk, analytical methods, polycyclic aromatic hydrocarbon, food analysis*

Acknowledgement: *This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [2021/02153-5; 2020/02728-5].*

MAJOR NUTRIENTS AND VITAMINS



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I1

SIMULTANEOUS DETERMINATION OF 49 AMINO ACIDS, B VITAMINS, FLAVONOIDS AND PHENOLIC ACIDS IN VEGETABLES BY LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY

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Amino acids, vitamins, flavonoids, phenolic acids, and other natural plant active ingredients contained in vegetables with important nutritional and medicinal values and functions have a protective potential for human health and have attracted considerable research interest. A sensitive and reliable analytical method was developed and validated for simultaneous determination of 49 metabolites based on a rapid metabolomic extraction procedure combined with liquid chromatography-tandem mass spectrometry (LC–MS/MS) in a single chromatographic run. Chromatographic and sample preparation conditions were thoroughly optimized, given the high diversity of the target analytes. Eight isotope-labeled standards were applied to validate the proposed method in terms of recovery, linearity, matrix effects, precision, and sensitivity.
In this study, a sensitive and reliable LC-MS/MS approach for the simultaneous rapid qualitative and quantitative analyses of 15 amino acids, 7 B vitamins, and 27 polyphenols in vegetables were developed, optimized, and validated. Since the selected analytes are natural active substances with antioxidant effect, a 0.2% vitamin C in 50% methanol aqueous solution is used during extraction to prevent oxidation of the analyte. A 20 min reversed phase gradient was used for separation followed by detection in MRM mode with positive and negative Electrospray. Method validation was carried out in terms of recoveries, linearity, matrix effects, precision, and sensitivity. The application of isotope-labelled standards guaranteed good method validation results. Most of the recoveries in four vegetable matrices ranged from 65.0% to 105.3% with associated RSDs< 20 %. Low LOQs was obtained, ranging from 0.06 to 17 µg/kg. Different range of linear calibration curves were established with R^2>0.993.
The proposed method has been successfully applied for accurate quantification of 49 compounds in 26 vegetables from different categories. The concentration of these compounds in vegetables were found highly species and variety dependent. Purple cabbage had the highest content of free amino acids and broccoli had higher levels of free B vitamins. *Compositae* and *Apiaceae* vegetables contained very high concentrations of chlorogenic acid, resulting in high total phenolic compound contents for these vegetables, especially red lettuce. This methodology was proved to be simple, reliable and high efficient for determination of the selected 49 nutrients and antioxidants in vegetables, which could be used for nutritional and metabolomics studies.

Keywords: *metabolites, vitamins, amino acids, LC-MS/MS, vegetable*

I2

THE EFFECT OF CULINARY TREATMENT ON THE CONTENT OF VITAMIN D IN UV TREATED MUSHROOMS

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Vitamin D plays an important role in many aspects of human health, such as skeletal and cardiovascular diseases, neuromuscular problems, diabetes, autoimmune and chronic diseases. This vitamin is currently mentioned in connection with the support of the immune system against Covid-19 disease. Although vitamin D can be partially synthesized by the human body from its precursor 7-dehydrocholesterol, vitamin D deficiency is currently a global health problem. We can increase the level of this vitamin by a diet rich in fish, eggs, milk and dairy products, or mushrooms with an increased content of vitamin D. Mushrooms, like human skin, can produce vitamin D when exposed to UV radiation. During this process, the content of ergosterol on the surface of the fungus is converted to ergocalciferol (vitamin D_2) by a series of photochemical and thermal reactions. The amount of vitamin D thus formed in mushrooms is considerably higher than the content of vitamin D in fortified food products. However, due to culinary adjustments, it can be lost again. The aim of the study was to determine the effect of culinary modifications of cooking, baking, frying, freezing and drying on the content of vitamin D in mushrooms treated with UV radiation. Sample preparation included repeated extraction with a mixture of *n*-hexane:ethylacetate (4:6, *v/v*) and analysis was accomplished with liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). For separation of ergocalciferol from ergosterol (both with molecular weight 396.65) was used chromatographic column ZORBAX Eclipse PAH and was applied gradient elution using 10mM ammonium formate in deionized water with 0.1 % formic acid and methanol:acetonitrile (1:1, *v/v*) also with 0.1 % formic acid. Limit of quantification for ergocalciferol was 10 ng/g of fresh mushrooms. Mushrooms used for these experiments were treated with UV-B radiation at an intensity of 3.25 mW/cm^2 for 6 seconds after harvesting. The content of vitamin D in the mushrooms thus treated averaged 21.5 ± 5.4 µg/100 g of fresh mushroom. The mushrooms were then cut and heat treated. It was found that culinary treatment causes losses of vitamin D in the range of 9.5–63 %. In the processing of mushrooms by cooking, depending on the pH of the water in which the mushrooms were cooked, there was a decrease in vitamin D content by 9.5–63 %. In the case of frying, the decrease in vitamin D in mushrooms was significantly higher at a lower temperature acting for a longer period of time, namely 61 %, at a higher temperature the decrease was only 17 %. In baking, the losses were more dependent on the time of its action than on used temperature and the losses ranged between 35–47 %.

Keywords: *mushroom, vitamin D, culinary treatment, LC-MS/MS, ergocalciferol*

Acknowledgement: *This work was supported from the grant of Specific university research – grants No A1_FPBT_2022_005 and A2_FPBT_2022_072.*

I3

DETERMINATION OF VITAMIN D AND ITS METABOLITES USING LC-MS/MS

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Vitamin D plays an important role in many aspects of human health, such as skeletal and cardiovascular diseases, neuromuscular problems, diabetes, and autoimmune and chronic diseases. In recent years, dietary intake of vitamin D has become an issue of a high concern as this bioactive molecule boosts the immune system and is presumed to provide some protection against Covid-19. Although vitamin D can be partially synthesized by the human body from its precursor 7-dehydrocholesterol, deficiency of this vitamin is currently a global health problem. Therefore, it is necessary to supply the body with vitamin D using fortified foods, such as UV-treated mushrooms. Because vitamin D is one of the lipophilic vitamins and its excessive intake can cause problems associated with hypervitaminosis (high blood pressure, bone loss, and kidney damage if not treated), it is important to monitor levels of this vitamin in fortified foods as well as in the blood. For this reason, it is necessary to have available accurate and fast methods for its quantification.
The aim of this study was to optimize and validate a fast and sensitive method for the determination of vitamin D_2 in fresh mushrooms and its metabolites 25(OH)D_2 and 25(OH)D_3 in the blood using liquid chromatography coupled to mass spectrometry (LC-MS/MS). As part of the study, both the parameters of the LC-MS/MS method and the extraction methods for fresh mushrooms and for blood were optimized. As optimal for extraction of desiccated mushrooms, solid-liquid extraction *n*-hexane:ethyl acetate, and *n*-hexane for blood plasma samples was chosen. Separation of target analytes was performed on a Zorbax Eclipse PAH column in combination with mobile phase consisting of acetonitrile:isopropanol:water. Satisfactory recoveries (> 80 %), repeatabilities (< 20 %) as well as limits of quantification (LOQs) were reached both for the control of a vitamin D_2 content in mushrooms (LOQ = 10 ng/g) and for the monitoring of vitamin D_2 and D_3 metabolite in human blood (LOQ = 2.5 ng/ml). For accurate quantification, isotopic dilution was employed.

Keywords: *vitamin D, LC-MS/MS, 25(OH)D, mushrooms, blood*

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I4

VITAMIN K (PHYLLOQUINONE AND MENAQUINONES) IN FOOD – LC-ESI-MS/MS METHOD DEVELOPMENT AND VALIDATION

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Vitamin K describes a group of fat-soluble vitamers; phylloquinone (PK) and menaquinones (MKs). As growing evidence of the importance of vitamin K in human health beyond blood coagulation and maintenance of healthy bone tissue, further research of vitamin K is necessary. Limited knowledge of the MK content in food exists which limits the study of the influence of MKs on the public health. At present only PK content has been stated in standard reference material (SRM), which causes uncertainty for the trueness of methods that quantify MKs. Additionally, standards of MKs are expensive and not readily available for all laboratories.
We have developed an LC-ESI-MS/MS method for quantification of 8 vitamin K vitamers (PK, and MK-4 to MK-10) using internal standards (ISs) (deuterium labelled (d7) PK, d7-MK-4, d7-MK-7 and d7-MK-9), and only 4 standards (PK, MK-4, MK-7 and MK-9). The validation of the method included assessment of matrix effect (ME) in cheese, natto and lamb liver, limit of quantification (LOQ), precision, and trueness. The LC-MS/MS method runtime is 9 min. The method was compared to an LC-FLD method (CEN 14148), for quantification of vitamin K in broccoli, cheese (cow and goat), natto, liver (lamb and veil) and microalgae[1].
LOQs of the method were 0.5 µg/100g for PK, MK-4, MK-6 and MK-7, 0.7 µg MK-5/100 g, 1.4 µg MK-8/100 g, 1.0 µg MK-9/100 g, and 4.0 µg MK-10/100 g. MEs were observed for all vitamers, but these were corrected for by the ISs. No significant differences between the quantified content by the LC-MS/MS method and the LC-FLD method were observed. Both methods quantified contents in SRM kelp (NIST 3232) within the acceptable range.
We then came into possession of standards of MK-5, MK-6, MK-8 and MK-10 and incorporated these in the method. Recovery tests were performed utilising pork spiked with 20 ng and 400 ng PK and the MKs. Significant matrix effects were observed causing recoveries of MK-5, MK-6, MK-8 and MK-10 of 87±6%, 77±4%, 191±26% and 17±3%, respectively. The recoveries of PK, MK-4, MK-7 and MK-9 where 106±7%, 95±9%, 95±2% and 96±7%, respectively. We then prolonged the method to a total runtime of 20, where after the recoveries of MK-5, MK-6, MK-8 and MK-10 improved with all vitamers achieving recoveries within the range of 80-120%. Furthermore, an improvement of the LOQs were obtained resulting in LOQs of PK, and MK-4 to MK-10 of 0.25 µg/100 g. We additionally quantified the content of PK in two SRM (kelp NIST 3232 and infant formula NIST 1849a) where contents within the acceptable range were obtained.
These results underlines the importance of recovery tests in a range of matrices to confirm that no matrix effect is significantly effecting the quantification of vitamin K when analyzing food.

1 Jensen, M. B., Rød, K. E., Ložnjak Švarc, P., Oveland, E., Jakobsen, J. (2022) Vitamin K (phylloquinone and menaquinones) in foods – Cost-effective quantification by LC-ESI-MS/MS. *Food Chemistry*, Volume 385, 132672.

Keywords: *menaquinones, recoveries, matrix effect, trueness, LC-MS/MS*

I5

DEVELOPMENT OF A SENSITIVE MICROBIOLOGICAL METHOD FOR QUANTIFICATION OF VITAMIN B12 IN PLANT FOODS

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Vitamin B12 has become a research topic of great interest in the face of several serious health consequences associated with VB12 deficiency, which is endemic among the rapidly increasing vegan population worldwide. VB12 is an integral cofactor in metabolism, and the deficiency of VB12 could lead to serious health conditions, including pernicious anaemia, cardiovascular disease and neurological disorders. Vegans have a high prevalence of VB12 deficiency as VB12 is found almost exclusively in animal-source foods. Several plant sources (including edible algae, tea and mushroom) have been reported to contain a relatively higher content of VB12 and could be the potential non-meat sources of VB12 for vegans. The microbiological assay based on the growth of a VB12 auxotroph bacteria, *Lactobacillus leichmannii* (ATCC®7830™), could be used to quantify VB12 as the bacterial growth is proportional to VB12 concentration under appropriate conditions. This study reports the optimization of a 96-well microtiter plate microbiological method capable of measuring trace levels of VB12 in different plant-based materials. The limit of detection (LOD) is 0.82 pg/ml, and the limit of quantification (LOQ) is 2.58 pg/ml, both of which were superior to other methods reported in the literature. VB12 levels were found to be considerably higher in several red and green seaweed species (15.3 to 64.7 µg/100g DW) than in mushroom and tea samples (≤ 2.5 µg/100g). Consumption of these seaweeds could provide a sufficient amount to meet the EU's recommended dietary allowance of 2.4 µg/day for VB12. The developed method with demonstrated excellent performance can be used to quantify VB12 in various food commodities.

Keywords: *vitamin B12, microbiological assay, seaweed, vegan*

I6

NOVEL MULTI-VITAMIN B METHOD FOR THE ANALYSIS OF SUPPLEMENTS OFFERING IMPROVED WORKFLOW WITHIN A LABORATORY

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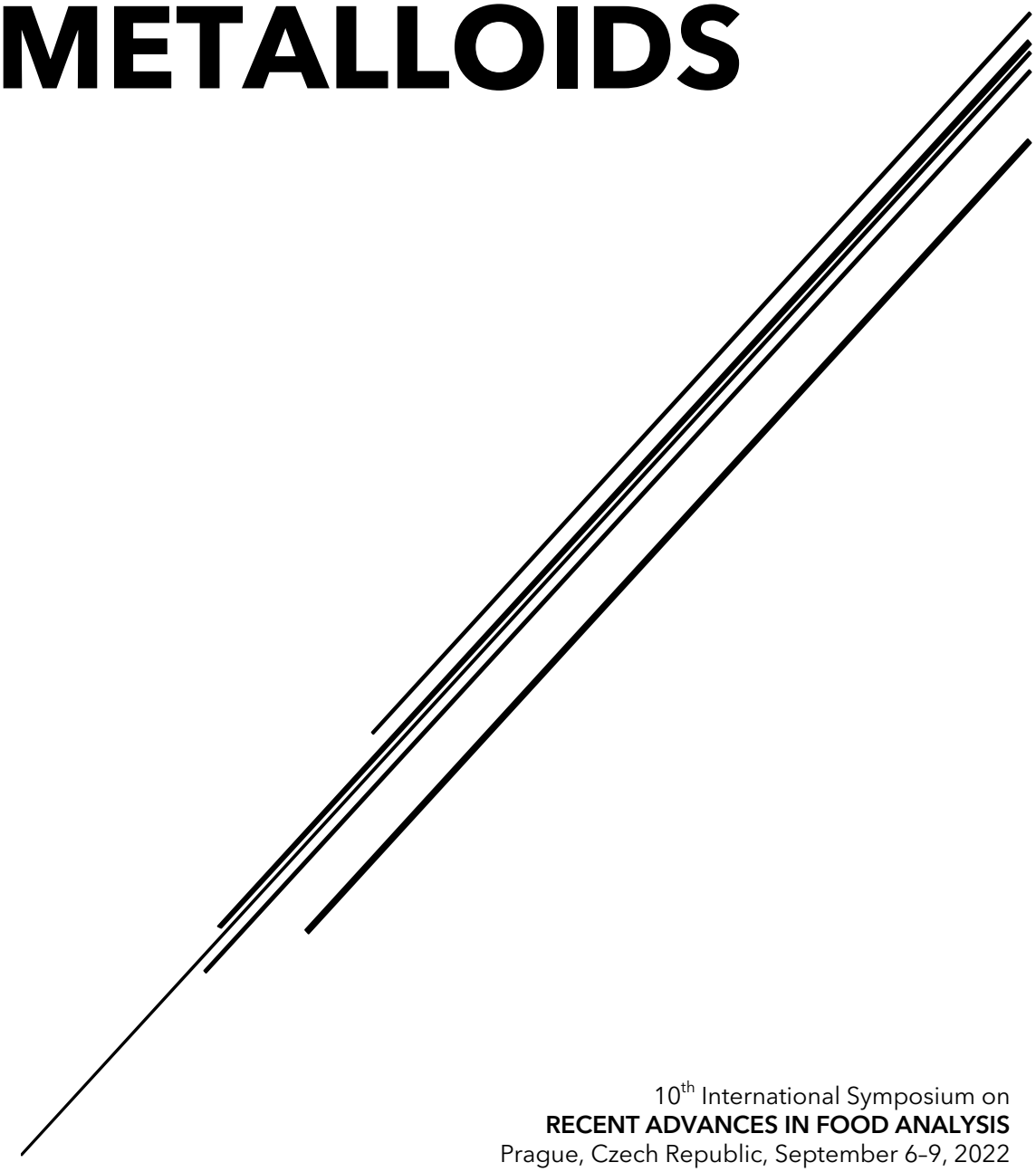
The health supplement industry is consistently expanding as interest and understanding in personal health becomes more widespread. Vitamin supplements take up the majority of the market and are taken by all age ranges for enhanced energy, weight management, immune support and filling nutritional gaps in restricted diets like veganism. Many supplements contain a plethora of vitamins aimed to fulfil all nutritional reference values, however some may only contain a high dose of one vitamin.
Supplements exist in many different forms such as tablets, capsules, chewables and liquids which aim to deliver a dose of vitamin conveniently to the user. These supplements are regulated to ensure the claim value of vitamin matches the actual content of the product until the end of its shelf life.
Analytical methods should be suited to deal with the relatively high concentration and mixtures of vitamins in these products and provide an accurate measurement within a sample within a reasonable timeframe. A novel mulit-vitamin immunoaffinity column, EASI-EXTRACT MULTI-VIT B (LGE), utilises a simple extraction procedure prior to LC-MS/MS detection which enables the simultaneous analysis of biotin (B7), folic acid (B9) and vitamin B12. The immunoaffinity column reduces the amount of unwanted sample reaching the LC-MS/MS, reducing matrix effects and maintenance requirements of the system.
The recovery of biotin, folic acid and vitamin B12 ranged between 79.89 – 120.68 for all samples tested and in all instances variation between triplicate IACs was <5% RSD, demonstrating good accuracy and precision. There were no significant matrix effects observed with any sample tested and analyte peaks were distinct and clearly resolved, demonstrating the benefit of immunoaffinity clean-up.

Keywords: *supplement, multi-vitamin, immunoaffinity column, easi-extract, LC-MS/MS*

METALS AND METALLOIDS



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J1

OPTIMIZATION OF AN IC-ICP-MS ANALYTICAL METHOD FOR DETERMINATION OF INORGANIC ARSENIC IN ALGAE AND ALGAE BASED-PRODUCTS

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Algae are known for their nutritional benefits and they are increasingly used as feed and food in EU countries (1). However, algae are also known to accumulate arsenic (As). Among the arsenic species, the health effects of inorganic forms are generally considered more toxic as compared to the organic forms (2). Arsenosugar compounds (AsSug) are generally the most abundant organic As species in algae and their potential toxicity is still being assessed. Precise determination of the inorganic fraction of As (Asi) is needed to assess the potential adverse effects in a given feed or food product. In this sense, two methods were approved by the European Committee for Standardization (CEN) for measuring Asi in food (EN 16802:2016) and feed (EN 17374:2020) by anion-exchange HPLC coupled to ICP-MS following waterbath extraction. However, as algae matrices contain complex As species, such as arsenosugars, the chromatographic separation of the current CEN standards may require optimization to improve the resolution and quantification of Asi. In addition, a robust method is needed which can be applied on different instruments.
In this study, we describe the optimization of a robust IC-ICP-MS analytical method for the determination of Asi in algae and algae based-products. The chromatographic separation was optimized on two different anion exchange columns with optimal gradient elution conditions to achieve a high peak resolution of the Asi with other adjacent arsenic species. For extraction of As species, two extraction procedures were compared in mildly acidic solutions (0.1 M HNO_3 and 3% H_2O_2): water bath extraction at 90°C during 1h & microwave-assisted extraction at 90 °C during 20 min. The microwave-assisted extraction seems to extract different arsenic species (methylated arsenic species (MMA; DMA) and arsenosugars) without altering them. The method detected common arsenosugars from extracts of reference materials (CRM NMIJ 7405b (hijiki seaweed) ; NIST SRM 3232 (Kelp Powder)), most with a good resolution peak providing semi-quantitative information on the AsSug levels. The waterbath extraction procedure seems to disrupt the integrity of AsSug but not the methylated As species and Asi. Both extraction methods resulted in similar Asi concentrations.
The proposed methods were validated intralaboratory for Asi determination. Some challenging algae products (*Fucus vesiculosum* and *Ascophyllum nodosum*) were selected based on their As speciation profile and results were compared in another laboratory to confirm the robustness of the method.

1. Sá Monteiro M, Sloth J, Holdt S, Hansen M. Analysis and risk assessment of seaweed. EFSA Journal. 2019;17:e170915.
2.EFSA. Panel on contaminants in the food chain (CONTAM). Scientific Opinion on Arsenic in Food. EFSA Journal. 2009;7:1351–5.

Keywords: *IC-ICPMS, algae, inorganic arsenic, arsenic speciation, arsenosugars*

Acknowledgement: *This work was partly funded by NEN, appointed by CEN and the European Commission (EC) to perform work in accordance with their Specific Agreement regarding standardization of algae and algae products. (EN/2019/ENER/C2/452-2019/SI2.832375).*

J2

PREDICTION OF METAL CONTENT (AL, AS, CU, HG, PB) IN TEA USING NEAR INFRARED SPECTROSCOPY

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One of the most consumed non-alcoholic beverages in the world is tea reaching a value of 44.3 billion dollars (Liang et al., 2021). Tea has a wide range of beneficial properties such as inhibiting the development of cancer, lowering the risk of cardiovascular diseases or reducing serum cholesterol. These beneficial properties are mainly related to polyphenols, but there are other compounds which are beneficial to human health, such as essential minerals (Cabrera et al., 2004). However, some metals can have both beneficial and adverse effects on human health and, therefore, their quantification is very important. The determination of the metal composition of tea is usually performed by ICP-mass analysis and also by Electrothermal Atomic Absorption Spectrometry (ET AAS) and Graphite Furnace Atomic Adsorption Spectrometry (GF AAS), all of which are complex and time-consuming methods. This work proposes a rapid method to determine the composition of some heavy metals in tea by using near infrared spectroscopy (NIR), as this analytical technique is fast, non-destructive and requires little or no sample preparation.
A total of 553 samples of black, green and red tea and their blends were analysed using the Foss NIRSystem 500 (Hillerod, Denmark) with transport device. Spectra were recorded from 1000-2498 nm every 2 nm and 32 spectra were performed at each recording. The heavy metals measured by ICP-mass analysis after nitric digestion were aluminium, arsenic, mercury, copper and lead. The modified partial least squares (MPLS) regression method was used to develop the NIR calibration model. The results showed that the capacity for prediction can be considered excellent with RSQ for both calibration (performed with 80% of the samples) and internal validation with values ranging from 0.888 for arsenic to 0.979 for mercury. The external calibration was performed with 20% of the samples that were not included in the calibration and there was no significant difference ($p>0.05$) between the reference and predicted values. This indicates that the NIR method is comparable to chemical methods.
The method is interesting for rapid prediction of heavy metals in tea in the ranges 1524.7-3169.8 ppm for Al, 0-1.93 ppm for Pb, 0-0.2563 for As, 0-0.0234 ppm for Hg and 4.209-27.685 for Cu.

Keywords: *tea, NIRS, heavy metals*

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J3

QUANTIFICATION OF ESSENTIAL AND TOXIC ELEMENTS IN HONEY BY ATOMIC SPECTROMETRIES

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Argentina is the fifth producer and second world exporter of honey. In terms of the quality of honey as foodstuff, the content of elements that are considered micronutrients (Ca, Na, K, Mg, Fe, Zn, Cu and Mn) is of interest, as well as those that can present toxicological risks (Pb, Cd, As, Sb and Hg). The elemental profile of honey varies with the area in which it is produced, receiving contributions of both nutritional and toxic elements from the environment. This work presents the methodology and results of the analysis of 160 honey samples produced in four locations of the province of Santa Fe, Argentina.

For the analysis, samples were digested in a microwave oven with HNO_3 and H_2O_2 and then quantified by flame atomic absorption spectrometry (FAAS) in the case of K, and inductively coupled plasma mass spectrometry (ICP-MS) for the rest of the elements. Adequate analytical recoveries were obtained for all analytes studied. The limits of quantification (LOQs) obtained for the elements considered micronutrients were sufficient to quantify them in all of the samples analyzed. On the other hand, the LOQs obtained for As (1.5 µg/kg), Cd (1.4 µg/kg) and Pb (22 µg/kg) are approximately 2 orders of magnitude lower than the maximum concentrations allowed according to the Mercosur technical regulations and in the case of lead one order of magnitude lower than the maximum established by the European Union. Although the content of Hg and Sb in honey is not currently legislated, the LOQs obtained were similar to those of the other toxic elements (7.9 and 7.3 µg/kg for Hg and Sb, respectively). Regarding the results obtained, all the samples presented levels below the LOQ for Hg and Sb, while Pb was only quantifiable in 11 samples and Cd in 5 samples. From the group of toxic elements, only As was quantifiable in a considerable number of samples (124, 78%), although at ultra-trace levels (mean 3.1 µg/kg). All the quantifiable elements presented highly significant statistical differences (ANOVA, $p<0.001$) with the place of origin of the honey, with the exception of Ca, which presented a more modest statistical significance (ANOVA, $p<0.02$). Correlations were observed between the concentrations of different elements, such as Cu and K, Mg and Mn, among others. The proposed method gave satisfactory results and can be applied to a large number of elements and is not necessarily limited to those presented here. The use of a closed system for the mineralization prevented the loss of volatile analytes during sample treatment, while also minimizing the risk of contamination. The detection systems used gave satisfactory LOQs, and ICP MS allowed the determination of a large number of elements with a high analytical frequency. Finally, the differences observed in trace elements for honey samples produced in different locations suggest that the elemental profile could be used for the classification of honey samples based on their place of origin.

Keywords: *honey, toxic elements, micronutrients, ICP-MS, FAAS*

Acknowledgement: *This research was funded by Agencia Santafesina de Ciencia, Tecnología e Innovación (ASaCTeI) through project IO-2018-00068-18.*

J4

ARSENIC SPECIATION IN FRUIT JUICES AND RICE-BASED PRODUCTS USING LC-ICP-MS

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Food contamination by heavy metals can come from various steps of production, packaging or preservation. They can naturally be present in the environment or can occur consequently to human activities. Among these metals, arsenic, which is naturally present in the environment, can also contaminate the food chain through human activity such as industrial discharges or the use of certain pesticides in agriculture. Thus, speciation of the different chemical forms of arsenic is of great importance to isolate inorganic compounds as arsenite As(III) or arsenate As(V), which are highly toxic and have carcinogenic properties.
This study aimed to develop arsenic speciation method using the Shimadzu quadrupole ICP-MS 2030 coupled to the inert Shimadzu LC-20Ai. Separation of arsenic species, including dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA), was achieved using an ion exchange column (Hamilton PRP-X100 10 µm 250 x 4.1 mm with guard column) and ammonium nitrate and phosphate based eluents as mobile phase (6.6 mM NH_4NO_3 and 6.6 mM $(NH_4)_2HPO_4$ at pH 6.2). The quadrupole mass analyzer was operated in the single ion monitoring mode (m/z 75) for detecting arsenic species. The method was fully validated on fruit juices and rice based products based upon international criteria and was found suitable to analyze arsenic derivatives in real samples at ppb level. A survey was carried out on numerous various juices and rice-based products from the Swiss market and all samples were in compliance with the legislation.[1] [

1] Swiss Ordinance of the Food Department of Home Affairs (FDHA) on the maximum levels of contaminants, RS 817.022.15, 1st May 2017.

Keywords: *LC-ICP-MS, speciation, arsenic, fruit, rice*

J5

MATRIX EFFECTS ANALYSING SELENIUM IN FEED BY ICP-MS FOLLOWING STANDARD METHOD EN 17053:2018

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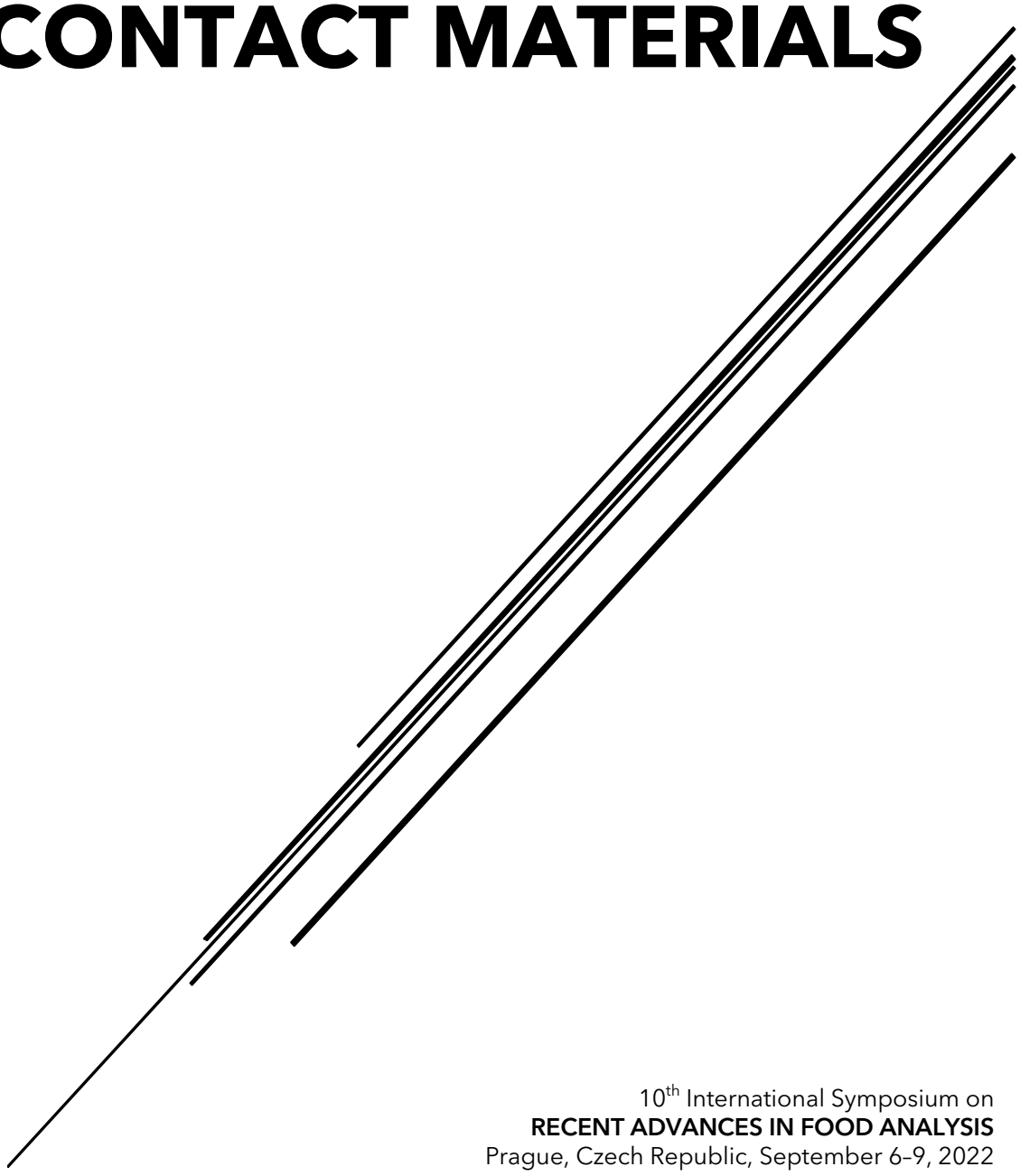
The European standard EN 17053:2018 is a currently used official method of analysis in the European Union (EU) for official control of selenium in animal feeding stuffs according to the method cascade laid down in article 34 of Regulation (EU) 2017/625. Selenium is used as feed additive. The above mentioned European standard describes a multi-method for the determination of selected heavy metals (e.g. lead and cadmium) and trace elements (e.g. iron, zinc and selenium) by ICP-MS proceeded by a microwave digestion procedure of the samples.
It has several suggestions for use of possible internal standards, which should be added prior to the ICP-MS measurement. The main suggestion as internal standard for all elements covered by the standard is rhodium. Additional suggestions for use as internal such as scandium or thulium are recommended for some elements. For example: In the case of thulium, it is recommended for elements with a high mass (e.g. uranium)
The German National Reference Laboratory for Additives for Use in Animal Nutrition has found during routine analysis that the measurement results (mass fractions) of selenium for some feed matrices are dependent on the respective dilution factor of the digested samples, although they always lie in the linear range of the calibration. Further investigations have shown that the possible cause for this are matrix effects as well as changes in instrument sensitivity during a series of measurements, which are not sufficiently compensated by rhodium used as internal standard. The compensation of matrix effects using ICP-MS as analysis technique have not yet been extensively studied. It is conceivable that the severity of matrix effects is related to the instrument configuration and design (e.g. ion optics).
The aim of the investigations is to identify a procedure that compensates for the matrix effects as simply and quickly as possible. In the course of these investigations, various parameters were tested. First results are presented and discussed here. Among other observations, the use of tellurium as an internal standard for the quantification of selenium instead of rhodium are promising. Standard addition is also an effective technique to compensate the matrix effects, but very labour-intensive in terms of routine analysis.

Keywords: *matrix effects, selenium, ICP-MS, standard method, calibration approaches*

MIGRANTS FROM FOOD CONTACT MATERIALS



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K1

ANALYSIS OF PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN PAPER AND CARDBOARD-BASED FOOD CONTACT MATERIALS

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Per- and polyfluoroalkyl substances (PFAS) are a broad family of organofluorine compounds, which includes more than 10,000 synthetic substances, containing at least one completely fluorinated methyl (–CF3) or methylene carbon atom (–CF2–) [1]. Due to their chemical amphiphilic composition, PFAS exhibit a number of interesting physicochemical properties, such as hydrophobic and oleophobic characteristics, providing resistance to moisture and oil, as well as chemical and thermal stability. For that reason, PFAS have been used for decades in industry as coatings in a broad variety of daily life applications and products, such as fire extinguishers, textiles, and paper, including paper and cardboard-based food contact materials (FCM). In this sense, PFAS can be released by the FCM into the food, joining the food chain, and causing potential risks to human health. For decades, it was thought that these substances were innocuous; however it is now known that long chain PFAS are persistent pollutants which present bioaccumulation, and are responsible of developmental and reproductive abnormalities, tumor formation, and suppressed immune response.
In this work, a novel analytical method has been developed and validated for the determination of 21 PFAS in paper and cardboard-based FCM. This method is based on a green ultrasound-assisted lixiviation followed by liquid chromatography coupled to high resolution mass spectrometry (LC-Q-Orbitrap HRMS). The method was validated in various paper and cardboard-based FCM obtaining good linearity (R^2 ≥0.99), method limits of quantification (12.5 μg kg^{-1}), accuracy (74-115 %), and precision (RSD <20 %). The eco-friendly characteristics of the proposed analytical method were assessed according to the Analytical Eco-Scale, demonstrating that it constitutes an excellent green analysis (Eco-Scale score >75). Finally, eight field samples of paper and cardboard-based FCM, including a pizza box, a popcorn box, a paper bag and a cardboard box for potato fries, an ice cream tub, and cardboard packaging for cooked Spanish omelet, fresh grapes, and frozen fish, were analyzed showing that they comply with current European regulations.

[1] Organisation for Economic Co-operation and Development (OECD), 2018. Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFAS): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances. Series on Risk Management No. 39.

Keywords: *food contact materials, high resolution mass spectrometry, liquid chromatography, paper and cardboard, PFAS*

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K2

ANALYSIS OF CONTAMINANTS IN BAMBOO- & OTHER BIO-BASED DISHES

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Bio-based food contact materials are nowadays frequently used as an ecological alternative for common plastic dishes. Unlike traditional fossil-based polymers, the biopolymers are biodegradable or compostable. Among many ´natural´ raw materials such as starch, cellulose or gluten, bamboo has become the most popular because of its short life cycle and good mechanical properties. In the production of dishes, the bamboo fiber is usually treated with various additives e.g. polyols, urea, melamine and/or formaldehyde, in some cases melamine is used as a main binder. Although bio-based dishes might be considered as safe by consumers, researchers have issued the warning on the risk of migration of some additives and other contaminants into foodstuffs. The aim of the first phase of the study was to implement non-target screening procedure enabling detection of potential migrants. To cover their entire polarity range, extracts of crushed samples were prepared by the use of three different solvents - 3% aqueous acetic acid, ethanol, isooctane. For extracts analysis, ultra-high-performance liquid chromatography coupled to high resolution tandem mass spectrometry (UHPLC-HRMS/MS) technique was employed. Chromatographic columns HSST3 C18, BEH C18 were used for separation, Q-TOF mass analyzer operated in positive and negative ionization mode for compounds detection. Target UHPLC-MS/MS analysis was used for quantification of melamine and synthentic pesticides screening.
33 samples of bio-based dishes from Czech, UK and China market were then analyzed. Melamine was detected in all but one bamboo-based product. The concentration of melamine ranged from 20.8 to 109.1 mg/kg. In case of samples from other bio-materials such as bagasse, sugar cane, coconut, starch, wheat and dough, melamine was detected in two samples only. The concentration was 43.7 mg/kg in corn starch based dinning set and 22 mg/kg in bowl from rice husks. In contrast, sample most contaminated by pesticide residues was wheat bran plate, where piperonyl butoxide, pirimiphos methyl, deltamethrin and tebuconazole were detected.

Keywords: *food contact material, bamboo, biomaterial, melamine, mass spectrometry*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727864 and from the Chinese Ministry of Science and Technology (MOST).*

K3

MIGRATION FROM RECYCLED PLASTIC MATERIALS: HS-GC-IMS AS RAPID METHOD TO ASSESS FOOD QUALITY

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Food Contact Materials (FCM) are all materials and articles intended to come into contact with food, such as packaging and containers, kitchen equipment, cutlery and dishes. Plastic represents in Europe one of the most common FCM; recycled plastics may also under certain conditions be used in FCM. The trend in Circular Economy, also considering the EU Green Deal program, will enlarge in the next future the use of recycled materials in food packaging. The safety of recycled plastics requires evaluation, as chemicals can migrate from the materials into food. All the plastic materials should be manufactured in compliance with EU regulations, including good manufacturing practices, so that any potential transfer to foods does not raise safety concerns; otherwise they could change the composition of the food in an unacceptable way or they could have adverse effects on the quality of foods (for instance, taste and/or odor). Some recycled plastic materials significantly impact the overall volatilome of foods, releasing off-notes, changing the typical profile during the shelf life. Mono- and multidimensional GC-MS were largely used in the past to evaluate the specific migration of volatiles compounds in foods. Head Space-Gas Chromatography coupled with Ion Mobility (HS-GC-IMS), underexploited technique in this area, should be considered a powerful tool useful to rapidly evaluate the release of off-notes as well as the change of the volatile profile in foods. The aim of this work was focused on the application of HS-GC-IMS to study volatile profiles of recycled plastics produced with different technologies, also investigating the release of off-notes in simulants, as well as in foods. 2D profiles allowed us obtaining some rapid molecular fingerprints, confirming in some cases the release of odorant and off-notes, confirming the usefulness of this rapid analytical approach.

Acknowledgement: *Work partially funded by Regione Piemonte and European Regional Development Funds within the Bioeconomy Platform "NUTRAcore" 333-151 (POR-FESR 2014-2020).*

K4

SAFETY OF FOOD CONTACT MATERIAL: WHAT ABOUT SUSTAINABILITY AND CIRCULAR ECONOMY?

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Sustainability is more than a public and political debate, as a result of the strategies promoted by the European Union: Circular Economy, Plastic Strategy and Single Use Plastic Directive. Packaging is strongly involved in these issues, but while awaiting the evolution of legislation and technologies to support sustainability, manufacturers are wondering about future perspectives about compostable, bio-based, reusable, recyclable materials. All these alternatives have a direct contact with circular economy principles: reducing waste and pollution, maintaining the value of materials and resources, regenerate natural systems. But besides all, food packaging has a strategic role: protection, information, promotion, preservation, but above all food packaging is an essential part of the food product: as food and packaging are purchased together by consumers. Therefore, the whole product is expected to be sustainable and safe.
Thus, new bio-based or compostable materials, recycling process, and repeated use items must have the challenge of safety: producers should be able to prove the sustainability of their packaging and at the same time to know which contaminants are necessary to monitor. To do that, risk assessment approaches have to take into consideration the challenges derived from the use of new materials. In this context, the investigation on predictable and unpredictable NIAS (Non intentionally added substances) is a fundamental tool to guarantee the safety. We need to investigate the story that we don't know about the given material, for example chemicals present in post consumer waste used as raw material, NIAS from recycling process, variability of material batches, unknown contaminants due to the use of natural biomass, etc. It should also be noted that, NIAS could derive from each part of the production chain. For that reason, a good knowledge of the materials and processes, together with an open approach which considers both target markers and untargeted screening will help to have a wider view avoiding possible risks. This poster will show examples and results of analytical applications developed by Mérieux NutriSciences which transform the above concepts into real life.

Keywords: *food contact material, NIAS, sustainability, circular economy, SAFETY*

K5

AUTOMATED SAMPLE PREPARATION FOR MOSH/MOAH ANALYSIS IN ACCORDANCE TO DGF C-VI 22 (20)

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For more than 10 years, the topic of potential migration of hydrocarbons, that originate from mineral oil into food, from packaging material of any kind, is widely discussed and still gets a lot of attention. The main groups, mineral oil consists of, are the groups of MOSH (Mineral Oil Saturated Hydrocarbons) and MOAH (Mineral Oil Aromatic Hydrocarbons). MOSH fractions are known to accumulate in human tissue, while MOAH even have a possible carcinogenic potential. MOSH/MOAH can be found in food (e.g. fats, chocolate, bakery products), cosmetics, and many more examples.
Food samples (fats/oils) can be processed according to DIN EN 16995:2017 and the JRC Guidance Document of 2019. The DIN EN 16995:2017 allows a LOQ > 10 mg/kg for MOSH/MOAH, while the health effects and accumulation in human tissue are also happening below this concentration. Since the actual method allows several optional steps in sample clean-up, which lead to different decision trees in laboratories and variations in round robin tests, a new method for MOSH/MOAH contents below 10 mg/kg (DGF C-VI 22 (20)) was published in December 2020 by the DGF (Deutsche Gesellschaft für Fettwissenschaft e.V.). This new method allows MOSH/MOAH analysis with online LC/GC (FID) for determination of 10x lower LOQs (1 mg/kg) in fatty matrices than before.
According to DGF C-VI 22 (20) a new mandatory step for clean-up is required, that involves silica gel in glass columns with subsequent evaporation, to eliminate disruptors of unsaponified samples prior to the established step of epoxidation in the still valid DIN EN 16995:2017. As a result of the new method, the chromatographic backgrounds are much better due to less m-CPBA usage. This mandatory clean-up step is time intensive and requires involvement of laboratory personnel, if carried out manually. The FREESTYLE SPE system from LCTech in combination with the EVAporation module for online evaporation can easily automate this labor- intensive clean-up step, even while running over night or over the weekend. This poster will present first results of an automated clean-up method for MOSH/MOAH according to DGF C-VI 22 (20) with very good reproducibility in fatty matrices.

Keywords: *MOSH, MOAH, sample preparation, MOSH/MOAH, DGF C-VI 22 (20)*

K6

STREAMLINED PFAS ANNOTATION AND VISUALIZATION WITH FLUOROMATCH FLOW AND VISUALIZER

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Greaseproof packaging often contains per and polyfluoroalkyl substances, often abbreviated as PFAS. They are comprised of a head group and a fluoroalkyl chain that can vary in length, creating homologous series. In fact, there are more than 7,600 PFAS compounds found in commerce. Despite the pervasiveness of PFAS compounds, there are a little over a hundred commercially available standards, and those tend to be expensive.
Tentatively identifying PFAS in food contact material (FCM) requires a screening instrument and annotation software. Typically, screening for these compounds in food contact materials (FCM) can be done by liquid chromatography based high-resolution tandem mass spectrometry. To date, no automated open source PFAS data analysis software exists to mine fluorine-containing organic compounds. We introduced FluoroMatch, which automates file conversion, chromatographic peak picking, blank feature filtering, PFAS annotation based on retention time, precursor masses and fragment masses, annotation ranking, and confidence assignment. The annotation software is built on a library that contains ~7,000 PFAS fragmentation patterns based on rules derived from standards and literature, and the software automates a process to add additional compounds.
To aid interpretation by making homologous series more identifiable, we have added a Visualizer tool to the FluoroMatch suite of software utilizing Microsoft PowerBI. It provides interactive mass defect plots, accurate mass vs. retention time plots, MS/MS fragmentation plots, annotation tables, and fragment screening. Selecting a feature in one graph will adjust what is displayed in other views. This interactive cross-filtering allows simplified evaluation of a feature, PFAS series, or other groups of features.
This is the first application of FluoroMatch automated PFAS annotation using in-silico PFAS fragmentation to food packaging.

Keywords: *PFAS, FCM, screening, contaminants, residues*

Acknowledgement: *The development of the FluoroMatch suite of software tools is in part funded by an Agilent ACT-UR grant. Also, in 2021, Stéphane Bayen has been recognized as an Agilent Thought Leader for his contribution to the understanding of both human and environmental food safety risk assessment. This work is a continuation of that quest. Jeremy Koelmel would like to specifically call out the Bowden group at the University of Florida and the Pollitt group at Yale. Jeremy also thanks SynQuest Labs for providing him PFAS standards. We would finally like to thank the unattributed Agilent, McGill, and Yale team members who made this work possible.*

K7

MIGRATION OF BISPHENOL S FROM FOOD THERMAL LABELS TO PACKAGED FOOD

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Bisphenol A (BPA) and structural analogues are commonly used in large quantities as a color developer in thermal papers. Among BPA structural analogues and functional alternatives, compounds such as bisphenol S (BPS), 4-hydroxyphenyl 4-isoprooxyphenylsulfone (D-8, also called BPSIP), bis(2-chloroethyl)ether-4,400-dihydroxydiphenyl sulfone copolymer (D-90), and bis-(3-allyl-4-hydroxyphenyl) sulfone (TGSA) have been frequently reported in thermal receipts for example. Several studies have flagged potential human health risks associated with chronic exposure to BPA and analogues. To prevent exposure from receipts, the European Union enforced in 2020 a limit for BPA concentration in thermal paper.
Although some studies have evaluated the occupational effects of those bisphenol analogues in thermal paper through skin exposure, no study has assessed food labels as a possible source of dietary exposure to those compounds in color developers. In its recent updated assessment of BPS, the European Food Safety Agency (EFSA) recommended the collection of data on the use of BPS in plastic food contact materials (FCMs) and on its occurrence and migration into food. A few studies have reported the occurrence of BPS in food. In a recent study in Canada (*Tian et al., Food Chemistry. 2020. 326:126942*), we reported a relatively higher frequency of detection for BPS in packaged fresh food (notably fish) compared to their non-packaged equivalent.
In this study, we hypothesized chemical migration from thermal label stickers on common packaging (cling wrapper films) could be a dietary source of BPS and other color developers. To test this hypothesis, multiple packaging samples (n=140) used for fresh food were collected from Montreal to assess the occurrence of bisphenols. BPA was not detected in any of the packaging samples; however, BPS, D-8, D-90, TGSA and Pergafast-201 were present in food thermal labels (n=40). Tests were then conducted on 24 packaged fish samples to assess the migration of bisphenols from labels into food. Relatively high migration of BPS (up to 1120 ng/g wet weight), D-8 (up to 226 ng/g ww) and PF-201 (up to 137 ng/g ww) were measured in fish wrapped in film with a thermal label (4˚C) for 5 days. The migration of D-90 into fish was minimal but detectable (up to 19.6 ng/g ww). This study shows, for the first time, that migration from thermal label stickers is a major source of bisphenols and other color developers in our diet; further detailed risk assessment is required.

Keywords: *bisphenol S, food contact materials, contaminants, endocrine disrupting chemicals, migration*

Acknowledgement: *We wish to acknowledge the financial support received from the Canadian Institutes of Health Research (CIHR) (IP3-150711: Endocrine disrupting chemicals: towards responsible replacements; Principal Investigator: Dr. B. Hales), the Canada Foundation for Innovation/John R. Evans Leaders Fund grant (Project #35318) of S. Bayen.*

K8

DEVELOPMENT OF QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIP MODELS ON MULTIPLE CHROMATOGRAPHIC COLUMNS TO IMPROVE THE IDENTIFICATION OF LEACHABLES IN FOOD PACKAGING USING NON-TARGETED ANALYSIS

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The chemical safety of food contact materials (FCMs) is critical considering their extensive usage in the food supply chain and the diversity of chemical residues which may migrate from the materials into food. On top of intentionally added substances, a range of non-intentionally added compounds may migrate from FCMs and have emerged as a concern for food industry due to a lack of detailed guidance for their assessment. Novel tools are needed to identify unknown or unexpected contaminants not captured by the conventional targeted methods. Quantitative structure-retention relationship (QSRR) models can be used to predict the chromatographic retention time of chemicals and facilitate the identification of unknown compounds, notably with non-targeted analysis.
In this study, QSRR models were developed from the data obtained for 178 pure chemical standards and four types of analytical columns (C18, phenylhexyl, pentafluorophenyl, cyano) in liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS). Different models were built to predict chromatographic retention times based on 2D molecular descriptors, and random forest models resulted in better predictive capacities. For each column, the resulting model was applied to identify leachables from actual plastic packaging samples. An in-depth investigation of the top 20 most intense molecular features revealed that all false-positives could be identified as outliers in the QSRR models (outside of the 95% prediction bands). Furthermore, analyzing a sample on multiple chromatographic columns and applying the associated QSRR models increased the capacity to filter false positives. Such an approach will contribute to a more effective identification of unknown or unexpected leachables in plastics, therefore refining our understanding of the behavor and the chemical risks associated with food contact materials.

Keywords: *mass spectrometry, non-targeted analysis, food packaging, plastics, non-intentionally added substances*

Acknowledgement: *We wish to acknowledge the financial support received from the Canadian Institutes of Health Research (CIHR) (IP3-150711: Endocrine disrupting chemicals: towards responsible replacements; Principal Investigator: Dr. B. Hales), the Canada Foundation for Innovation/John R. Evans Leaders Fund grant (Project #35318) of S. Bayen. Z. Xu was funded in part by a scholarship under the Natural Sciences and Engineering Research Council of Canada (NSERC) CREATE Project grant (Advanced Technological Training network on the risk and remediation of Pollution in URban Environments. PI: K.J. Wilkinson).*

K9

DETERMINATION OF MINERAL OIL HYDROCARBONS BY GCXGC-FID

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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]. More recently (April 2022) the Standing Committee on Plants, Animals, Food and Feed (SCOPAFF) report issued in a draft statement strict limits for the MOAH as a sum, with a demand for withdrawal from the market in case of violation, for various food products: 0.5 mg/kg for dry foods with a low fat/oil content (≤4% fat/oil), 1 mg/kg for foods with a higher fat/oil content (>4% fat/oil) and 2 mg/kg for fats/oils. However this limits will have to be approved by an upcoming EFSA statement to be mandatory [2].
Contamination with petroleum hydrocarbons is detectable in many foods [3]. There are a variety of possible sources for an input of mineral oil hydrocarbons. For the food industry, in the event of a positive finding, the identification of the source of contamination and the initiation of appropriate measures are the first priority. Between the cocoa bean and the chocolate bar there are long transport routes and many processing stages and thus a variety of entry routes. For example, the use of jute bags treated with "batching oil" (crude petroleum fraction) for transport and storage purposes of cocoa beans is a known source of contamination. In addition to cocoa beans, masses and butters, other ingredients of chocolate such as sugar, milk powder, lecithin and flavorings are possible sources of mineral oil components.Depending on the raw material, different matrix interferences appear [4], which cannot be eliminated despite a wide variety of modern purification techniques such as epoxidation, and thus make the quantification of the mineral oil hydrocarbons complicated. By means of two-dimensional GCxGC-TOF(MS), the findings can be qualitatively validated, but quantification is still important for the evaluation. Here we present a further development of the analytics, with which a quantification of MOAH is possible despite interference. Possible interferences can be separated by GCxGC-FID and additionally the MOAH content can be quantified.

[1] EFSA Rapid risk assessment on the possible risk for public health due to the contamination of infant formula and follow-on formula by mineral oil aromatic hydrocarbons (MOAH), 15th. November 2019
[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, MOSH/MOAH, GCxGC-FID*

K10

BISPHENOL A IN DRINKING WATER – OCCURRENCE IN DOMESTIC DISTRIBUTION SYSTEMS AFTER COATING OF PIPES WITH EPOXIDE RESINS

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Corresponding to the recommendation of the Regional Office for Europe of the World Health Organization (WHO) from 2017, three representative endocrine-disrupting compounds, Bisphenol A (BPA), Nonylphenol and Beta-estradiol, may be considered as benchmarks in drinking water. For BPA, a value of 0.1 µg/L was established for assessing the occurrence and the treatment efficacy. According to directive (EU) 2020/2184 on the quality of drinking water [1], and based on the 2015 opinion of EFSA [2], a health based parametric value of 2.5 µg/L has been set for BPA. By 12 January 2026, EU member states shall ensure that drinking water complies with this value [1].
In our work, we show results of recent analyses of drinking water from domestic distribution systems, present latest insight into contamination with BPA during the very last meters of the pipes and discuss the reasons for it. The analyses were carried out with regards to official control of drinking water in Baden-Württemberg, Germany. Our results indicate that in many cases in which pipes have been reconstructed using coatings of BPA-based epoxide resins, also BPA can be found in the corresponding drinking water. BPA levels were found to be higher the older the coating was and the higher the temperature of the water that flew through, or if the coating was technically inadequate.
The highest BPA levels with more than 200 µg/L were found in hot water samples from an epoxy coated pipework that had additionally been disinfected thermally, due to the occurrence of *Legionella*. Such drinking water must be regarded as unsafe for human consumption and does not comply with requirements of article 4 of the directive (EU) 2020/2184. With respect to the ongoing re-evaluation of BPA by EFSA [3], an expected further reduction of the tolerable daily intake (TDI) from 4 µg/kg body weight (2015 assessment) to only 0.04 ng/kg body weight will mean even more conspicuous samples and must also lead to tighten measures. Generally, coating procedures of old pipes using BPA-based epoxide resins are far from being safe and homeowners should be aware of doubtful methods, dubious procedures for reconstruction works, and providers that claim obsolete techniques as still being valid.

[1] Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption.
[2] EFSA CEF Panel (2015). Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. EFSA Journal 2015;13(1):3978.
[3] EFSA (2021). Bisphenol A: EFSA draft opinion proposes lowering the tolerable daily intake. Available via: https://www.efsa.europa.eu/en/news/bisphenol-efsa-draft-opinion-proposes-lowering-tolerable-daily-intake, accessed 15 June 2022.

Keywords: *bisphenol A (BPA), drinking water, epoxide resins*

K11

NON-INTENTIONALLY ADDED SUBSTANCE (NIAS) SCREENING FROM POLYMERIC FOOD CONTACT MATERIALS BY THERMAL DESORPTION GC-MS AND SEMI-QUANTIFICATION BY PTV-GC-MS

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Polymeric food packaging is essential since protected packaged food facilitates their transport and increases their shelf life. However, packaging introduces a safety concern as beside the known additives used as well Non-Intentionally Added Substances (NIAS) might be present in food contact materials (FCMs) and could therefore migrate into the packed food. NIAS substances are not added for a technical reason during the production process but are degradation/reaction products related to the FCM manufacturing. Often their presence is not known by the consumer and not even by the producer.
Polymeric food contact articles are regulated within Europe under the Commission Regulation (EU) 10/2011 on plastic materials and articles intended to come into contact with food. This regulation describes in its table 1 a whole list of constituents which are allowed to be used if fulfilling, beside the overall migration, the specific migration limit (SML) in pre-set food simulants (e.g. 10, 20, 50 and 95 (v/v) % ethanol, 3 % acetic acid, isooctane, vegetable oil or poly(2,6-diphenyl-p-phenylene oxide) for dry foodstuffs) having a pre-defined migration ratio corresponding to 6 dm^2/kg food (simulant). NIAS substances should be traceable at a level of 0,01 mg/kg food simulant. A 2-step approach has been developed for the evaluation of FCMs on NIAS substances.
Step 1: Thermal desorption GC-MS analysis; enabling to screen unknown constituents in polymeric material up to 1000 Da (higher threshold for potential absorption) covering both the polar and non-polar migration media. By this knowledge in advance, the traceability of certain substances in the migration solutions is facilitated during the GC-MS evaluation. The thermal desorption GC-MS method covers as well the volatility of the substances (volatile, semi-volatile range and selected non-volatile); the nature of packed food or food simulant (aqueous or fatty: covering all as a worst case); the level of determination (e.g. trace or major substances); the functionality/chemical character of certain substances e.g. isomers, homologous series.
Step 2: PTV-GC-MS analysis of migration solutions; for this evaluation isooctane and 95 (v/v) % ethanol migration solutions are measured by the semi-quantitative analysis approach. An external standard has been selected for semi-quantification. In order not to additionally contaminate the migration solutions a matrix/fitted external calibration has been used. As the method is a screening method electron impact ionization has been applied with a relevant mass range up till 700 M/e.
The outcome is a full evaluation of potential migration substances, the semi-quantitative evaluation of each single substance found in the migration solution. This data is then used for the final safety assessment of the FCM.

Keywords: *NIAS, food contact materials, mass spectrometry, contaminants, screening*

K12

OCCURENCE OF PHOTOINITIATOR TYPE CONTAMINANTS IN OAT FLAKES FROM CZECH MARKET

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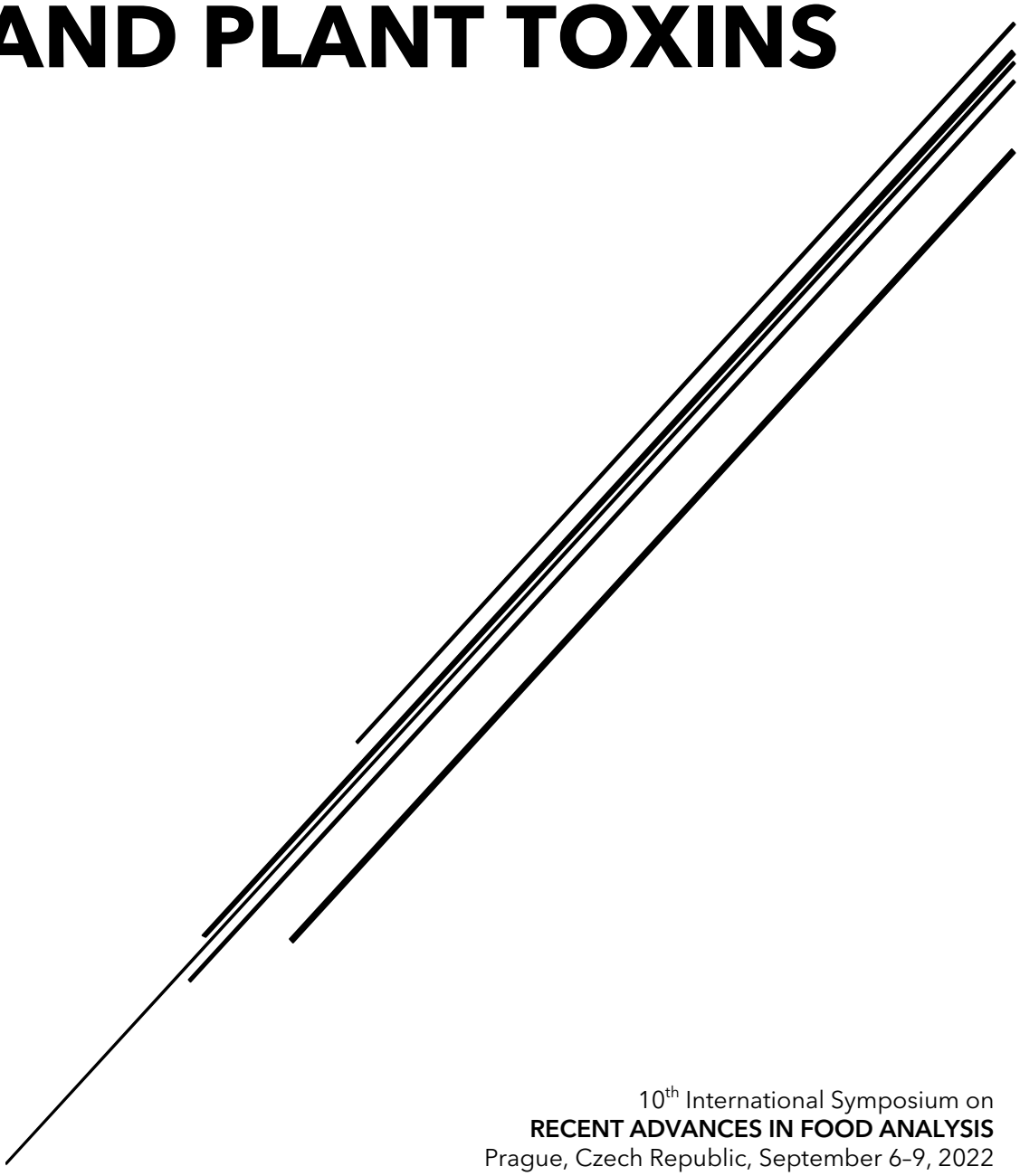
Photoinitiators are highly photoactive compounds, that are able to absorb UV radiation and start polymerization reaction and are commonly used during UV curing of printing inks. Benzophenone or 2-isopropyl thioxanthone are examples of these substances. Modern photoinitiators are often used during printed food packaging production. However, these substances can migrate into the food inside the packaging. Toxicity of these substances was not yet fully evaluated and they possibly degrade into many compounds for which significant toxicity cannot be ruled out. It is therefore important to monitor the presence of these substances in food products.
In this work the presence of 10 analytes in oatflakes and its packaging was evaluated. These included photoinitiators, co-initiators, their degradation products and a plasticizer diethylhexyl maleate. Photoinitiators in the packaging were determined after their migration from packaging into food simulants. For extraction a modified QuEChERS method was used followed by analysis by ultra-high-performance liquid chromatography with tandem mass spectrometric detection. Recovery of the method was 77-119 % and repeatability lower than 12 %. For majority of analytes the limit of quantification of 2 ppb was reached. Matrix effects were also evaluated in oatmeals and for most analytes were insignificant.
Oatflakes samples from Czech market were analyzed for the presence of analytes. Most frequently detected analytes were benzophenone and 4-methylbenzophenone. Concentrations of benzophenone ranged from 0.4 to 17.4 ppb, 4-methylbenzophenone was detected below limit of quantification. Frequent detection of benzophenone allowed for statistical evaluation of the influence the type of packaging has on the concentration of benzophenone in oatflakes. The detected analytes did not exceed legislatory limits except one case when diethylhexyl maleate was detected at 39 ppb level. In parallel, migration of analytes from the packaging materials was measured for all the analytes. Benzophenone was also detected most frequently in food packaging samples and its concentration ranged from 156 to 345 ng/dm^2 of packaging. Based on these results, the influence of packaging material on the amount of benzophenone in oatflakes could be evaluated.

Keywords: *photoinitiators, food packaging, benzophenone, liquid chromatography, mass spectrometry*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

MYCOTOXINS, MARINE AND PLANT TOXINS



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M1

ADVANTAGES OF SELECTIVE ANALYTE CLEANUP FOR MYCOTOXIN TESTING OF CANNABIS AND HEMP PRODUCTS

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Cannabis and cannabis products consists beside oft he THC and cannabiol by products of unwanted secondary fungal metabolites which are produced during drying of the cannabis plants or seeds and storage thereof. To fulfill the low regulatory limits special sample cleanup procedures need tobe undertaken or matrix match or internal standard usage is required to overcome the massive impact of matrix burden influences in the chromatography and downstream impacts in the analytical devices e.g. mass spectrometry. To overcome this we processed hemp seed and flower by solvent extraction and after sample dilution a analyte specific and selective binding cartridge was used. Easy to handle and fast but efficent in sample cleanup. The AflacLEAN SMART and OtaCLEAN SMART catridges allow purification of aflatoxins B/G or ochratoxin A or if combinde both toxin groups could be copurified very easy in less than 10 minutes and fulfilling the regualtory limits. Analysis coudl be peroformed by LC-MS/MS or HPLC-FLD with chromatography time less than 10 minutes , allowing high sample throughput and fast analysis. The possible full automation oft he sample cleanup procedure allows a sequential analysis of more than 70 samples per day, 24/7 with highest reproducibility and maximum sensitivity, this technology is called FREESTYLE ThermELUTE and allows by thermal elution and chromatographical focussing in combination with a selective antibody a purification oft he analytes to such high degree, that even limits of babyfood could be achieved witout further concentration of analytes and the risk of loss of analytes prior to chromatographical analysis. The use of identical protocols for extraction , sample dilution and just by combining the the sample to the right antibody cartridge a flexible aflatoxin or ochratoxin A analysis within the same cleanup device without long maintenance and downtime. The risk of crosscontamination could be erased by the assignment of sample tot he individual column, which is exchanged for each individual sample, allowing always a high throughput without changes in performance or quality oft he analysis. The purification effect and recoveries are excellent and according tot he regulated and expected limits of detection and recovery.

M2

AGARITINE AND ITS DEGRADATION PRODUCTS IN FRESH AND TREATED MUSHROOMS

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Agaritine, β-N-[γ-L-glutamyl]-4-hydroxymethylphenylhydrazine, is a natural toxin found in mushrooms, especially in champignons. Reported values of agaritine in *Agaricus spp*. are 80–1730 mg/kg fresh mushroom; however, the level may vary depending on the species, growth stage and analyzed parts of the fungus. Precursors and metabolites of agaritine may be more toxic than the parent compound alone, some are considered potential carcinogens (as well as agaritine). Due to the potential carcinogenicity, presence of these compounds in *Agaricus spp*. is monitored. It is known that agaritine is unstable and processing may reduce its content. Therefore, the main aim of this study was monitoring of agaritine and its toxic degradation products content in fresh and processed champignons.
U-HPLC-HRMS method for the determination of agaritine, its precursors and degradation products in fresh and frozen samples, UV-treated samples and culinary-treated samples was developed and validated. Separation was achieved using reverse phase column ACQUITY UPLC HSS T3, detection was performed employing a time-of-flight mass spectrometer. Also, the stability of the agaritine was monitored during storage of the samples and sample extract under different conditions. From the results, it is clear that both processing and storage affect agaritine content in champignons. Several precursors and degradation products were found in frozen, UV-treated and culinary-treated samples. Agaritine was detected in all fresh, UV-treated and culinary-treated samples; frozen samples, on the other hand, contained significantly lower amounts of agaritine. The effect of UV radiation on the agaritine content in champignons has not been clearly demonstrated and the most effective culinary treatment for reducing the agaritine content in champignons was oven baking.

Keywords: *agaritine, mushroom, LC-MS*

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M3

DEVELOPMENT OF A CONFIRMATORY METHOD FOR HYDROXYANTHRACENES QUANTIFICATION IN ALOE VERA JUICE BY UHPLC-MS/MS

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Hydroxyanthracene derivatives (HADs) are secondary metabolites present in plants such as the Aloe genus comprising approximately 250 species of plants. Aloe vera plants are used for different purposes: for instances, as ornamental plants, in cosmetics (shampoo and gel for skin care), extracts as food supplements and also in drinks and juices. Juices of Aloe vera are made up from the sap of the pressed, whole and unpeeled leaves. The claimed interests of these food supplements, that can be bought in supermarkets, bioshop and online shops, are their potential beneficial effects on the immune, digestive and gastrointestinal systems. However, the abusive use of this category of food supplements can cause harmful effects on human health such as electrolyte imbalance, impaired function of the intestine and dependence on laxatives. In addition, HADs may have genotoxic and carcinogenic effects according to the scientific opinion of the EFSA Panel in 2018. Seeing this context and following the concern of EFSA in 2018, a new regulation has been published enforcing the control of hydroxyanthracene derivatives (EU 2021/468). It is therefore crucial to develop a method able to detect and quantify these compounds. Importantly, the standing committee of EFSA on plants, animals, food and feed from the 5th of October 2020 stated that 'products ready for use after preparation in accordance with the manufacturer's instructions containing an analysed level higher than or equal to 1 ppm aloe-emodin and/or 1 ppm emodin and/or 1 ppm aloin A+ aloin B provide clear evidence of presence of these substances in the products and are therefore of concern for public health'… 'The Commission stressed that the level of 1 ppm for aloe-emodin/emodin and the level of 1 ppm for the sum of aloin A and aloin B are for the time being the lowest levels that can be reliably quantified in laboratories across the EU and can therefore be put forward as limits of quantification in an EU harmonized risk management approach.' Therefore, a Liquid Chromatographic-tandem Mass Spectrometric (LC-MS/MS) method was develop for routine purpose in order to maintain the risk as low as possible. The present method aimed at validating a sensitive and reliable UHPLC-MS/MS method accurately and sensitively detecting and quantifying emodin, aloe-emodin, aloin A and aloin B and danthron. The mass spectrometric parameters have been optimized for each compounds, as well as the liquid chromatographic conditions. Different extraction and purification processes have been assessed and refined. To finalize the method development, validation of the method has been carried out and several parameters have been verified such as limit of detection and quantification, linearity, specificity, overall recovery, precision (within and between days) and measurement uncertainty.

Keywords: *hydroxyanthracenes, aloe vera, UHPLC-MS/MS, plant toxins*

M4

LECTIN ACTIVITY IN COMMONLY CONSUMED PLANT-BASED FOODS - CALLING FOR METHOD HARMONIZATION AND RISK ASSESSMENT.

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Lectins are ubiquitous proteins characterized through their ability to bind different types of carbohydrates. It is well known that active lectins from insufficiently prepared legumes can cause adverse human health effects. The objective of this research was to determine the activity of lectins in samples across plant families representing commercially available edible plants, and the feasibility of inactivating lectins through soaking and boiling. Lectins were extracted from 46 samples withing the plant families of Adoxaceae, Amaranthaceae, Cannabaceae, Fabaceae, Gramineae, Lamiaceae, Linaceae, Pedaliaceae, and Solanaceae. A hemagglutination assay based on non-treated or trypsin treated rabbit erythrocytes was used to measure the lectin activity. The results showed the highest lectin activity in species from the Fabaceae family and demonstrated that soaking and boiling have an effect on the levels of active lectins. The presented research combines lectin activity obtained from two different assays with raw and processed edible plants. In addition, we examined the current risk assessment, and regulations necessary for an adequate official reporting of results. We therefore encourage the scientific community to further explore this field and agree on harmonized methods for analysis and interpretation, and hope that our methodology can initiate this development.

Keywords: *active lectins, disease, hemagglutination, plant-based food, risk assessment*

Acknowledgement: *A. Adamcová acknowledges the financial support of Charles University project No. SVV 260 548. Laboratory technicians at the Plant and Soil Science Section, Department of Plant and Environmental Sciences, University of Copenhagen are acknowledged for assistance with the nitrogen analysis. The Danish Veterinary and Food Administration is acknowledged for financing the experiments with lectins.*

M5

AUTOMATING ANALYSIS OF OTA IN ANIMAL FEED SAMPLES WHILST IMPROVING QUALITY AND REDUCING ANALYTICAL TIME

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Ochratoxin A (OTA) is known to be naturally present in a variety of animal feed samples at considerably high levels. With the current EU legislative limits for OTA in animal feed as high a 100 ppb it is important that animal feed is regularly screened for natural contamination. R-Biopharm have developed an automated animal feed clean-up method. This uses immunoaffinity cartridges, IMMUNOPREP® ONLINE OCHRATOXIN, in conjunction with an automated handling system, CHRONECT Symbiosis RIDA®CREST, to allow for complete online automated mycotoxin analysis enabling high-throughput and accurate determination of OTA in animal feed.
To assess the developed method, control animal feed samples (pig feed pellets, dog food and corn feed) were spiked at maximum legislative levels according to current EU legislation for OTA (100 ppb). LOQ and LOD were also assessed at 10 ppb (1/10th legislative level) and 3.33 ppb (1/3rd LOQ) respectively. Samples were extracted according to the developed animal feed method prior to automated analysis with CHRONECT Symbiosis RIDA®CREST system. For all samples assessed, % recovery and % RSD were reported.
Recovery for all animal feed samples met with European method performance criteria (EC 401/2006) with acceptable recovery >90 % for all samples assessed. An LOQ at 1/10th of the EU legislative level and an LOD at 1/3rd of the LOQ was successfully obtained. Chromatography was also acceptable with well resolved peaks in each case.

Keywords: *automation, immunoaffinity cartridge, online, handling systems, RIDA CREST*

M6

ANALYSIS OF AFLATOXIN AND OCHRATOXIN IN VEGAN FOOD PRODUCTS

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Pseudo cereals are seeds of non-grasses and are a staple in vegan diets. Examples of pseudo cereals include quinoa, millet, bulgur wheat and spelt grain. They are gluten-free and can therefore serve as a substitute for true cereals that cannot be consumed by people with gluten intolerances. Pseudo cereals have a high nutritional value, acting as a good source of protein, vitamins, and starch.Dairy-free alternative milks are also popular item for vegans and lactose-intolerant individuals alike. Examples of popular dairy-free alternative milks include hazelnut, almond, soya, pea, coconut, and oat milk.Both pseudo cereals and dairy-free alternative milks are susceptible to contamination with aflatoxin and ochratoxin and so methods are required for these commodities to ensure EU regulations pertaining to maximum mycotoxin concentrations are adhered to.
This poster looks at methods using immunoaffinity clean-up with either single or multi-toxin columns; EASI-EXTRACT® AFLATOXIN, OCHRAPREP® and AFLAOCHRA PREP®, to analyse the concentration of aflatoxin and ochratoxin in pseudo cereals and dairy-free alternative milks.Results demonstrate that the developed methods meet the relevant acceptance criteria for each product with all %RSDs being below 20%. These methods are therefore suitable for the analysis of the relevant food products.

Keywords: *dairy free, pseudo cereals, alternative milks, gluten, intolerances*

M7

ACETONITRILE EXTRACTION FOR THE ANALYSIS OF MULTI-TOXINS IN ANIMAL FEEDS

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EU regulations for mycotoxins are complex with varying limits applied to specific commodities. This has resulted in an increasing trend in multi-mycotoxin analysis within the food and feed industry. As a result, there has been greater demand for multi-toxin immunoaffinity columns to effectively remove sample matrix from complex commodities such as animal feeds to ensure compliance with EU method performance criteria.
This study summaries the validation of a new acetonitrile extraction prior to clean-up using a multi-toxin immunoaffinity column; 11^{+}Myco MS-PREP® for animal feeds such as silage and forage. A multi-toxin dried distillers grains (DDGS) reference material (Trilogy Analytical Lab, USA) was also included to assess the accuracy and reliability of this clean-up method. Samples were extracted and passed through n=3 replicate IAC and calculated contamination, % recovery and % RSD were reported. For the reference material, the calculated contamination was corrected for recovery prior to reporting. Solvent-based calibration standards were used for quantification throughout and were compared against matrix-matched calibration standards to assess matrix effects.
Excellent recoveries were obtained for all spiked animal feed samples and ranged from 75 to 103 % with % RSD generally <10 % demonstrating an accurate and reliable method that complies with EU method performance criteria (EC 401/2006). Matrix effects were <10% for all analytes, demonstrating excellent clean-up with 11^{+}Myco MS-PREP®.

Keywords: *multi-toxin, complex commodities, animal feed, EU method, food*

M8

PYRROLIZIDINE AND TROPANE ALKALOIDS IN HONEY – RESULTS OF THE PRELIMINARY STUDY

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Pyrrolizidine alkaloids (PAs) and tropane alkaloids (TAs) are toxins of natural origin produced by various plant species. Both PAs and TAs containing plants can be found almost all over the world. Often, they are perceived as invasive and noxious weeds, which can infest cultivated fields, meadows, pastures or open ranges replacing nutritious plants. As the presence of scopolamine and atropine has been confirmed in the floral nectar of *Datura* species and also pyrrolizidine alkaloids are present in flower nectar and pollen, both types of alkaloids can be transferred into honey through bee foraging and endanger consumers. The main health concern in the case of the alkaloids is related to chronic disease that can be initiated by even low-level dietary exposure. The consequences of chronic or intermittent exposure include cancerous diseases, progressive liver disorders leading to cirrhosis, congenital anomalies, and pulmonary arterial hypertension in the case of PAs. TAs can prevent the interaction of acetylcholine to its receptor which may affect the heart rate, respiration and functions of the central nervous system.
As the consumption of honey is constantly increasing worldwide, it is crucial to provide a safe product for the consumers. However, procedures for the simultaneous determination of PAs and TAs in honey could only be found in inconsiderable number. For that reason, a sensitive method for the determination of both pyrrolizidine and tropane alkaloids in honey was developed.
The analytical protocol used sulphuric acid extraction, solid-phase extraction purification with the MCX cartridges and liquid chromatography - mass spectrometry detection. The developed procedure was subjected to validation in terms of linearity, selectivity, repeatability, reproducibility, limits of quantification and determination, matrix effect and uncertainty. All validation parameters fulfilled the requirements of the European Commission Decision 2002/657/EC.
A total of 29 honey samples were analysed for the determination of PAs and TAs. Honey samples were collected as regular veterinary inspection procedure, and represented various types of honey: monofloral including buckwheat, rape, and acacia; multifloral and honeydew.
In 15 out of 29 samples, at least one of the analysed PAs was detected. The total content of determined PAs was in the range 2.2 - 147.0 µg/kg. The most abundant alkaloids were echimidine, intermedine, lycopsamine and senecionine. Echimidine was present in 31% of all analysed honeys, while intermedine, lycopsamine and senecionine contaminated 24%. Seneciphylline, retrorsine, senecivernine and erucifoline were the other detected alkaloids. However, scopolamine and atropine were not determined in the analysed samples.

Keywords: *pyrrolizidine alkaloids, tropane alkaloids, honey, LC-MS*

M9

DETERMINATION OF ATROPINE AND SCOPOLAMINE IN FEED BY LC-MS METHOD

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Tropane alkaloids (TAs) are produced as secondary metabolites and naturally occur in plants of several families including *Brassicaceae, Solanaceae* and *Erythroxylaceae*. They are distributed globally and *Datura stramonium* is an invasive weed across many temperate and tropical regions. Already, contamination with this weed is increasingly being identified in crops such as maize, millet, buck-wheat, flax, sunflowers, sorghum, and soybeans, which are often used as feed constituents. As the recognition and avoidance of TAs containing plants in the processed feed is practically impossible, contaminated feeds were identified as the main source of animals intoxications.
Visual methods used for screening of botanical impurities in unground materials have been used to comply with Directive 2002/32/EC of the European Parliament and of the Council. However, visual methods are focused on seeds, disregarding the fact that alkaloids levels in some parts of TA-containing plants such as leaves and flowers can be equally high or even higher than in the seeds. Additionally, visual methods cannot be used for ground materials and compound feeds, and do not yield information on the actual TAs content in the sample, thus might provide insufficient level of animals protection.
To be able to assure the proper control of the tropane alkaloids, especially in processed feed materials and compound feeds, adequate and sensitive analytical methods are required.
The developed protocol used extraction with the solvent mixture containing methanol, water and formic acid (60:40:0.4, v/v/v), and subsequent purification with the polymeric mix mode SPE cartridges – PCX. For the elution of TAs from the cartridges a mixture containing ethyl acetate, methanol, acetonitrile, triethylamine and ammonia (8:1:1:0.1:0.1, v/v/v/v/v) was used. Purified samples were subjected to the LC-MS analysis and the separation was performed on C18 column.
After validation, the developed method was applied to the analysis of 42 feed samples. Among the tested samples were compound feeds including complete and complementary feed, and feed materials such as soybean meal, rapeseed meal and maize.
A total of 67% of all analysed samples contained at least one of the monitored tropane alkaloids. The highest concentration of the sum of investigated TAs was 147.9 µg/kg. The average content of TAs was 20.5 µg/kg and median concentration was 5.2 µg/kg. The presence of scopolamine and atropine was found in 55% of complete feeds and 60% of complementary feeds, with the highest TAs concentrations of 31.0 and 32.9 µg/kg in complete and complementary feed, respectively. Soybean meal was the most often contaminated feed commodity.

Keywords: *tropane alkaloids, scopolamine, atropine, feed, LC-MS*

M10

OCHRATOXIN A AND STERIGMATOCYSTIN IN GRATED GRANA CHEESE: OCCURRENCE AND STRATEGIES FOR CONTROLLING THEIR INCIDENCE

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The presence of toxigenic fungi on cheese wheels represents a risk for consumers, due to the potential production of different mycotoxins. In the last years, the consumption of grated cheese is continuously growing in Europe, with about the 25% of total hard cheese production in Italy which is currently destined to become grated cheeses. Because of the inclusion of cheese rind during the grating step, grated cheeses have been reported as foods potentially contaminated by mycotoxins. In the present work, a survey was carried out on ochratoxin A (OTA) and sterigmatocystin (STC) contamination in grated cheese products obtained from grated grana cheeses, collected in several supermarkets of Northern Italy over the period 2018–2020. OTA and STC were respectively found in the 49 % and 94% of the samples, in a range from

Keywords: *ochratoxin A, sterigmatocystin, grated cheese, long-ripened grana cheese*

M11

PERFORMANCE ASSESSMENT OF THE EU REFERENCE METHOD FOR LIPOPHILIC MARINE BIOTOXIN DETECTION IN MARINE GASTROPODS – A NON-TRADITIONAL NON-FILTER FEEDING VECTOR SPECIES

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The recognised official methods for the detection of regulated marine biotoxins in bivalve molluscs are listed in Annex V of EU Commission Implementation Regulation (CIR) 2019/627. These methods must be used for official control testing of bivalve molluscs to check compliance with EU established limits as laid down in Regulation (EU) 853/2004. There are no established official methods for the testing of other marine species, sometimes referred to as 'non-traditional vectors' of marine biotoxins. These may also accumulate marine biotoxins (including non-filter feeders) and are regulated in CIR 2019/627. Competent authorities must declare fishery products unfit for human consumption if "live bivalve molluscs, echinoderms, tunicates or marine gastropods contain marine biotoxins in total quantities exceeding the limits referred to in Regulation (EC) No 853/2004".
The official / reference method for lipophilic toxin (LT) detection is the EURL LC-MS/MS method. This method was assessed for testing of the non-traditional vector and non-filter feeding marine gastropod species - Periwinkle. A within laboratory validation study was carried out for the regulated okadaic acid (OA), pectenotoxin (PTX), azaspiracid (AZA), and yessotoxin (YTX) group toxins along with pinnatoxin G, an emerging toxin and recognised marker for pinnatoxin group toxins. Validation data was assessed against CIR 2021/808 method performance criteria for confirmatory methods. The study incorporated both spatial and temporal variation, with samples obtained across a number of regions within the UK. Validation data will be presented along with the results of natural levels of biotoxins that were detected in several of the sub-samples obtained.

Keywords: *biotoxin, emerging, non traditional vector, lipophilic, validation*

Acknowledgement: *UK Food Standards Agency (FSA-UK) and Food Standards Scotland (FSS)*

M12

DEVELOPMENT OF A MULTI-METHOD FOR QUINOLIZIDINE ALKALOIDS AND ITS APPLICATION TO A VARIETY OF LUPINE-BASED FOOD PRODUCTS

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Quinolizidine alkaloids are toxic secondary plant metabolites in lupins. Lupin seeds have a high protein content and are thus an interesting protein source for vegetarians and vegans. The alkaloid content of lupins can be reduced by breeding of sweet lupin varieties or a debittering step prior to their use as food ingredient. Over the past years, not only lupin flour and semolina but also more and more processed food products, many where lupin extracts replace milk as ingredient (e.g. yoghurts, puddings and ice creams), have entered the market. Currently, only limited data is available concerning the occurrence of quinolizidine alkaloids in lupin-based food products. This is partly due to the lack of robust multi-methods.
The development of a rapid and sensitive multi-method encompassing twelve quinolizidine alkaloids suitable for both dry and liquid/pasty foods will be presented. Two challenges were encountered in the process: First, some quinolizidine alkaloids are isomeric and their separation poses some challenges. Secondly, the analyte albine exhibited a "strange" chromatographic behaviour. Both issues were addressed by studying the influence of the pH of the mobile phase. With an optimised mobile phase all isomers could be separated on a C18 column in less than twelve minutes. The validation of the method yielded excellent recovery and precision data as well as sufficiently low limits of quantitation for both dry and liquid/pasty food products.
The method was applied to a variety of lupin-based food products and the found quinolizidine alkaloid patterns will be presented. The high amounts of quinolizidine alkaloids observed in some products suggest that further monitoring of products on the market should be carried out, as this is a prerequisite to assess the risk of lupin-based food products with greater accuracy.

Keywords: *quinolizidine alkaloids, LC-MS/MS, isomer separation, lupine food products, multi-method*

M13

DIETARY SUPPLEMENTS AS A SOURCE OF MYCOTOXINS?

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According to the Act of 25 August 2006 on food and nutrition safety (Journal of Laws of 2015, item 594), a dietary supplement is a foodstuff, therefore legal regulations of these products are less restrictive than pharmaceutical products. The current legal status allows anyone to place a supplement on the market, provided they declare its composition to the sanitary authorities; in Poland it is the GIS, on the so-called Notification. The NIK report, from 2021 states that many supplements do not show features declared by producers.
The aim of this study was to evaluate mycotoxins contamination in dietary supplements available in Poland. The material consisted of dietary supplements based on: *Plantago psyllium* (n = 6), *Crataegus oxyacantha* L. (n = 16), *Lepidium meyenii* Walpers. (n = 16), *Silybum marianum* (L.) Gaertn (n = 13), *Stevia rebaudiana* Bertoni (n = 15) and *Epilobium parviflorum* L. (n=23), which occurred in the form of dried plants. Mycotoxins determination was performed using HPLC-FLD method (immunoaffinity column AflaTest from Vicam-AF; immunoaffinity column OchraPrep from R-Biophram Rhône Ltd–OTA) and HPLC-MS/MS method (Bond Elut®Mycotoxin column from Agilent - DON, NIV, ZEN, T-2 and HT-2).
The most frequently detected mycotoxins were PAT (50%), ZEN (46%) and T-2 toxin (36%). The highest content of these mycotoxins was: PAT_{max} = 93.2 ppb, ZEN_{max} = 1048.4 ppb, $T\text{-}2_{max}$ = 297.7 ppb. Of all the assortment examined, the most contaminated were dietary supplements based on Milk Thistle (100%) and Hoary willowherb (87%).
The conducted research confirms the presence of mycotoxins in dietary supplements in the form of dried plants. Producers should take into account and monitor their level of contamination in these types of products.

Keywords: *dietary supplements, mycotoxins, contaminations*

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M14

SIMULTANEOUS DETERMINATION OF ALTERNARIA TOXINS, ERGOT ALKALOID EPIMERS, AND OTHER MAJOR MYCOTOXINS IN VARIOUS FOOD MATRICES BY LC-MS/MS

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Various food commodities are vulnerable to different types of fungal pathogens and could be contaminated with differential classes of mycotoxins as a result. It is Ideally to implement a generic method for simultaneous determination of multi-mycotoxins in different food matrices or agricultural products. In this study, a simplified sample preparation procedure and a reliable LC-MS/MS analytical method was developed for comprehensive measurement of 37 regulated and emerging mycotoxins including 5 Alternaria toxins, 6 major ergot alkaloids and their corresponding epimers. Four different food matrices (baby wheat cereal, peanut, tomato puree, and blended flour) were chosen for method validation to demonstrate the applicability of this analytical method to a wide range of food types. Sample extraction was performed using a formic acid-acidified 80:20 acetonitrile:water solution followed by extract dry-down and reconstitution in a 50:50 water:methanol solution for injection analysis on a Biphenyl LC column. Chromatographic analysis was performed using LC-MS friendly acidic mobile phases and completed with a short 11-minute cycling time for proper separation of ergot alkaloid epimers. Accurate quantification was achieved using matrix-matched calibration standards at the range of 0.4 to 400 µg/kg. The recoveries of all mycotoxins (except citrinin) in fortified samples were from 70% to 120%, and the relative standard deviation (RSD) was less than 20%. For the vast majority of analytes, the limit of quantification was at 0.4 µg/kg which was satisfactory to meet the regulatory levels.

Keywords: *mycotoxins, alternaria, ergot alkaloids*

M15

IMPLEMENTATION OF THE SAMPLE PRETREATMENT FOR THE ISOLATION AND PURIFICATION OF TETRODOTOXIN ANALOGUES TO BE FURTHER ANALYZED BY HILIC-LC-MS/MS

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Tetrodotoxins are a group of powerful neurotoxins present in seafood, which are being considered an emerging risk for human health, acting selectively on voltage-gated sodium channels. LC-MS/MS has been proposed as the analytical method to carry out the control of these neurotoxins, being HILIC Chromatography an alternative for an efficient separation of the TTX analogues. The complexity of the biological matrix in which TTXs are present, makes strictly necessary the development of an efficient sample pre-treatment for the isolation and purification of the toxins involved in the seafood contamination, as well as an efficient interferences removal, in order to compensate the lack of standards and reference materials commercially available, which would have been necessary to optimize the LC-MS/MS parameters, prior to the application of this technique for further confirmation purposes. The objective of this work has been therefore focused on these optimizations, and with this aim, chromatographic parameters such as mobile phase composition, change in gradient slope, flow or sample injection volume, etc. have been evaluated and optimized in order to obtain the adequate selectivity on the fractionation step. The optimization of the parameters of the mass spectrometry analyzer (such as fragmentor potential and collision energies) has been also critical in order to obtain an increased sensitivity for the detection of TTX and its analogues, in particular when they are present at trace level in naturally contaminated samples.

Keywords: *HILIC, LC-MS/MS, chromatoghraphy, TTX analogs, tetrodotoxin*

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M16

SIMULTANEOUS MULTI-MYCOTOXIN DETERMINATION OF 6 MYCOTOXINS IN WHEAT USING BIOCHIP ARRAY TECHNOLOGY ON THE EVIDENCE INVESTIGATOR

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The Myco 6 biochip array was developed for the detection of Aflatoxin B_1 (AFB_1), Ochratoxin A (OTA), Deoxynivalenol (DON), Zearalenone (ZEA), T-2/HT-2 Toxins (T-2/HT-2) and Fumonisin B_1 (FB_1) in wheat. Using a single sample preparation method, the measuring ranges were AFB_1 1 - 80 ppb, OTA 1.5 - 160 ppb, T-2/HT-2 10 - 500 ppb, ZEA 12.5 – 1,600 ppb, DON 150 – 3,000 ppb and FB_1 200 – 12,000 ppb. Precision and within laboratory repeatability performance was within the criteria set by Commission Regulation (EU) No. 519/2014 for confirmatory methods. The rate of false suspect results for all assays was ≤0.03% much below ≤5% criterion specified under Commission Regulation (EU) No 519/2014. The Myco 6 Array was subjected to Proficiency Testing rounds with all reported results for UKGTN Proficiency Test rounds within the Z-score $|-2|Z|+2|$. The method performance data generated in this study has shown that the Myco 6 Array is *Fit-For-Purpose* for the simultaneous detection of six mycotoxins in wheat.

Keywords: *biochip array, evidence investigator, multi-mycotoxins*

M17

DETERMINATION OF MULTICLASS CYANOTOXINS IN SPIRULINA-BASED DIETARY SUPPLEMENTS BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Cyanobacteria are a diverse group of oxygenic photosynthetic prokaryotes which are believed to be one of the oldest life forms on Earth. They live in a wide range of ecosystems and withstand extreme environmental conditions. An important proportion of cyanobacteria are known to be producers of harmful cyanotoxins, which are toxic secondary metabolites that can impact on ecosystem and human health. The oral route is one of the main ways whereby humans can be exposed to cyanotoxins. Therefore, the consumption of contaminated algae-based food supplements is becoming more relevant due to its upsurge, which underlines the importance of controlling these toxins in this kind of products. Currently, the main challenge is to be able to determine different families of cyanotoxins at the same time.

In this respect, this work describes the simultaneous determination of seven cyanotoxins belonging to three different classes: the cyclic peptides microcystin-LR (MC-LR), microcystin-RR (MC-RR) and nodularin (NOD); the alkaloid anatoxin-a (ANA); and three non-protein amino acids isomers such as β-methylamine-L-alanine (BMAA), 2,4-diaminobutyric acid (DAB) and N-(2-aminoethyl)glycine) (AEG). These are determined in spirulina-derived food supplements using a novel solid-liquid extraction coupled with a tandem-solid phase extraction procedure for clean-up and preconcentration (SLE-tandem-SPE). Extracts were analyzed by hydrophilic interaction liquid chromatography with mass spectrometry detection (HILIC-MS/MS). A SeQuant ZIC-HILIC column was employed to achieve the chromatographic separation in less than 12 min using water and acetonitrile, both acidified with 0.3% of formic acid, as mobile phase. Previously a SLE was developed using 4 mL of aqueous 5% formic acid to extract the most polar compounds, followed by 4 mL of 80% MeOH. Both extracts were combined and submitted to a tandem-SPE using mixed-mode cation exchange (MCX) and Strata-X cartridges. Elution from both cartridges was performed using 10% $NH_3 \cdot H_2O$ in MeOH.

Method validation was carried out in terms of linearity, limit of detection (LOD) and quantification (LOQ), recoveries, matrix effect and repeatability and intermediate precision. LOQs in the range of 50-300 $\mu g \cdot kg^{-1}$ and recoveries ranging between 64.2% and 102.9% with an associated RSD<19.2% were achieved. Satisfactory precision was obtained with RSD values lower than 19.6% in all cases, except for BMAA, which reported the highest RSD values, reaching 25.1%. The method was satisfactorily applied to determine the occurrence of cyanotoxins in nine blue green algae (BGA) dietary supplements. DAB was the most frequently detected cyanotoxin, at concentrations up to 2408 $\mu g \cdot kg^{-1}$ and AEG was found in two samples at concentrations up to 194 $\mu g \cdot kg^{-1}$. In addition, MC-LR and MC-RR were found in one sample at concentration levels higher than 5 $mg \cdot kg^{-1}$, which illustrates the need to provide for tighter control of these substances in this kind of matrices.

Keywords: *cyanotoxins, HILIC-MS/MS, spirulina-based dietary supplements, tandem-SPE*

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M18

INFLUENCE OF CULTIVATION METHODS - CONVENTIONAL AND ORGANIC - ON THE CONTENT OF MYCOTOXINS AND PESTICIDES IN GRAPE MUSTS AND POMACE

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Grapes are one of the most widely grown crops globally, with production in 2018 of nearly 80 million tons. About 75% of the grapes produced are intended for wine production, of which 20-30% are waste products. They are also called grape pomace and consist of skins, stalks, seeds, and remaining pulp. These by-products end up in landfills or are used to produce wine alcohol, as fertilizer, or as animal feed. Grapes, as well as by-products, can be contaminated with mycotoxins (mainly ochratoxin A). It is reported that more than 90% of OTA can be retained in pomace. Fungicides are used to limit mold growth and consequently, contamination with mycotoxins. It is an effective means of limiting the growth of fungi, but on the other hand, it is a frequent cause of chemical contamination of grapes, juices, and wines. The question arises as to how the wine-growing system, i.e., organic and conventional, affects the risk of contamination with mycotoxins in the first case, and with fungicide active substances in the second. The present study aimed to determine the contents of mycotoxins in grape must and pomace and the contents of active substances of pesticides used in conventional cultivation.
Two German grape varieties, *Vitis vinifera* L. Solaris and Hibernal, were used in the research. In both cases, from organic and conventional plantations, collective fruit samples were taken in three replications. Both must and grape pomace were used for the research. The plantation is located in northwest Poland in the Rajkowo Palace vineyard. Ochratoxin A and aflatoxins were determined using HPLC-FLD methods. Trichothecenes (DON, NIV, DAS, T-2, HT-2), zearalenone, fumonisins, and patulin were determined using HPLC-MS/MS methods. The samples clean-up was performed using: Ochraprep (for OTA), AflaTest (for AFs), MycoSep 228AflaPat (for PAT), MultiSep 211Fum (for FUM), and Bond Elut Mycotoxin (for trichothecenes and ZEN) columns. To detect pesticides (280 analytes, e.g. cyflufenamid, cyprodinil, fludioxonil, fluopyram, metalaxyl-M, mancozeb, tebuconazole, trifloxystrobin), gas chromatography-tandem mass spectrometry method (according to PN-EN 15662: 2018-06-GC-MS/MS) was used. No mycotoxins were detected in the analyzed samples, except for three must and two pomace samples with low levels of zearalenone (values below 0.2 µg/kg). Significant contamination of cyprodinil (minimum 14.7±7.4 mg/kg) and fludioxonil (minimum 15.3±7.7 mg/kg) was detected in must samples from the conventionally cultivated plantation.

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M19

THE PRESENCE OF OCHRATOXIN A IN BREAST-MILK, URINE AND SERUM OF LACTATING WOMEN

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Mycotoxins are secondary metabolites of molds. Ochratoxin A (OTA) is the most common in the Polish climate. It is produced by fungi of the genera *Aspergillus* and *Penicillium*. It is produced as a result of improper food storage. It is present in many products, that are consumed both by humans and animals: cereals, wheat gluten, coffee, dried fruit, wine, grape juice, spices, beer and products based on them. OTA is nephrotoxic, hepatotoxic, potentially carcinogenic and teratogenic. OTA mainly enters an organisms by oral intake.
The aim of the study was to detect the presence of OTA in milk, urine and serum of lactating women. A survey was also conducted regarding the daily diet of women. The research group consisted of 32 lactating women (11 were the donors from the Milk Bank in Toruń, the other 21 were recruited for this study).
Results of the analysis showed the occurrence of OTA only in 3 milk samples (9.38%). The minimum level was 0.01 ng/ml, while maximum 0.018 ng/ml and mean 0.0013 ng/ml. Twenty-six urine samples (81.25%) were OTA positive, with minimum level 0.013 ng/ml, maximum level 0.117 ng/ml and mean 0.0192 ng/ml. Also, all 32 serum samples (100%) were contaminated by OTA, with minimum level 0.099 ng/ml, maximum level 2.38 ng/ml and mean 0.4649 ng/ml.In the case of 3 women, OTA was present in all tested body fluids. Based on the results, the following conclusions can be drawn: the breast-milk of women in the study group is slightly contaminated with ochratoxin A. Ten samples of urine contained ochratoxin A above its average content in tested samples. Moreover, serum of 8 women contains ochratoxin A at a level above the average content of this mycotoxin in tested samples. The average ochratoxin A level in serum in the presented studies was 0.4649 ng/ml and is much lower than the average serum ochratoxin A level established in several countries in the world, i.e. 0.7 ng/ml.

Keywords: *lactating women, Ochratoxin A, contamination*

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M20

INHIBITION OF GROWTH AND MYCOTOXIN PRODUCTION OF FUSARIUM CULMORUM BY TRICHODERMA

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Fusarium fungi can infect field crops and consequently cause mycotoxin contamination, leading to yield losses and deterioration in the quality of agricultural products, and poses a huge threat to food safety. The study aimed to assess the ability of selected *Trichoderma viridescens*, *T. viride* and *T. atroviride* strains to inhibit mycelial growth and the biosynthesis of mycotoxin by selected strains of *Fusarium culmorum*. A Potato Dextrose Agar (PDA) double agar plate bioassay was used for antagonistic evaluation of *Trichoderma* strains. These studies showed that all *Trichoderma* strains used in the study significantly influenced the growth of *Fusarium* mycelium. A qualitative evaluation of inter-colony interactions after 5 days of co-inoculation at 25 ± 2 °C showed that all *Trichoderma* strains outgrown from 75% to 100% of the area. In contact with all tested pathogens, the *Trichoderma* strains were characterized by a degree of inhibition ranging from 27 to 67%. To assess the degree of inhibition of the biosynthesis of selected mycotoxins, joint cultivation of *Trichoderma* and *Fusarium* on rice was carried out. In the studied cultures, the content of trichothecenes, zearalenone, and their modified forms was analyzed and their content was compared with the control (*Fusarium* culture on rice). The mycotoxin content was analyzed using ultra-high-performance liquid chromatography high-resolution mass spectrometry. On the basis of the conducted research, *Trichoderma* strains turned out to be effective suppressors of the mycotoxins tested. The degree of inhibition of deoxynivalenol biosynthesis was on average from 80 to 99%, sums of 3- and 15-acetyl-deoxynivalenol from 92 to 100%, nivalenol from 28 to 100%, and fusarenon X from 87 to 100%. However, inhibition degree of zearalenone was on average from 46 to 98 %, zearalenone-14-sulphate from 97 to 100%, α-zearalenol from 49 to 100%, and β-zearalenol from 12 to 97% compared to the control where only *Fusarium* was grown. In co-cultures of *Trichoderma* strains of F. culmorum 846, deoxynivalenol 3-glucoside was detected in an amount about from 1 to 8 mg/kg of sample and almost twice as much β-zearalenol as compared to the control, when *F. culmorum* 846 was co-cultured with *T. viride* 355. *Trichoderma* spp. Is a genus of fungi with enormous potential antagonistic to *Fusarium* pathogens. They may also have the potential to be used in the biological control of cereal plants against *Fusarium* - one of the most important pathogens of these plants.

Keywords: *biocontrol, trichoderma, fusarium, mycotoxins, food safety*

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M21

VALIDATION OF A FLOW-THROUGH RAPID TEST FOR A QUICK AND EASY DETECTION OF OCHRATOXIN A IN WINE WITH A CUT-OFF VALUE OF 1 µG/L

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Ochratoxin A (OTA) is a nephrotoxic and hepatocarcinogenic toxin produced by fungi. OTA has been shown to occur in various cereals and plant products such as coffee and grapes. OTA also occurs in wine produced from contaminated grapes. In the European Union the maximum limit for OTA in wine is 2 µg/L in accordance with Commission Regulation 1881/2006. Wine products should be tested with appropriate methods to assure the contaminated wine is not offered to customers. The screening target concentration (STC) for a screening method should be ideally set at half of the maximum limit for the given contaminant. In order to reach the detection level of 1 µg/L of OTA in wine a new immune-based flow-through rapid test was developed and validated.
The performance of the method was tested with a set of 21 blank wine samples and a set of 21 wine samples spiked at 1 µg/L with OTA. The samples were analysed using three different batches of ochratoxin A wine test kit. Additionally, sets of samples were also spiked at lower and higher level, that is 0.5 µg/L and 1.5 µg/L. Visual interpretation of each result (in total 252 results) was performed by three different analysts and the results were classified as screen negative (2 lines visible) or positive (only 1 line visible). The specificity was determined to be 100% meaning all samples not containing any OTA (negative) gave correctly negative result. The overall sensitivity was 98% meaning 2% of samples gave false negative result. This false negative rate was lower than the acceptable false negative rate for a screening method (≤5%). The test was also shown to be highly robust and small deviations from the recommended testing protocol did not affect the test result. The test is applicable for analysis of red, white or rosé wines in just 15 min and can be applied on site at wineries for in-process and final products testing. It can be used by unexperienced users and does not require any additional reagents or equipment.

M22

MONITORING OF PYRROLIZIDINE ALKALOIDS IN FOODSTUFFS ON THE ITALIAN MARKET COLLECTED BETWEEN 2019-2022

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Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by over 6000 plant species worldwide. The PA-content of plant material depends on a large number of factors (species, plant organ, harvest, climatic conditions). In animal studies, 1,2-unsaturated PAs have proven to be genotoxic carcinogens. According to the 2017 EFSA scientific opinion, the food most exposed to the presence of PAs is honey, tea, herbal infusions and food supplements. Following the EFSA's recommendations, during the last years we began monitoring and collecting analytical data on the presence of PAs in relevant food.
The aim of this study is to report the data obtained from the monitoring of 602 samples of different matrices collected on the Italian market between 2019-2022, in view of the new Commission Regulation (EU) 2020/2040 put into force from 1 July 2022. This Regulation sets maximum levels for the sum of Pyrrolizidine alkaloids in certain foodstuffs. The samples included 48 tea (dried products), 41 herbal infusions, 24 dried herbs (like oregano and rosemary), 13 fresh borage leaves, 320 honey, 130 pollen and 26 herbal food supplements.All samples were analysed for their PAs content by a LC-MS/MS method developed and validated in-house, that detects up to 35 analytes (tertiary amines and corresponding N-oxide). Limit of Quantification depended on the matrix (1 µg/kg in honey and 5 µg/kg in other matrices) and was considered fit-for- purpose.
The following PAs concentration ranges have been found: 11-4678 µg/kg in dry herbs, 5-3410 µg/kg in fresh Borage leaves, 5-1346 µg/kg in tea (*Camellia sinensis*), 5-1171 µg/kg in herbal infusions, 5-667 µg/kg in herbal food supplements, 6-10168 µg/Kg in pollen and 1-121 µg/kg in honey. In these samples the contamination was due mainly to the presence of one prevailing alkaloid: Lycopsamine N-oxide in borage and dry herbs, Echinatine N-oxide in pollen, Lycopsamine in herbal food supplements, Retrorsine N-oxide in tea and herbal infusions and Echimidine in honey. The analysis revealed that percentage of contaminated samples has increased from 2019 to present day. In particular, 36 of contaminated samples exceed the maximum levels established by the new EU Commission Regulation and were more represented by dry herbs and tea.
In conclusion, a lot of food items can contain substantial amounts of PAs and contribute significantly to the human exposure, and in particular to the vulnerable population groups. Ongoing efforts should be made to minimize or prevent PAs occurrence in foodstuffs by the application of good agricultural and harvest practices to ensure a high level of human health protection.

Keywords: *monitoring, pyrrolizidine alkaloids, foodstuffs, LC-MS/MS*

M23

CERTIFICATION OF MARINE TOXINS BY QUANTITATIVE NMR AT THE HIGHEST METROLOGICAL LEVEL

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Marine toxins generated by various species of algae can become a major problem of food safety since these toxins are accumulating through the food chain and can lead to harmful levels of contamination in shell fish and other sea food. In recent years, fast and sensitive LC-MS methods were established to detect marine toxins in sea food.[1] Therefore, the access to well characterized reference materials for a precise and accurate quantitation of these different toxins has become an increased need of the testing laboratories. Unfortunately, the isolation, synthesis and purification of these compounds is very difficult and available quantities of material are typically in the range of a few milligrams. This is a major challenge for the certification and production of reference materials for these compounds. In order to achieve certification of very small quantities according to ISO/IEC 17025 and ISO 17034, a special quantitative NMR (qNMR)[2],[3] / LC-MS workflow was established. The setup combines all the crucial aspects and parameters that were elaborated in recent years during the accredited characterization of pure substances as qNMR standards and qNMR solutions. This workflow is shown on our poster.

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[3] M. Weber, Ch. Hellriegel, A. Rueck, J. Wuethrich, P. Jenks, Journal of Pharmaceutical and Biomedical Analysis, 93, 102-110, 2014.

Keywords: *marine toxins, certified reference materials, seafood safety, quantitative NMR (qNMR)*

M24

PERFORMANCE DATA OF AN LC-MS/MS BASED MULTI-METHOD IN PROCESSED GRAIN-BASED PRODUCTS

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The improved sensitivity and robustness of modern LC-MS/MS instruments has led to a continuously increasing number of analytical methods covering multiple mycotoxins (and even different contaminant classes) with only minimal or even without any clean-up. The majority of the published methods focuses on raw agricultural commodities (such as grains, nuts and dried fruits) however, whereas data on method performance in processed foodstuffs is rather scarce.
In this work, we have applied a previously developed "dilute and shoot" method to four matrices of processed cereal products, i.e. noodles, biscuits, crackers and musli. Method performance data was retrieved for 800 compounds (mostly mycotoxins and other fungal secondary metabolites, but plant toxins such as pyrrolizidine alkaloids, cyanogens and glycoalkalolids were included was well). In order to introduce heterogeneity to the sample set used for validation we chose 7 individual brands / samples per matrix and different additives/flavors (nuts, dried fruits, chocolate, yoghurt) in case of the musli samples.
Official criteria for recovery of the extraction and for repeatability were met for the majority of the analytes (92-94% and 93-96%, respectively, depending on the matrix) and both absolute and relative matrix effects were reduced compared to raw grains. The intermediate precision obtained on technical replicates over a period of 7 weeks was < 10% for approx.. 90% of all compounds. We speculate that processing removes/transforms part of the matrix constituents that cause matrix effects whereas additives such as anti-oxidants stabilize particularly labile analytes that exhibit low and irreproducible extraction efficiencies in raw commodities.

Keywords: *mycotoxins, plant toxins, LC-MS/MS, method validation*

M25

METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN PLANT-BASED FOODS AND HONEY USING LC-MS/MS

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Pyrrolizidine alkaloids (PAs) are toxins exclusively biosynthesised by plants. They are typical plant secondary metabolites against herbivores.[1] 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 'PA-containing plants', which is mainly due to cross contamination during harvesting.[2] PAs are regarded as undesirable substances in food and feed, due to their genotoxic and carcinogenic properties, and for that reason were the subject of an EFSA opinion in 2011.[3] Based on the outcomes of various risk assessments, the fact that teas/herbal infusions and other food items could contain substantial amounts of PAs has to be considered as a relevant food safety issue. Based on these studies, the European Commission has set maximum levels of PAs in certain foodstuff, such as herbs, spices, teas, herbal infusions and pollen products. Maximum levels refer to the "lowerbound" sum of 35 PAs, and are set in EC Reg. (EU) 2020/2040[4] enforced from 1st July 2022 and amending Regulation (EC) 1881/2006. Due to the risk posed to human health, food testing laboratories need to develop and validate suitable confirmatory methods for the quantitative determination of PAs. Certainly, the main challenge of this analysis is the presence of a large number of isomers that present the same MRM transitions and are extremely challenging to resolve in the chromatographic dimension.

In this work, we describe a simplified approach for the quantification of 35 EU regulated PAs in plant-based food and honey using UPLC-MS/MS and we address the separation of a number of the isomers in a single chromatographic run. The proposed method has been validated in-house and the performance parameters, including trueness and precision, have been assessed. Matrix effects were evaluated, and the sample clean-up efficiency was studied. Limits of detection and quantification across all matrices investigated were found to be significantly below the maximum limits set by the EU Regulation, allowing the method to be employed for testing of food intended for infants and young children.

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4. Commission Regulation (EU) 2020/2040 of 11 December 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of pyrrolizidine alkaloids in certain foodstuffs.

Keywords: *pyrrolizidine alkaloids, natural toxins*

M26

DEVELOPMENT OF A MULTI-TOXIN UPLC-MS/MS METHOD FOR 50 MYCOTOXINS AND TROPANE ALKALOIDS IN CEREAL COMMODITIES

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Mycotoxins are naturally occurring, notoriously toxic compounds to both humans and animals. They can occur in high frequency and concentrations in cereals, and in other food and feedstuffs. The demand for testing for masked, modified, and emerging mycotoxins has significantly increased over the last decade, as ongoing studies provide a steady stream of insights about newly discovered mycotoxin metabolites - as do plant breeding efforts adapting to a changing climate. Hence, there is a need to extend the scope of analysis to cover these compounds not already legislatively regulated.

A multi-toxin UPLC-MS/MS method for 50 regulated and emerging mycotoxins, atropine, and scopolamine in cereal-based products was developed. A mixture of wheat, barley, rice, and maize flours were extracted using a simple "dilute-and-shoot" protocol, without clean-up or internal standards. Calibration curves were plotted using solvent standards and matrix-matched calibration. Coefficients of determination (R^2) were almost all > 0.99. The calibration range covered three orders of magnitude for most analytes, and values for relative standard deviation (%RSDr) were ≤10% for all analytes. Matrix effect ranged from -100% to +83 %. Data was imported into the *waters_connect for quantitation* software and processed with the *MS Quan* app for an improved efficiency in data processing and review. Ion ratios and retention times from the spiked test portions agreed well with the criteria specified in the SANTE guidelines 12089/2016 for all compounds.

The sensitivity of the Xevo TQ-XS allows considerable dilution of the sample extract while still reaching extremely low limits of quantification - the lowest method limit of quantification (m-LOQ) was for aflatoxins (0.1 µg/kg).

M27

MONITORING OF TROPANE ALKALOIDS IN GLUTEN-FREE FOODS AND THEIR FATE DURING PROCESSING OF CONTAMINATED BUCKWHEAT

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Occurrence of tropane alkaloids (TAs) in food crops and products thereof is high concern. Maximum limits have been recently established in EU[1] for atropine and scopolamine in selected commodities. The available data show buckwheat as one of possible sources of dietary intake of these natural toxins. The presented study focused on monitoring of TAs in glute-free products collected at Czech market, in the next phase, their fate across a processing chain was investigated. For samples 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 21 TAs were monitored. Target TAs were detected in 3 out of 35 samples analyzed, the highest contamination 31 µg/kg (atropine) was determined in a sample of buckwheat flakes. When considering ARfD 16 ng per kg of body weight (estimated by EFSA for sum of atropine and scopolamine), then, consumption of 100 g of such flakes would result in case of consumer with bod weight 80 kg, ARfD would be exceeded by 242%. In the next part of the study, biscuits baked from flour artificially contaminated by extract from *Atropa bella-dona* and *Datura stramonium* (TA content in flour was 5.6 mg/kg atropine, 3 mg/kg scopolamine, others at lower levels) were analyzed by ultra-high performance liquid chromatography coupled with tandem high-resolution tandem mass spectrometry UHPLC-HRMS/MS) technique which enables identification of possible degradation products. While the content of parent tropane alkaloids in biscuits decreased, significant increase of apoatropine and aposcopolamine signals was recorded.

[1] Commission regulation (EU) 2021/1408 of 27 August *2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of tropane alkaloids in certain foodstuffs*. L 304/1, 30.08.2021, p. 4.

Keywords: *tropane alkaloids, buckwheat, liquid chromatography, mass spectrometry*

Acknowledgement: *This work was supperted from the grant of Specific university research - grant A2_FPBT_2022_019.*

M28

LEVELS OF LIPOPHILIC MARINE BIOTOXINS REGISTERED IN BLACK SEA MUSSELS FROM BULGARIAN COAST

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Marine biotoxins are produced by certain microalgae species. Based on their chemical structure they are devided into lipophilic and hydrophilic. Both could cause human illness and therefore represent a serious threat to public health. Shellfish such as mussels are the main dietary source of marine biotoxins. The digestive gland (hepatopancreas) is the organ where toxins accumulate and concentrate. The aim of this study was to estimate the levels of multiple lipophilic marine biotoxins in mussel (*Mytilus galloprovincialis*) from the Black Sea and to compare them with the regulatory levels. Harvested were most consumed mussels (N = 17) - from natural populations as well as from aquaculture regions. Sampling was performed in 2021. In order to develop the worst-case scenario, the hepatopancreas (hp) of mussels dissected und subjected to further analysis. The samples were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The most abundant toxin was PTX-sa (0,001- 0,030 µg/g hp), followed progressively by 7-epi-PTX-sa (0,001-0,14 µg/g hp), hydroxy-YTX (0,026-0,075 µg/g hp), YTX (0,028-0,122 µg/g hp) and PTX-2 (0,002 µg/g hp). Although in all samples were detected at least two of the determined biotoxins the regulatory levels of both toxins from pectenotoxin group (160 µg/kg) and yessotoxin group (3,75 mg/kg) were not exceeded. Indeed, the registered concentrations were much lower than the levels set in the EU legislation. Thus, as toxicokinetics, oral toxicity and relative potency of individual PTX and YTX-group is still being investigated, monitoring on their levels in most preferred shellfish is required to keep the consumers' health safe.

Keywords: *Black Sea, mussels, lipophilic biotoxins, LC-MS/MS*

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M29

OCHRATOXIN A IN CHEESE: DEVELOPMENT AND VALIDATION OF AN LC-METHOD WITH FLD- AND MS/MS-DETECTION AND ITS APPLICATION ON HARD CHEESE SAMPLES FROM THE GERMAN MARKET

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EFSA published a risk assessment on ochratoxin A (OTA) in food in 2020 (1). Data on OTA in cheese (n = 15) were also included in the exposure assessment, which indicate that cheese could be among the most relevant sources of exposure to OTA from food. The mean OTA levels in cheese were 2.24 µg/kg (lower bound) and 18.54 µg/kg (lower bound) in the 95th percentile. However, the database was very small with 15 samples, especially since the type of cheese could not be specified in all samples, so that EFSA recommends generating further content data to reduce uncertainty. OTA has so far been detected mainly on traditionally produced cheeses with a long ripening period (principally on or near the rind) and most frequently on cheeses labeled 'Parmigiano Reggiano' and 'Grana Padano', so the investigations initially focused on these foods (grated and in one piece).
Since there is no standardized method for analyzing OTA in cheese, our lab developed and validated a method based on the tentative standard DIN EN 17641:2021-02 (Foodstuffs - Multimethod for the determination of aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, T-2 toxin, HT-2 toxin and zearalenone by LC-MS/MS) for the determination of OTA and optional aflatoxin M1 in hard cheese and soft cheese.
After QuEChERS extraction of milled hard cheese or an aqueous slurry of soft cheese, we established and validated three different variations based on LC-FLD analysis after clean up with immunoaffintiy columns (IAC) as well as LC-MS/MS analysis with and without IAC clean up. For OTA the linear working range was validated from 0.063 – 6.3 µg/kg for all three variations, while the limit of quantification was determined to be 0.2 µg/kg for LC-FLD and 0.25 µg/kg respectively 0.5 µg/kg for LC-MS/MS with and without IAC clean up.
We utilized the LC-FLD variation of the method to analyse 42 hard cheese samples labeled with 'Parmigiano Reggiano' or 'Grana Padano' from the German market purchased from local retailers or from internet retailers. 5 samples were bought as flakes of hard cheese, 10 samples consisted of grated hard cheese and 24 samples were bought as packaged pieces of hard cheese. 70% of the grated hard cheese samples resulted in quantifiable OTA contents above the LOQ of 0.2 µg/kg, whereas only 12.5% of the samples of packaged pieces and none of the flake samples resulted in OTA contents above the LOQ. Positive samples were confirmed by LC-MS/MS. A further evaluation and discussion of the results of this survey will be presented in this contribution.
1 EFSA (2020) Scientific Opinion: Risk assessment of ochratoxin A in food; EFSA Journal 2020;18(5):6113; 150pp.

Keywords: *mycotoxins, ochratoxin A, cheese, LC-FLD, LC-MS/MS*

M30

OCCURRENCE OF DEOXYNIVALENOL (DON) AND ITS MODIFIED FORMS IN CEREALS AND CEREAL PRODUCTS IN THE GERMANY 2000-2021

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Deoxynivalenol (DON) is one of the most frequently detected mycotoxins in cereals and cereal products. Several modified forms have been detected as well, among which 3-acetyl-DON (3-ac-DON), 15-acetyl-DON (15-ac-DON) and DON-3-glucoside (DON-3-G) are considered the most relevant ones. While DON is regulated in the European Union in food and feed, the modified forms are not addressed in current legislation.
In 2017, the European Food Safety Authority (EFSA) assessed the risk to animal and human health related to DON and these three modified forms in food and feed. Since the modified forms can be transformed to the parent compound after ingestion, the same toxicity was assumed. Accordingly, EFSA extended the tolerable daily intake (TDI) value for DON and established a group-TDI for DON, 3-ac-DON, 15-ac-DON and DON-3-G.
In the present study, data gathered by German official control laboratories within the German National Monitoring Programme from 2000-2021 were evaluated. Data on the occurrence of DON was available for more than 27000 cereals and cereal products intended for human consumption. For more than 7300 of these samples, the level of one or both of the acetylated forms was reported, while only 194 results were available for DON-3-G. A quantified level of DON was reported for 45% of all samples. For more than 95% of the samples, all three forms were below the limit of detection (LOD) or quantification (LOQ).
To assess the co-occurrence of DON and its three modified forms, the ratios of the modified forms to the parent compound was calculated for all samples. A modified medium bound approach was conceived to take into account the high percentage of left censored data: If the modified form was reported to be below LOD or LOQ, the level of the modified form was assumed as 50% of LOQ (for results below LOQ) or 50% of LOD (for results below LOD). Furthermore, samples were only considered, if the level of DON in the same sample was quantified to be at least 10-20 times as high as the LOQ of the corresponding modified form. This approach was found to yield sufficient numbers for statistical analysis, while maintaining quality of data allowing to draw meaningful conclusions.
In summary, the study shows that the acetylated forms of DON are present at low to negligible levels relative to DON in most types of cereals and cereal products. Exceptions are 3-ac-DON in oats and 15-ac-DON in maize which were found at 5-10 fold higher median relative levels compared to all other types of cereals. The median ratio of DON-3-G to DON was the highest median ratio for all modified forms investigated. No conclusions regarding the distribution of DON-3-G in different types of cereals could be drawn.

Keywords: *modified mycotoxins, DON-3-glucoside, 3-acetyl-DON, 15-acetyl-DON, food monitoring*

M31

TOWARDS THE DETERMINATION OF DEOXYNIVALENOL IN WINTER AND DURUM WHEAT USING A HANDHELD NEAR INFRARED SPECTROMETER

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Mycotoxins are a diverse group of hazardous omnipresent fungal secondary metabolites. Among those toxins deoxynivalenol (DON) is found in 60% of analyzed grain samples (Eskola et al., 2020). In fact, it is the most prevalent mycotoxin in wheat (Khaneghah et al., 2018). The frequent occurrence of DON and the resulting health and economic impacts of this *Fusarium* toxin call for the development of rapid methods, ideally enabling on-site analysis. Current screening methods are mainly based on immunoanalytical approaches like ELISAs. These methods require laboratory equipment, significant amounts of disposables and skilled personnel. Thus, on-site implementation is often challenging or not possible. Infrared (IR) spectroscopy holds great potential as an alternative to those established screening methods (McMullin et al., 2015). This technique allows for the analysis of intact kernels, no disposables are needed, and operation of a routine spectrometer is straightforward. Moreover, several robust handheld devices are available on the market. With IR spectroscopy, fungi induced sample changes are measured and by using chemometric methods linked to the mycotoxin value obtained by external reference analysis. While IR measurements are straightforward, the implementation of IR spectroscopy as indirect screening method is not. This is linked to the need for a sufficient amount of naturally contaminated samples, the usage of chemometric methods, and the reliance on other analytical techniques to obtain the needed mycotoxin concentration. In this study, we have evaluated the usability of a commercially available handheld near IR (NIR) spectrometer for the screening of DON in durum and winter wheat. We used the obtained NIR spectra and the corresponding mycotoxin concentrations measured by LC-MS/MS for the classification of high and low contaminated samples at a threshold of 1250 µg kg^{-1}. Our preliminary results on exploring different machine learning approaches, strategies on counteracting class imbalances in naturally contaminated wheat samples, as well as the influence of milling the samples will be presented.

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Keywords: *mycotoxins, infrared spectroscopy, machine learning*

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M32

LC-MS/MS ANALYSIS OF PYRROLIZIDINE ALKALOIDS IN HERBS, HERBAL INFUSIONS AND TEA WITH A SIMPLE "DILUTE AND SHOOT" APPROACH

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Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by many plant species. Some PAs can cause liver damage, and genotoxic and carcinogenic potential has been shown in animal experiments. When PAs are found in products like herbs and spices, it is mainly due to contamination during harvest with plants that contain PAs. Some food crops, like borage, naturally contain PAs.
A new EU Regulation (EU) 2020/2040 came into effect on 1st July 2022. The regulation amends Regulation (EG) 1881/2006 and sets maximum levels (MLs) for PAs for the first time. In total, 35 PAs are named in the regulation. Depending on the matrix, the MLs vary between 75 and 1000 µg/kg (total PAs).
The analysis, especially the chromatography, is challenging due to the number of compounds and their similar structures, which leads to co-elution of isomeric compounds in many cases.
We present a highly sensitive LC-MS/MS method for PAs with good separation of isomeric compounds. A dilute and shoot approach was used for sample preparation, demonstrating that even with a simple extraction procedure, the method is highly sensitive for different matrices such as peppermint, black tea or fennel.
Details of sample preparation procedures and instrument set up are described. Matrix effects and recoveries are discussed, as are limits of detection/quantification, which proved to be below the MLs of the regulation.

Keywords: *pyrrolizidine alkaloids, new EU Regulation (EU) 2020/2040, LC-MS/MS method for PAs, dilute and shoot approach*

M33

ENHANCEMENT OF AGRI-FOOD BY-PRODUCTS: REDUCTION OF MYCOTOXIGENIC FUNGI GROWTH AND THEIR MYCOTOXIN PRODUCTION

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Food waste extracts represent a potential source of natural compounds with biological, biostatic and biocidal activity against fungi. Using extraction processes with high technological level and high sustainability, by-products of food production of fruit, wine and beer could be used as environmental friendly alternatives to protect plants from mycotoxigenic fungi and mycotoxin contamination, limiting the use of chemical products.
In this study, in *vitro* tests were carried out to verify the potential use of several extracts obtained from by-products of the agri-food chain in reducing the growth of the main mycotoxigenic fungi and the production of their relative mycotoxins: *Aspergillus flavus*-aflatoxin B1 (AFB_1), *Fusarium verticillioides*-fumonisin B_1 and B_2 (FBs), *Fusarium graminearum*-deoxynivalenol (DON) and zearalenone (ZEA), *Aspergillus carbonarius*-ochratoxin A (OTA), *Alternaria alternata*-alternariol (AOH), alternariol mono ether (AME), tenuazonic acid (TeA) and tentoxin (TEN).
Fungal strains were centrally transferred on Petri dishes containing Potato Dextrose Agar (PDA) and 1 mL of a solution of the different extracts at 1000 mg/l. After 14 days of incubation at 25°C, fungal growth was determined measuring fungal colony along two orthogonal lines, while the quantification of mycotoxins was performed using HPLC-FLD, LC-MS/MS and GC-MS systems. The reduction percentages for fungal growth and mycotoxin production were calculated comparing results obtained with those of the same fungi cultivated on PDA medium without the presence of the extracts. The results obtained were very interesting; fungal growth was affected by natural extracts with maximum percentage of reduction close to 48% in comparison with untreated thesis and depending on the fungus considered. More effective and promising results were obtained on mycotoxins, with reduction close to 70% for AFB_1 and DON and to 95% for AME and ZEA. A different efficacy was obtained by each natural extract in relation with the target fungus-mycotoxin considered.

Keywords: *mycotoxins, food waste, bioactive compounds*

M34

OPTIMIZATION OF DETERMINATION OF FREE AND TOTAL DEOXYNIVALENOL IN URINE

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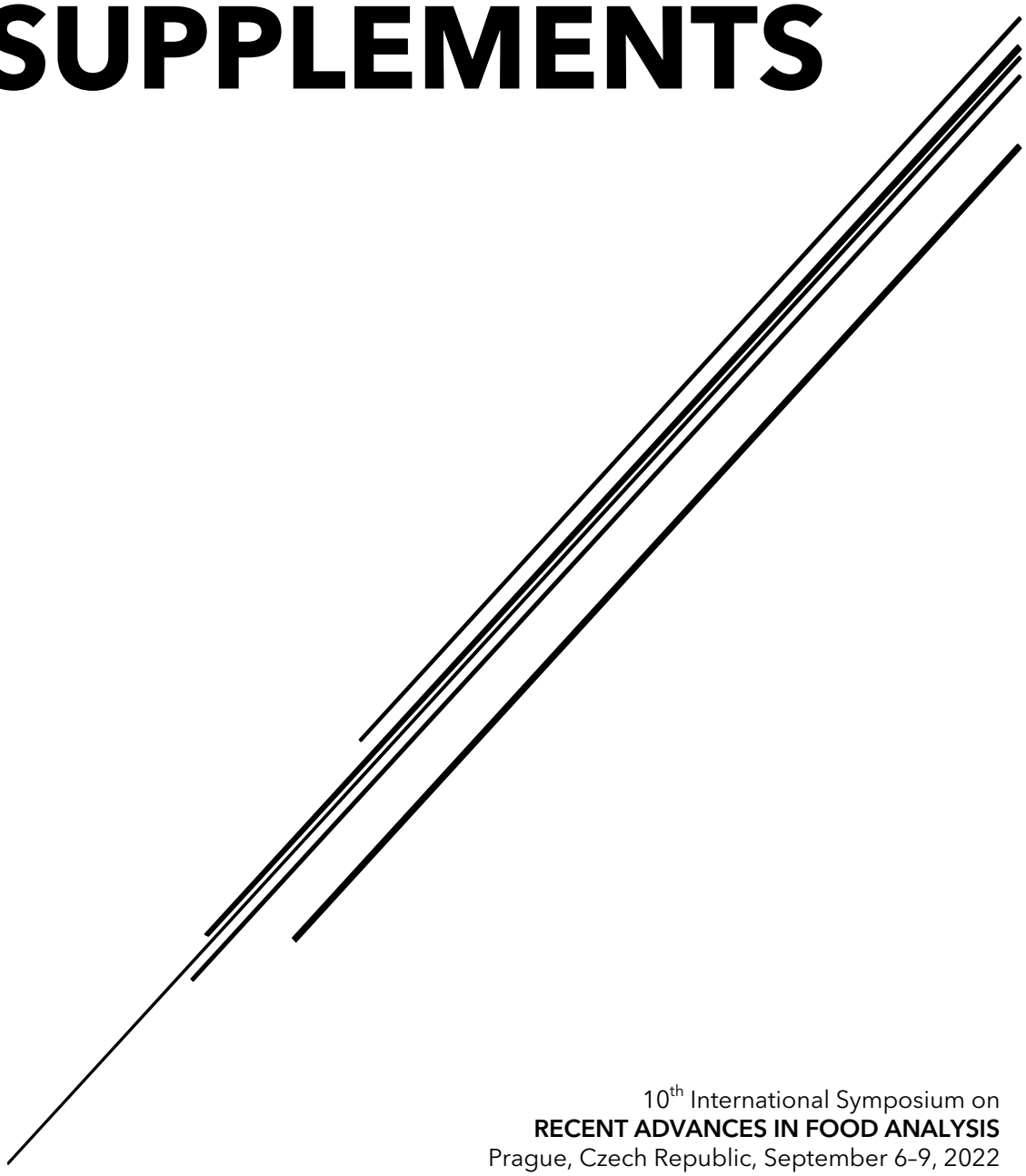
Deoxynivalenol (DON) and other structurally similar trichothecene mycotoxins are rapidly metabolized and excreted in humans. Despite this fact, DON shows strong emetic effects and from the view of chronic effects also pose a risk due to protein synthesis inhibition and cytotoxicity. Therefore it is regulated in EU and many other countries worldwide and European Food Safety Authority (EFSA) set a tolerable daily intake (TDI) for DON at 1 μg/kg body weight/day (including its major metabolites DON-3-glucoside, 3- and 15-acetyl-DON). DON is predominantly excreted in urine mainly after glucuronidation as DON-3-glucuronide (DON-3-GlcA) and DON-15-glucuronide (DON-15-GlcA) and to a lesser extent in a free form. Detection of other minor human urinary metabolites of DON such as deepoxy-DON, DON-7-glucuronide and DON-sulfate has been also described recently. As regards analytical determination of total DON in urine, e.g. free DON and DON associated in DON-glucuronides, enzymatic deconjugation using beta-glucuronidase is typically used in a combination with clean-up step employing immunoaffinity columns (IAC) and liquid chromatography coupled with mass spectrometric detection (LC−MS).
In this study, (i) excretion rate of free and glucuronidated DON was evaluated within a small scale study using artificial contamination of a beverage to the TDI level of a volunteer followed by (ii) optimization of enzymatic hydrolysis of DON-glucuronides (hydrolysis pH, temperature, time and number of enzyme units) contained in urine using beta-glucuronidase from *Helix pomatia* and subsequent (iii) instrumental analysis optimization (ionization and detection parameters) using ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (U-HPLC−HRMS/MS) employing Q-Exactive Plus. The aim of the study was to achieve low limit of quantification (LOQ) without the need utilize time consuming and costly IAC approach which, on the other hand, allows to obtain practically arbitrarily low LOQ depending on the amount of sample pushed through the IAC cartridge. Our approach resulted in acceptable LOQ of 1 μg/L.

Keywords: *human biomonitoring, urine, mycotoxins, deoxynivalenol, liquid chromatography – high resolution mass spectrometry*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

NOVEL FOODS & SUPPLEMENTS



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N1

THE INFLUENCE OF NON-GLUTEN PROTEIN ON CONVENTIONAL-BAKED AND OHMIC-BAKED BREAD PROPERTIES

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Foaming and emulsification properties of proteins are the most important functional properties for obtaining a high gluten-free (GF) bread quality, especially bread volume and crumb properties. Additionally, batter stabilization becomes a great challenge in GF bread, as CO_2 dissipates easily through the batter, resulting in small, dense, and crumbly breads. The extent of gas stabilization is closely related to the functional properties of protein. This study attempted to assess the effect of non-gluten proteins from different sources (plant and animal) on the rheological behavior of GF batter (pasting properties,and foam stability) and on bread quality after baking (conventional oven and ohmic heating). To achieve this purpose, the functional properties of selected non-gluten proteins relevant for foam stabilization were evaluated. Amongst these proteins, egg albumin and potato protein exhibited favorable functionality in GF bread; in particular, potato protein produced breads with the highest volume in both baking methods and could be a good candidate for replacing egg albumin. According to the correlation matrix, protein solubility was found to be crucial in influencing foaming and emulsification behavior, leading to improved GF bread properties.

Keywords: *gluten-free bread, non-gluten protein, ohmic baking, batter stabilization, protein*

Acknowledgement: *The authors acknowledge companies partners who provided materials, namely hydroxypropyl methylcellulose (HPMC; Metolose®) from HARKE Services GmbH (Muelheim an der Ruhr, Germany), potato protein from Avebe (Veendam, The Netherlands), soy isolate protein from Almi GmbH (Vienna, Austria), and lupin isolate protein from Prolupin GmbH (Grimmen, Germany).*

N2

QUALITY, SAFETY AND AUTHENTICATION OF INSECT-DERIVED PRODUCTS

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Insects constitute an alternative source of proteins in the current economic and environmental context of European livestock. Several insect rearing industries have appeared in the EU to produce insect meals. The FARMYNG project (FlAgship demonstration of industrial scale production of nutrient Resources from Mealworms to develop a bioeconomY New Generation, BBI-H2020, 2019-2024) takes the challenge to develop methods for quality and safety testing but also for the authentication of insect-derived products.
For quality testing, it is important to have tools for a rapid characterization of the insects or insect meals. The major value of the insect meals is their high protein content. This parameter needs to be estimated with caution because the exoskeleton of insects is made of chitin which contains nitrogen that might be counted in the estimation of the protein content. In the FARMYNG project, chemical and near infrared (NIR) methods for a fast characterization (proteins, humidity, fat, ashes and chitin) of insect-derived products are developed and compared to reference methods.
Concerning the method development for safety testing, efforts focused on a method allowing the detection of *Clostridium perfringens* in 1 g of product as this criteria falls under EU regulation. We took part to the inter-laboratories trials organized by the ISO to determine the most suitable media and characterize the performances of the method referred as ISO/CD 15213-3. We then verify the applicability of the method to insect meals. The ISO/CD 15213-3 method should be published as a technical specification (TS) in 2023.
When aiming at insect species identification, a traditional morphological examination is not adapted as insects are processed into meals. Genomic methods were then considered in the FARMYNG project. Among them, real-time polymerase chain reaction (PCR) has shown to be effective to detect insect species. Methods for *Tenebrio molitor, Hermetia illucens* and *Alphitobius diaperinus* were developed and published. Methods are now ready for *Acheta domesticus, Gryllus assimilis* and *Bombyx Mori*. The method validation is based on performance criteria recommended in international guidelines as specificity, sensitivity, applicability and robustness. We have also observed that some commercialized insect species were mislabeled e.g. as *Alphitobius laevigatus* instead of *Alphitobius diaperinus*. However, real-time PCR is limited to the targeted species and does not permit an untargeted approach allowing to identify a mix of different insect species or potentially contaminating insect species. For this purpose, we use metabarcoding approaches based on high-throughput sequencing (HTS). A big work is done to select the most appropriate barcodes and to improve the bioinformatic treatment of the information coming from classical Illumina platforms (providing very high quality sequencing reads), as well as from Oxford Nanopore technologies (allowing longer sequence reads).

Acknowledgement: *This research was financially supported by the European Commission in the frame of Horizon 2020 Public-Private Partnership Bio-Based Industries Joint Undertaking (topic BBI.2018.F2 – Large-scale production of proteins for food and feed applications from alternative, sustainable sources) through the FARMYNG project.*

N3

FATE OF POLYCHLOROBIPHENYLS IN THE INSECT TENEBRIO MOLITOR: CONSEQUENCES FOR FURTHER USE AS FOOD AND FEED

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There is a growing demand for animal protein while the resources used for animal feeding are limited or may raise environmental issues. Thus, there is a need to find alternative and sustainable protein sources. Edible insects may be an interesting solution with a good nutritional value. Learning form the BSE prion crisis, it is essential to ensure the safety of insect products and the chemical contamination is a crucial issue. Among priority contaminants, persistent organic pollutants (POPs) such as polychlorobiphenyls (PCBs), dioxins (PCDD/Fs) or polycyclic aromatic hydrocarbon (PAHs) are of serious concern in food supply. In this context, the aim of this study was to explore the ability of yellow mealworm (*Tenebrio molitor*), the most widely bred and traded insect species in Europe, to bioaccumulate PCBs from their feeding substrate during rearing.
Based on a miniaturized experimental farming, *Tenebrio molitor* larvae were fed during 20 days with wheat bran artificially contaminated with PCBs at a concentration of 0.67 ppb, 4 ppb or 24.4 ppb (n=9). Thanks to the set up of an extraction procedure including accelerated solvent extraction (ASE), centrifugal evaporation and gel permeation chromatography (GPC) coupled to an analysis by gaz chromatography hyphenated with a micro-Electron Capture Detector (GC-µECD), the larvae PCB content was then measured.
The bioaccumulation factors (BAF = concentration of PCBs in larvae / concentration of PCBs in wheat bran) obtained with dried larvae, the most common commercially available form, ranged between 1.1 and 2.9 and were significantly higher with the highest contamination level for PCBs 138, 153 and 180.
This study highlights the ability of *Tenebrio molitor* to bioaccumulate PCBs from their diet during rearing and the significant impact of the drying process on the bioaccumulation factors. This demonstrates the importance of considering the quality of the substrates used for farming insects as food and feed in terms of content in chemical contaminants including POPs like PCBs.

Keywords: *PCB, insect, yellow mealworm, bioaccumulation, GC-µECD*

Acknowledgement: *This study has been implemented in the R&D Booster program funded by the Auvergne Rhône Alpes Region (INSECT2FEED project).*

N4

CRITICAL ASSESSMENT OF JAMAICAN FIELD CRICKET METABOLOME AFTER RAPESEED MEAL ADDED TO FEED

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Constantly growing world population puts enormous pressure on food systems. One of the solutions how to supply the population with enough nutrients and especially proteins in the future is the production of edible insects. The main advantages of edible insects are high conversion of feed, a small environmental footprint, and small demands on space and water. These advantages make edible insects a possible challenging sustainable alternative to traditional animal protein sources and make it a possible future common ingredient of food and feed. This potential usage of insects makes this commodity a growing concept and draws the attention of researchers who mainly focus on the impact of rearing conditions such as temperature on biomass gain, feed conversion, and nutritional properties. However, the effects of feed composition on the quality of edible insects are still quite unexplored, especially in terms of bioactive compounds and micronutrients profiles. Complementing knowledge in this field is particularly desirable for the future production of quality and safe edible insects.
In this study, the effect of using by-product from rapeseed oil production (rapeseed meal) on the metabolome of Jamaican field cricket (*Gryllus assimilis*) was investigated. Rapeseed meal was used in this experiment as a substitution for protein component (soya) in feed. Five groups of crickets differing in feed composition were analyzed. Attention was paid especially to the transfer of bioactive compounds. For the evaluation of the changes in cricket biomass, the metabolomic analysis using ultra-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (U-HPLC-HRMS/MS) with subsequent use of chemometric tools (PCA, PLS-DA) was performed. Target screening of selected bioactive compounds such as sinapine or antinutritious glucosinolate goitrin was also performed.
It was possible to distinguish the groups of Jamaican field cricket according to the feed composition and possible markers of rapeseed meal and soya in feed were identified. Transfer of sinapine into the cricket tissue was observed by target screening. Although this compound has antioxidative and anti-inflammatory properties, its bitterness can negatively affect the taste of the final product. On the other hand, antinutritious glucosinolate goitrin was not confirmed in any of the tested samples. In addition, markers of soya (daidzein, genistein, saponins) were observed in cricket tissue. This study comprehensively describes changes in cricket metabolome after modifying the feed composition with rapeseed meal as a by-product of rapeseed oil production. The results can contribute to the optimization of edible insect breeding to produce safe and quality products and to contribute to the functioning of the circular economy and the processing of by-products of food production.

Keywords: *edible insects, novel food, metabolomics, mass spectrometry*

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N5

EXPLORING THE ECO-FRIENDLY PRODUCTION OF MICROALGAL-DERIVED BIOACTIVE PEPTIDES

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Food-derived bioactive peptides have shown strong potential for application in functional food formulations and nutraceutical products as health-promoting agents. Over recent years, microalgae have been investigated as new and sustainable sources of proteins as an alternative to the common animal-based proteins. More recently, microalgae are gaining importance as powerful sources of bioactive peptides with potential health benefits, including antioxidant properties. Furthermore, consumer interest in natural and sustainable food products is increasing globally. Therefore, the eco-friendly production of proteins and food-derived peptides from novel sources needs to be studied. In this work, the use of ultrasound-based extraction methods (UAE) was evaluated as green approaches to extract the proteins from the microalgae *Nannochloropsis gaditana*. Moreover, the enzymatic production of protein hydrolysates was investigated to produce bioactive peptides with potential antioxidant properties. In a first step, greener alternatives to the traditional method commonly used for the extraction of proteins (5 h, 60 °C, 5 % NaOH) were investigated. For that purpose, different extraction conditions and solvents were evaluated to reduce the time, temperature, and volume of alkaline solution used in the conventional approach. With the environmental-friendly method developed using ultrasounds and water (1h, 60 °C), a protein yield of 14.5 ± 1.0% was reached, comparable to the traditional method (17.2 ± 2.6%) ($p > 0.05$) but by reducing the extraction time (1 vs 5 h of the conventional method) and using a greener extraction solvent. In a second step, protein hydrolysis was performed with the extracted proteins using different enzymes (Papain, Flavourzyme®, and Alcalase®). The protein extracts after enzymatic treatment showed a degree of hydrolysis up to 52% and 69% using Alcalase® and Flavourzyme®, respectively, while the papain-hydrolyzation did not have any effect. Additionally, the bioactivity of the produced microalgal-derived peptides is currently under investigation by a spectrum of in vitro cell-based assays measuring potential endpoints of interest like cytotoxicity and antioxidant activities. In conclusion, this study provides eco-friendly alternative methods to extract proteins from microalgae and produce enzymatic protein hydrolysates with potential biological activities for novel food ingredients.

Keywords: *microalgae, green approaches, protein hydrolysates, antioxidant peptides, novel food ingredients*

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N6

DETERMINATION OF ELEMENTAL NUTRIENTS AND MICRONUTRIENTS IN FUNCTIONAL FOOD BY ICP-OES

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The functional food and beverage industry has experienced rapid growth over the last decade, furthered by consumer response to the COVID-19 pandemic (1,3). Some of these products are marketed to boost immune functionality that extends beyond basic nutritional value, claiming prevention and treatment of disease (2,4). These therapeutic claims are frequently related to the elemental content of the product.
ICP-OES is an ideal tool for verifying the concentration of elements listed on the labels of functional foods and beverages. This study investigated the efficacy, speed and simplicity of this technique for the determination of calcium, magnesium, potassium, sodium, phosphorous, iron, copper, manganese and zinc in foods.
This study utilized the US FDA Elemental Analysis Manual (EAM) method 4.4 for food and related products on ICP-OES. Three elderberry-based functional food samples (2) were analyzed using an Agilent 5900 ICP-OES.
The use of Agilent's IntelliQuant software feature streamlined method development and simplified wavelength selection by allowing the prescreening of samples for possible interferences. The IntelliQuant function was also able to recommend a suitable calibration range. The SVDV mode (Synchronous Vertical Dual View) of the Agilent 5900 ICP-OES was then used to analyze the samples. The advantages of this mode include removing the need to individually select radial or axial viewing modes depending on the sensitivity of each element. This capability delivered excellent detection limits for all elements in a single analytical run.
The accuracy of the method was confirmed by the analysis of a certified reference material and a spike recovery test for all 9 elements. All recoveries were excellent, detection limits were all within the nominal FDA method requirements and the instrument displayed exceptional stability over time. This study demonstrates the suitability of this instrument and method for confirming the elemental aspects of nutritional labelling.

Keywords: *ICP-OES, functional food*

Acknowledgement: *Agilent Technologies*

N7

DETERMINATION OF HEAVY METALS AND NUTRIENT ELEMENTS IN ALTERNATIVE PROTEINS USING ICP-MS

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Demand for alternative proteins, derived from plants, fungi and other biological sources, is booming and is expected to grow considerably due to consumer interest in health, sustainability, and food ethics (1, 2). The ICP-MS technique is widely used in the food industry for the elemental analysis of trace metals and contaminants in foods to ensure food safety and quality assurance. This study shows the analysis of the heavy metals; arsenic, cadmium, chromium, lead, mercury, and other elements in different protein-based food samples. Heavy metals were measured in the ppb levels and the nutrient elements at the ppm levels. All the metals were measured at the same time.
Agilent ICP-MS with ultra high matrix technology (UHMI) was used for this study due to the high matrices typical of these samples type. These advantages of this technology include lowering the risk of contamination introduced by sample dilution and not having to use matrix-matched calibration standards. Using the collision cell of the ICP-MS in helium mode, combined with double-charged ion correction remove polyatomic and double-charged ion interferences, simplified the analysis.
The study used 4.7 in the US FDA Elemental Analysis Manual (EAM) for food and related products. The accuracy of the method was evaluated by analyzing two food-based standard reference materials and conducting a spike recovery test for all elements in the four protein-based food samples. Excellent recoveries were achieved in all cases. The instrument also exceeded the nominal detection limit requirements specified in the EAM method and showed excellent stability over time. The study demonstrated that the instrument and method are suitable for the routine, multi-element screening of trace level elements in alternative protein foods, making them ideal for food safety programs.

Keywords: *ICP-MS, alternative protein, food safety*

Acknowledgement: *Agilent Technologies*

N8

CHANGES IN THE PROFILE OF HYPERCHOLESTEROLEMIC FATTY ACIDS IN COW COLOSTRUM DURING THE FIRST DAYS OF LACTATION

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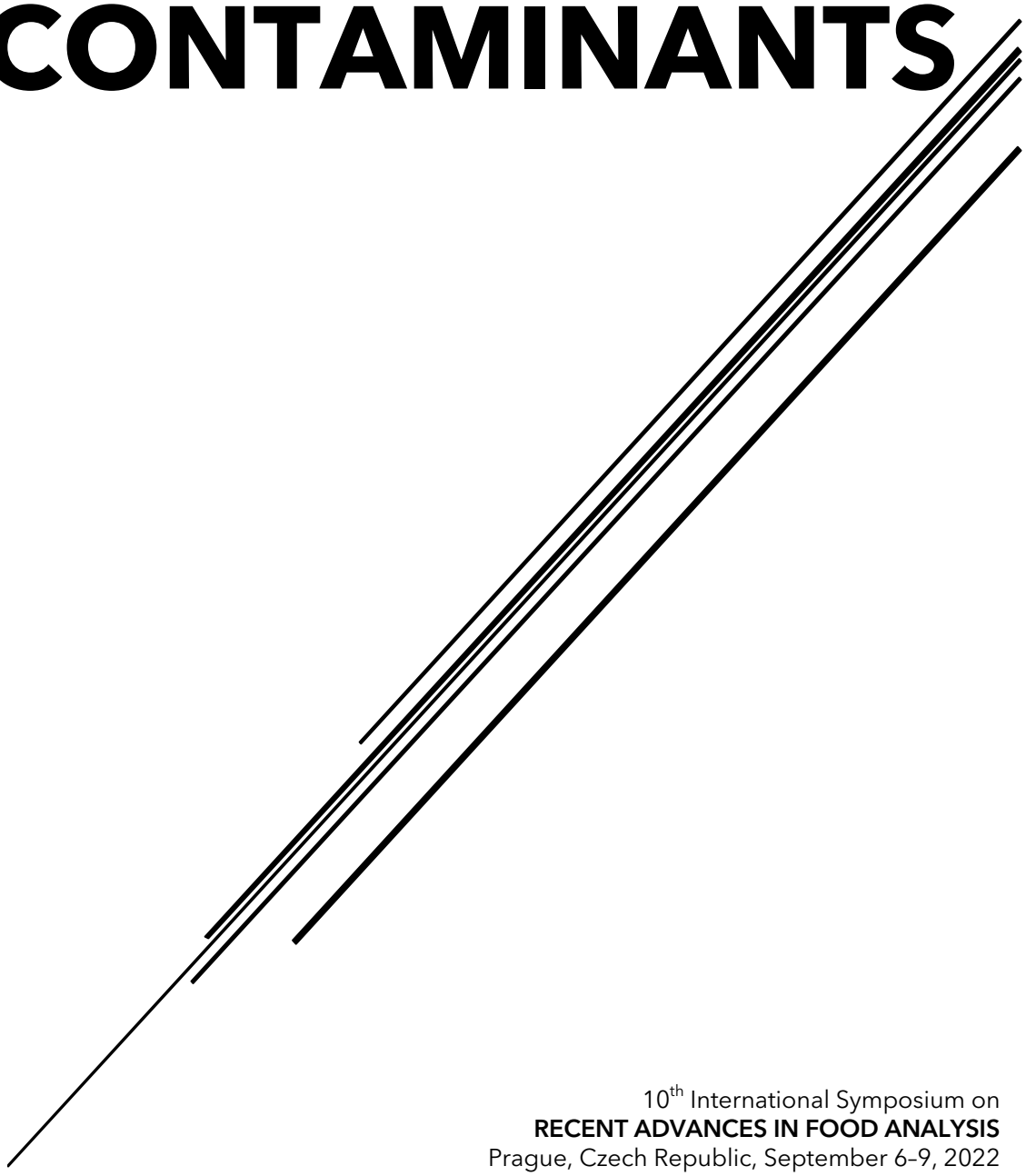
Bovine colostrum is a secretion of the mammary gland produced by cows within the first 72 h after parturition; later, the secretion is called immature or transitional milk. It is an important source of a highly concentrated complex of nutritional and biologically active compounds needed for the early nutrition of a newborn calf. For its unique composition, bovine colostrum and colostrum-based products are recently used in humans as dietary, nutraceutical, and medicinal supplements for the prevention and healing of cardiovascular and immunity-related diseases, allergies, neurological and skin disorders, and type-2 diabetes. Although the variation in the content of many nutritional and bioactive compounds has been widely studied, the information on fatty acids (FA) profile changes during the first days of lactation is lacking.
Yet, FAs in colostrum are considered important components. The most important FAs in colostrum include saturated FAs, mainly palmitic, myristic, and lauric acid. Studies showed that these three FAs could have a hypercholesterolemic effect, which can cause increased levels of LDL cholesterol and risk of cardiovascular diseases, hypertension, and arthritis. On the other hand, colostrum also contains hypocholesterolemic FAs that are represented by oleic acid and polyunsaturated FA. A ratio between hypo- and hypercholesterolemic FAs can be used for the evaluation of the health properties of bovine milk fat.
The aim of our work was to study changes in the levels of hyper- and hypocholesterolemic FAs in bovine colostrum and immature milk during the first four days of lactation to evaluate their possible impact on human health. The colostrum was obtained from eight Czech Fleckvieh cows kept on a private dairy farm (Klíčová, Božice, CZ). The samples were taken from morning milking during the first four days of lactation, and FA profiles were analyzed using comprehensive two-dimensional gas chromatography with a vacuum ultraviolet spectroscopy detector (GC×GC-VUV), which provided a better separation for such complex samples.
The sum of hypercholesterolemic FAs was the highest on day 1; it was 54.81% in milk and declined $p<0,05$ to 38.98% in milk found on day 4. A detailed look at the profiles of individual FAs revealed that palmitic acid had the highest concentration while lauric acid had the smallest representation out of the hypercholesterolemic FAs. The sum of hypocholesterolemic FAs showed the opposite trend, with the lowest value on day 1 (27.83%) and the highest on day 4 (37.51%). The hypo-/hypercholesterolemic ratio (h/H index) calculated for day 1 exhibited a low value of 0.51, gradually increasing to 0.96 on day 4. This demonstrates that consumption of colostrum from later phases is more advantageous for human health. Our results suggest that day of lactation has a great impact on the health properties of milk fat and should be considered in the production of colostrum-based products for nutraceutical and medicinal purposes.

Keywords: *bovine colostrum, fatty acids, hypercholesterolemic effect, GC×GC-VUV*

PROCESSING CONTAMINANTS



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O1

INVESTIGATION OF THE IMPACT OF RAW MATERIALS AND BAKING CONDITIONS ON ACRYLAMIDE CONTENT IN BISCUITS

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Acrylamide (AA) is a chemical process contaminant that is found at different levels in carbohydrate-rich foods, processed at high temperatures. Biscuits are one of the main sources that contribute to AA exposure, especially for children, and therefore it is important to evaluate the raw materials, the ingredients added in the manufacturing recipe and the baking conditions, from the point of view of this contaminant.
The aim of this study was to investigate the influence of the type of sweetener (sugar, fructose) and type of fat (butter, palm oil) used in the formulation and the baking conditions (200°C, 210°C/20, 30 min) on the AA content and color parameters of biscuits. The AA content was determined by GC-MS/MS, using purification of AA derivatives on a chromatographic column with activated florisil. LOD and LOQ of the method were 1.23 and 3.7 µg/kg, respectively. All parameter validation results fulfilled the criteria set by EC 2017/2158. Correlation between the AA content and CIE color parameters *L**, *a**, *b** were studied.
Results showed that for the biscuits with butter, the AA content was significantly smaller. The AA content increased at higher temperatures and longer durations of baking, the highest level being found in biscuits baked at 210°C/30 min, in the case of butter varying between 12.28–262.77µg/kg, while for palm oil it was between 52.37–843.79 µg/kg, the maximum content being determined when fructose was used. The AA content of biscuits was significantly correlated with the color parameters *L** (R^2= 0.92-0.99) and *a** (R^2= 0.87-0.99). The results suggests that the AA level of biscuits is influenced by the type of sweetener and fat used in the manufacturing recipe and the baking conditions, a significant lower content being determined when sugar and butter were added and when the baking process was realized at a lower temperature (200°C) and a shorter duration of time (20 min).

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O2

INFLUENCE OF FRYING CONDITIONS ON ACRYLAMIDE CONTENT IN FRENCH FRIES

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Acrylamide (AA) is a chemical process contaminant that is formed at temperatures above 120°C and low moisture conditions, being considered a genotoxic carcinogen. French fries are one of the most consumed potato products, being one of the main sources of acrylamide in foods due to the high content of asparagine and reducing saccharides, precursors in acrylamide formation, and the fact that are exposed to frying process.
In this study, potatoes of Queen Anne variety, 9 mm sliced, were deep fried in palm oil at different temperatures and time combinations (150, 170 and 190°C for 6, 8 and 11 min) and the acrylamide content and color parameters were determined. The AA content was determined by GC-MS/MS, using liquid-liquid extraction and purification of AA by solid phase extraction, followed by derivatization. LOD and LOQ of the method were 10.29 and 30.87 µg/kg, respectively. Correlations between the acrylamide content and CIE color parameters *L**, *a**, *b** were investigated.
Results showed that the AA content is significantly influenced by the frying conditions. As the frying temperature and frying time increased, the AA content of French fries increased as follows: for 150°C it increased from 118.47 µg/kg (6 min) to 232.05 µg/kg (11 min), for 170°C from 188.38 µg/kg (6 min) to 684.37 µg/kg (11 min), while for 190°C it increased from 192.36 µg/kg (6 min) to 1141.41 µg/kg (11 min). In the case of potatoes fried for 11 min at 170°C and 190°C, respectively, the AA content of French fries exceeded the benchmark level (500 µg/kg) established by Commission Regulation (EU) 2017/2158. By increasing the frying time from 6 to 8 min, an increase of AA content (% d.m.) with around 48%, 25% and 98% was obtained for 150, 170 and 190°C, respectively. When increasing the frying time from 6 to 11 min, the increase was around 58%, 175% and 361% for the same frying conditions investigated. The AA content of French fries was negatively correlated with the color parameters *L** (R^2= 0.67-0.99), and positively correlated with the color parameters *a** (R^2= 0.93-0.99) and *b** (R^2= 0.40-0.98). This study indicates that the AA content is directly influenced by the frying conditions, frying potatoes at a lower temperature for a shorter time, until a golden yellow color is obtained determining a lower AA content.

Acknowledgement: *This study was funded through the following projects funded by the Romanian Ministry of Research, Innovation and Digitalization: PN 19 02 03 01/2019-2022, Contract 17 PFE/2021 and METROFOOD-RO (Grant no. SMIS 2014 + 136213, Competitiveness Operational Program 2014-2020).*

O3

ETHYLENE OXIDE IN SESAME SEEDS: HIGH-THROUGHPUT SENSITIVE HEADSPACE ANALYSIS BY DIRECT MASS SPECTROMETRY

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The use of ethylene oxide as a fumigant for food products is banned in the EU, yet still practiced in some other countries. The current threshold for ethylene oxide and its degradation product chloroethanol is 50 µg/kg, and this may probably decrease further soon. The current industry standard of the QuEChERS method is a quite complex liquid extraction and derivatization protocol that includes 10 different steps and takes ca. 3.5 h per sample. Here, we present a novel, direct headspace approach using direct mass spectrometry without any sample prep step and without any chromatographic separation.
A Voice200ultra SIFT-MS (Selective Ion Flow Tube Mass Spectrometer, Syft GmbH, Germany) was coupled with an MPS Robotic headspace autosampler (Gerstel, Germany). The MS method was developed by identifying product ion peaks of ethylene oxide, chloroethanol and interfering acetaldehyde. Following this, the headspace method was optimized. The effect of grinding and cryogrinding sesame seeds, the necessary amount of sample per vial, incubation time and temperature were investigated. Finally, the method was validated according to the ICH (Q2) protocol.desinfectants
Results showed that ethylene oxide can easily be measured directly in the headspace from intact sesame seeds. Cryogrinding just increases the formation of chloroethanol but does not improve the precision of the method. Incubating 5 g sesame in a 30 mL headspace vial for 20 min at 40°C were ideal incubation conditions since unwanted roasting of sesame seeds starts beyond these conditions. Matrix interference from three different samples of sesame seeds were negligible except for acetaldehyde. Its interference was resolved by subtraction using one specific acetaldehyde signal. Even a 10-fold excess of acetaldehyde (500 µg/kg acetaldehyde and 50 µg/kg ethylene oxide on sesame) did not change ethylene oxide measurements significantly. Precision of six samples at 50 µg/kg was at ±20%, recovery rates were at 80% due to the degradation to chloroethanol. The response was linear from the limit of detection of 5 µg/kg to >5 mg/kg with $R^2 = 0.9996$. Changing the matrix of three different sesame samples did not significantly influence the data accuracy and precision. The calibration is stable at least over several weeks, so quality control samples can be minimized during routine analysis of ethylene oxide in sesame.
This illustrates how SIFT-MS can be used as an easy and fast ethylene oxide detection method. With only two manual steps (weighing sesame seeds into the headspace vial and writing a sequence to start the SIFT-MS measurement) up to 290 samples/day can be measured. This significantly speeds up analysis and dramatically decreases potential errors during sample preparation while maintaining the robustness and sensitivity of current state-of-the-art methods.

Keywords: *ethylene oxide, sesame seeds, high-throughput, chloroethanol, desinfectant*

O4

DETERMINATION OF PESTICIDES RESIDUES IN RAPESEED–DERIVED PRODUCTS BY QUECHERS - M-SPE CLEAN-UP FOLLOWED BY GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Rapeseeds and their derived products are known as difficult matrices making pesticide residue analyses a bit challenging for the official laboratories due to the high matrice interferences. A common approach for the determination of residues is QuEChERS acetonitrile extraction method with dispersive solid phase extraction (d-SPE) clean-up followed by instrumental analyses. In this work, the manual dispersive clean-up is replaced by automatic sample clean-up. A method using µ-Solid Phase Extraction (µ-SPE) clean-up method of QuEChERS citrate buffer extracts of rapeseeds and rapeseed cake and meal was validated. A customised workflow was designed and implemented on a stand-alone Thermo Scientific™ TriPlus™ RSH™ multi-purpose autosample. Compatible µ -SPE cartridges containing magnesium sulfate, primary-secondary amine, C_{18}, and graphitized carbon X is used for the automatic clean-up process. Cleaned extracts were diluted with acetonitrile and injected on a Gas Chromatography Tandem Mass Spectrometry. The validation was performed according to SANTE/11312/2021 guideline. Rapeseed, rapeseed cake and rapeseed meal blanks were spiked at three different concentration levels (5, 10 and 50 µg/kg) with 136 GC-amenable pesticides and metabolites. More than 100 compounds met the performance requirements at the lowest spike level, resulting in a LOQ of 5 µg/kg, (108 in rapeseeds, 113 in rapeseed meal and 121 in rapeseeds cake). Satisfactory overall recoveries (70−120 %) and relative standard deviations of ≤20% were achieved for 75 % of pesticides and/or metabolites in rapeseeds, 83.8 % in rapeseed meal 89 % in rapeseeds cake. Rapeseed cake EUPT-CF15 was analysed using the validated method. Pesticides residues measurements were also very accurate, further demonstrating that the automatic µ -SPE can be successfully implemented to save time and resources compared to current d-SPE method to cover the same analytical scope.

Keywords: *gas chromatography tandem mass spectrometry, GC-MS/MS, micro-SPE, µ-SPE, pesticides residue, rapeseeds*

Acknowledgement: *The authors thank Thomi Preiswerk from CTC Analytics for his technical support.*

O5

ANALYSIS OF FURAN AND ALKYLFURANS IN FOOD COMMODITIES USING HEADSPACE SPME ARROW AND GC-MS

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Furan and alkylfurans are produced during heating of foodstuff as a result of the thermal degradation of compounds such as carbohydrates, ascorbic acid, and derivatives, as well as some lipids. The International Agency for Research of Cancer classified furan as a possible carcinogenic compound, and there is a general concern on the possible health risks associated with the occurrence of furans and alkylfurans in food. Methods reported for the analysis of these volatile organic compounds include static headspace (HS) and solid phase microextraction (SPME) in combination with GC-MS. The use of SPME for the analysis of these highly volatile analytes has demonstrated improved method sensitivity and higher S/N for some of the alkylfurans. However, the fragility of traditional SPME fibers can be a concern. In this work, we developed a HS-SPME-GC-MS method for the analysis of furans and alkylfurans in baby formula and coffee using an SPME arrow. The SPME arrow geometry allows for a much better mechanical robustness of the extraction device and enhanced method sensitivity. Different experimental conditions such as coating chemistry, incubation temperature, and extraction time were evaluated. Two calibration ranges were covered, a low concentration range from 1.25 to 150 µg/kg, and a high concentration calibration range from 25 to 8000 µg/kg. For the analysis of highly concentrated samples, different conditions of split (1:100), extraction time (1 min) and sodium chloride solution (30%) (5 mL) were selected. The method was evaluated in matrices spiked at two concentration levels: 5 and 50 µg/kg for baby formula, and 1000 and 4000 µg/kg for coffee. Satisfactory results in terms of linearity, accuracy and precision were obtained in the majority of the cases. Accuracy values above 111% in coffee samples spiked at 4000 µg/kg could be due to sample handling, but additional experimental work may be needed to further understand this bias.

Keywords: *alkyl furans, furan, SPME Arrow, SPME*

O6

HIGHLY SENSITIVE ANALYSIS OF P-PHENETIDINE, IMPURITY OF ETHOXYQUIN, IN FISH MEAL AND ANIMAL FEED

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Ethoxyquin was approved as a feed additive in accordance with Directive 70/524/EEC and entered in the Register of Feed Additives in accordance with Article 10 (1) of Regulation (EC) No 1831/2003. Its use was suspended by the Regulation (EU) 2017/962 [1]. Usually, ethoxyquin products contain more than 91 % ethoxyquin, the contents of ethoxyquin polymers and p-phenetidine (4-ethoxy-aniline) are less than 8 % and 3 % respectively [2]. P-phenetidine is a possible mutagen. That is why in September 2021 EFSA Standing Committee on Plants, Animals, Food and Feed published its current report about p-phenetidine (section A.09. J) [3]) and accepted this decision: „p-phenetidine: based on the information provided by the EURL for feed additives, an LOQ of 125 ng/kg for determining p-phenetidine in fish meal and compound feed is achievable and could be set as ML in Directive 2002/32/EC to enforce the suspension of use of ethoxyquin in feed" [3]. On the other hand it is also a pesticide and regulated by the Regulation (EU) 396/2005, but there is no specific MRL for ethoxyquin in animal feed. The typical LOQ for ethoxyquin in food and feed is 0.01 mg/kg. Considering the possibility of a max. 3% content of p-phenetidine in ethoxyquin a LOQ significantly lower than 1 µg/kg is needed to screen animal feed products and to avoid serious health consequences for animals and humans. Complex or complete animal feed samples are a very difficult kind of matrix to analyze for traces, because it usually contains all typical macromolecules like fat and lipids, carbohydrates, proteins and also many kinds of micronutritions e. g. vitamins. It is also usually dried. We modified the QuEChERS extraction approach [4] for the analysis of p-phenetidine with an LOD of 0.1 µg/kg in fish meal and animal feed by changing the ratio of water/acetonitrile. The analysis is done with LC-MSMS after a stepwise clean up from all matrix components with different SPE materials.

[1] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0962&from=HR
[2] https://www.efsa.europa.eu/en/press/news/151118
[3] https://ec.europa.eu/food/system/files/2021-12/reg-com_ani-nutrit_20210920_sum.pdf
[4] Official collection §64 LFBG: determination of pesticide residues in fruit and vegetables using GC-MS and/or LC-MS/MS after acetonitrile-extraction/distribution and cleaning with dispersive SPE (QuEChERS) (acc. to DIN EN 15662); L 00.00-115

O7

DETERMINATION OF 22 BISPHENOL A SUBSTITUTES IN FOOD, COSMETICS AND CONSUMER PRODUCTS - A NEW APPROACH WITH GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (GC-MS/MS)

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Bisphenol A (BPA), the best known bisphenol, is used as a monomer in the production of polycarbonates and epoxy resins, but is also added to various polymers as a plasticizer, and its production levels continue to increase [1]. Its effect on humans is well studied: it acts as an endocrine disruptor by mimicking or blocking receptors, resulting in a change in hormone balance [2]. This endocrine effect and ubiquitous presence led to a ban on baby products and a listing as a substance of very high concern (SVHC) in 2011 [3]. This has caused the growing use of BPA substitutes with structural analogues that are already increasingly found in human matrices [4]. Some BPA structural analogues, such as bisphenol S (BPS) and bisphenol F (BPF), have already been shown to have effects comparable to those of bisphenol A [5]. Thus, there is an urgent need for further research and monitoring of potential sources of contamination for bisphenols. Many studies focus on only a few BPA structural analogues. We developed a measurement method which covers a broad range of 22 bisphenols. To identify the major routes of contamination, we established sample preparation methods for different types of foods (fats, oils, dry and moist products) and also for personal care and consumer products. The sample preparations are based on simple extraction techniques combined with minimal purification steps to enable fast and simple analytical procedures. Gas chromatography-tandem mass spectrometry (GC-MS/MS) is used to measure the silylated compounds.

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4. Zhang, B. *et al.* Concentrations of bisphenol A and its alternatives in paired maternal–fetal urine, serum and amniotic fluid from an e-waste dismantling area in China. *Environ. Int.* 136, 105407 (2020).
5. Molina-Molina, J. M. *et al.* In vitro study on the agonistic and antagonistic activities of bisphenol-S and other bisphenol-A congeners and derivatives via nuclear receptors. *Toxicol. Appl. Pharmacol.* 272, 127–136 (2013).

Keywords: *bisphenol A substitutes, GC-MS/MS*

O8

FREE AND BOUND MCPD AND GLYCIDYL ESTERS IN PLANT-BASED FOODS

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Free 3-MCPD is a well-known food processing contaminant that often presents in soy sauce or acid-hydrolyzed vegetable protein. Its fatty acid esters (MCPD esters or bound MCPD) together with glycidyl fatty acid esters (GEs) are also food processing contaminants predominantly found in refined vegetable oils and products thereof. These compounds are of health concern due to their carcinogenic properties. Currently, the European Union has established the maximum limits for the sum of free and bound 3-MCPD, as well as GEs in vegetable oils, infant formula, or baby foods. While bound MCPD and GEs are usually analyzed together due to their similarity in physio-chemical properties, there is no official method to also determine free 3-MCPD together with the other two compounds in food products .
In this study, a method for determination of both free and bound 3-MCPD and GEs in composite foods by GC-MS/MS was developed. Vegetable oils and infant formula have been used as matrices for method validation which showed excellent linearity, precision, and accuracy, reproducibility, and repeatability. The validated method was then successfully applied to analyze various plant-based foods such as seaweed, vegan sausage, or vegan burger. For GEs, the concentrations ranged from ND to 50 µg/kg. In case of bound 3-MCPD, this range was 0.1-212 µg/kg. Free 3-MCPD was also detected in many samples, albeit at low levels of less than 30 µg/kg. Interestingly, rather high level of free 3-MCPD was found in a seaweed sample, up to 200 µg/kg.

Keywords: *free 3-MCPD, bound 3-MCPD, glycidyl esters, analytical method, plant-based foods*

O9

RESULTS FROM A COLLABORATIVE STUDY ON 4 POLYCYCLIC AROMATIC HYDROCARBONS IN PLANT-BASED FOOD SUPPLEMENTS

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Determination of carcinogenic polycyclic aromatic hydrocarbons (PAHs) in food supplements is challenged by the presence of other e.g. heterogeneous PAH-like compounds in the matrix. A collaborative study with 12 participants was conducted in order to assess performance characteristics of a fast method intended to analyze the four regulated PAHs (PAH 4) benzo[*b*]fluoranthene [BbF], benz[*a*]anthracene [BaA], chrysene [CHR] and benzo[*a*]pyrene [BaP] in five different plant-based food supplements in form of capsules, powder, and tablets. The principle of the method includes the extraction of PAHs with ethylacetate:cyclohexane followed by a two-step SPE clean up and final analysis by GC-MS or LC-FLD. The method was validated for the regulated PAH 4 in the analytically challenging concentration range from 2.5 µg/kg to 6.9 µg/kg. The performance criteria for the method set in European Regulation No 333/2007 for the overall repeatability, reproducibility (HorRat values below 2) and recovery (range 50-120%) were fulfilled. Based on the statistical evaluation of the results it was concluded that the method is a suitable alternative to existing methods and should be studied for additional matrices.

Keywords: *EURL-PC ring trial, PAH 4, food supplements, GC-MS, LC-FLD*

Acknowledgement: *We would like to thank the EU Commission for financial support during parts of the work in the European Reference Laboratory for Processing Contaminants. All participating laboratories are thanked for their voluntary participation in the collaborative trial and for their comments to the method.*

O10

A STUDY ON THE IMPACT OF THE HARVESTING OPERATIONS ON MINERAL OIL CONTAMINATION OF EXTRA VIRGIN OLIVE OILS

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Mineral oils (MOH) are complex mixtures of thousands of hydrocarbons of petrogenic origin, being some of the most spread contaminants in food matrices. They comprise saturated (MOSH) and aromatic (MOAH) hydrocarbons. From a toxicological point of view, their impact on human health is raising concern especially in relation to the MOAH, due to the proven carcinogenic and genotoxic action of some alkylated compounds with more than 3 benzene rings. Further information in this regard will be revealed soon, as an updated evaluation by EFSA is expected by the end of the year. Very recently a limit of 2 mg/kg for total MOAH in vegetable oils, has been indicated by the EC (https://ec.europa.eu/food/system/files/2022-05/reg-com_toxic_20220421_sum.pdf).
Liquid chromatography (LC) coupled with gas chromatography (GC) equipped with flame ionization detector (FID) is the analytical reference method. GC coupled to mass spectrometry (MS) may help in confirming mineral oils contamination by verifying the presence of specific markers. Comprehensive two-dimensional gas chromatography (GC×GC) with parallel MS/FID detection allows to improve separation and provides a better characterization of the contamination.
The presence of MOH in vegetable oils, including olive oil, is the result of their lipophilic character. A systematic study along the olive oil supply chain to precisely identify the contamination sources revealed that the harvesting operation may be the most critical step. In olive oils extracted from samples of olives hand-picked directly from the tree, levels of MOSH were found to range from
In conclusion, what differentiated the samples with a significant increase in contamination, compared to the others, appeared to be the degree of mechanization of the harvesting procedures. Finally, for confirmation and further characterization, the olive oil samples and related lubricants were subjected to GC×GC-FID/MS analysis. The FID signal allowed to confirm, by a fingerprinting approach and through the quantification, the origin of the contamination, as already verified with the LC-GC-FID. From a qualitative point of view, the complexity of the matrices did not allow for a detailed characterization by MS, even if some interesting information regarding the qualitative composition of the samples could be obtained.

Keywords: *olive oil, mineral oil contamination, harvesting operations, HPLC-GC-FID, GCxGC-FID/MS*

O11

SIMULTANEOUS DETERMINATION OF 5-HYDROXYMETHYLFURAL, FURFURAL, 4-HYDROXY-2,5-DIMETHYL-3(2H)-FURANONE AND 5-METHYLFURFURAL BY DILUTE-AND-SHOT AND HPLC-DAD IN BRAZILIAN COMMERCIAL CAKES

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5-hydroxymethylfural (HMF), furfural, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) and 5-methylfurfural (5MF) are polar chemical compounds reported to be potentially toxic to human health. They can develop in the industrialization of cakes during heating at about 180°, or from the ingredients and additives used. In Brazil, this bakery product is widely consumed by children and adolescents. The aim of this work was to validate a method for the quantification of four furan derivatives in industrialized and commercialized cakes in Brazil. 120 samples were evaluated. Sample preparation was based on the dilute-in-shoot strategy, using 10mL of the solvent methanol:water (70:30 v/v) in 1g of sample. High performance liquid chromatography coupled to a diode array detector was used for quantification. In a 15 minutes method, the analytes were analyzed at a wavelength of 284 nm. The validation parameters evaluated were limits of detection (LOD), limit of quantification (LOQ), recovery, inter-day and intra-day precision, linearity and matrix effect follow the provisions of SANCO, 2020. Linearity was evaluated on a curve of seven concentrations, starting at the LOQ, midpoint (5ppm) and end point (12.5ppm). Recovery and inter- and intra-day precision were analyzed at the LOQ, midpoint and end point of the concentration curve. The method was validated in a matrix free of analytes and obtained LOD of 0.03ppm, LOQ of 0.15ppm for the four furan derivatives. The recovery in LOQ was 113% for HMF, 86.44% for furfural, 80% for HDMF and 87% for 5MF. At 5ppm, recoveries were 98%, 101%, 97.7% and 110.3% for HMF, Furfural, HDMF and 5MF, respectively. At the end point of the curve the recoveries were 107% for HMF, 104% for furfural, 102% for HDMF and 106% for 5MF. The linearity analysis indicated R^2 greater than 0.99 for the four analytes. The analyzed samples have different compositions, different flavors and this influenced the quantification results obtained. Ingestion of HMF (possible genotoxic and mutagenic) and HDMF (nervous system disorder) is a concern, especially when consumers are children, who have a weaker immune system when compared to a healthy adult.

Keywords: *analytical methods, infant food, food analysis, furan*

Acknowledgement: *This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq [156398/2019-2].*

O12

HIGHLY SENSITIVE ANALYSIS OF FURAN, ALKYLFURANS AND BENZENE IN BABY FOOD USING HS-GC/MS WITH ADDITIONAL SAMPLE PRECONCENTRATION IN THE COLD INJECTION SYSTEM

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Furan and alkylfurans are formed from reducing sugars, amino acids, ascorbic acid, unsaturated fatty acids and carotenoids during heating processes (sterilization, baking, etc.). This is relevant in the preparation of roasted coffee, canned and jarred foods, baked goods and jarred baby foods. Benzene can be formed in foods by thermal decomposition of benzoic acid, e.g. in the production of carrot juice or carrot-based baby food.
Animal studies with furan have shown that this compound can cause liver damage and liver cancer. Based on consumption data, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EFSA conclude that the level of exposure to furan and alkylfurans in food raises health concerns [1]. Benzene is classified by IARC as a group 1 carcinogen, which means it is considered a definite cause of cancer in humans.
The method developed at the Federal Office of Consumer Protection and Food Safety allows the analysis of all analytes included in the recent EU Monitoring Recommendation 2022/495 (furan, 2- and 3-methylfuran, 2-ethylfuran, 2,5-dimethylfuran and 2-pentylfuran). In addition, 2,3-dimethylfuran, 2-butylfuran and benzene can be detected in parallel.
Up to now, furan and alkylfurans have been analyzed by static or dynamic Headspace (HS)-GC/MS and SPME-GC/MS. Due to the limited injection volume, 2-pentylfuran in baby food cannot be detected with sufficient sensitivity by static HS-GC/MS. In the newly developed method, additional sample preconcentration in the cold injection system using a carbon-based absorbent increased the sensitivity by 50-fold allowing a LOQ of 3 µg/kg or lower.
Using an optimized GC method, all analytes can be baseline separated on a DB624 column and selectively and specifically detected by MS.
Since the method is to be used for the characterization of proficiency test or reference materials, it was optimized especially with regard to precision. The use of silanised HS syringes and HS vials resulted in a significant reduction of adsorption effects. The addition of grinding balls before incubation of the samples enhances equilibration between sample, extraction solvent and gas phase and thus improves method precision.
In summary, the validation results for fruit-based baby food show that the requirements for method performance set out in EU Monitoring Recommendation 2022/495 are fully met for furan and the five alkylfurans listed in the recommendation. In addition, method performance for 2,3-dimethylfuran, 2-butylfuran and benzene also complies with these criteria.

[1] EFSA Panel on Contaminants in the Food Chain (CONTAM), *EFSA Journal*, 2017, 15(10), 5005

Keywords: *furan, benzene, alkylfurans, GC-MS, headspace*

O13

A STUDY ON THE TRANSFER OF MINERAL OIL CONTAMINANTS FROM THE DIETS TO THE PIGS

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Mineral oil hydrocarbons (MOH) are a complex mixture of liposoluble compounds of petrogenic origin, which can be divided into saturated (MOSH) and aromatic (MOAH) hydrocarbons. Both types of MOH can have a negative impact on human health, given the bioaccumulation of MOSH in tissues and organs and being especially relevant the carcinogenic, mutagenic and genotoxic behaviour associated to some MOAH. Due to this reason, concern about human exposure to MOH through the diet has increased and their transfer inside the food chain should be studied for a proper understanding of their behaviour. Thus, the purpose of this work was to evaluate the MOSH and MOAH content in pig diets formulated with different added fats and their possible transfer to the animals.

Female pigs were fed for 60 days with two different basal diets depending on the experimental period (growing or finishing). These basal diets were common for all the pigs, but a 5% (w/w) of different lipid sources were added: crude palm oil (CPO); crude olive pomace oil (COPO); olive pomace acid oil (OPAO) and blend of CPO and OPAO (1:1, w/w), resulting in 4 different dietary treatments. After slaughter, back fat samples from 9 different pigs were taken and samples from the same dietary treatment were pooled and divided to obtain two different replicates, which were separately homogenized. MOSH and MOAH content was determined by liquid chromatography (LC) coupled with gas chromatography (GC) with a flame ionization detector (FID). To analyse the diets, 5 g of homogenized sample were subjected to microwave-assisted saponification (MAS) and following epoxidation before the instrumental determination. For the back fat, 1 g of homogenized sample was subjected to MAS and injected.

Higher concentrations of MOSH (37.9 mg/kg and 34.1 mg/kg) were obtained for diets with CPO, which were due to a greater contribution of MOSH with C_{25} – C_{50} distribution, while the levels in the other diets were in the range of 20.3 - 29.3 mg/kg. MOAH levels observed for the diets were between 1.0 and 1.9 mg/kg. MOSH and MOAH profiles resulted to be similar for both diets with the same added fat. MOSH found in the back fat showed a different profile than in the diets, i.e. distributed over a narrower molecular weight range, but common among all the back fat samples, suggesting the accumulation of hydrocarbons of specific molecular weights as the result of animal metabolism. Similar to the diets, higher levels of MOSH were found in CPO back fat (29.8 mg/kg) than in the other samples (10.0 – 14.0 mg/kg). MOAH were not present in back fat probably because they are not bioaccumulated. The presence of MOH in the diets can be due to the added fat, other ingredients (as cereals) or the manufacturing process. Thus, special attention should be paid to these contamination sources, as they can affect the levels of bioaccumulation of MOSH in the animals, leading to a different extent of human exposure.

Keywords: *mineral oil hydrocarbons, pig diets, pork back fat, bioaccumulation, HPLC-GC-FID*

Acknowledgement: *This study has been supported by the Spanish Ministry of Universities through the pre doctoral contract within the FPU program (FPU18/01010).*

O14

DEVELOPMENT OF A SIMPLE METHOD FOR DETERMINATION OF ACRYLAMIDE IN BABY FOOD MARKETED IN BRAZIL BY LC-MS/MS

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The International Agency for Research on Cancer (IARC) classified acrylamide in the category of substances "probably carcinogenic to humans", so the presence of acrylamide in food is a risk to human health. Due to this, several analytical methods have been proposed for food control, although many steps for extraction and cleanup are needed. Therefore, in this study, a simple method for the determination of acrylamide in baby foods marketed in Brazil based on LC-QqQ-MS/MS was developed and validated. In relation to sample preparation, our main aim was the application of a simple extraction method. For this purpose, a traditional solid-liquid extraction (SLE) was employed to extract acrylamide using a mixture of acetonitrile:water: formic acid (69/30/1, v/v/v) as extraction solvent. Moreover, a cleanup step has been added due to matrix interferences. Thus, the supernatant was submitted to clean up with 50 mg of alumina. Suitable performance criteria were achieved, with a LOQ of 20 μg kg^{-1} for baby foods. With the aim of checking the applicability of the developed method, 50 commercial baby food samples available in Brazilian markets were analyzed. For fruit-based baby food, in 13% of the analyzed samples, acrylamide was detected at levels between < LOQ (20 μg kg^{-1}) and 37 μg kg^{-1} (Apple and plum). In samples of baby food composed of meat and/ or vegetables, higher levels of acrylamide than for fruit-based baby foods were observed. For instance, baby food composed of Stroganoff and rice provided higher acrylamide content (90 μg kg^{-1}) followed by chicken risotto (56 μg kg^{-1}). Furthermore, the results found allowed us to establish that the acrylamide level in baby food depends on the different ingredients used during the processing. Thus, this study can contribute to lower consumption of foods with high levels of acrylamide. This can be done through the selection of infant foods whose ingredients provide less acrylamide formation

Keywords: *processing contaminant, heat-induced compound, LC-MS/MS method, food analysis, baby food*

Acknowledgement: *Authors are grateful to São Paulo Research Foundation (FAPESP) for financial support (Process n° 2017/11635-8) and for the scholarship awarded to RP (process numbers 2019/04727-9 and 2020/01974-2). Authors gratefully acknowledge to the Spanish Ministry of Science and Innovation, Spain, and FEDER-EU (project ref. PID2019-106201RB-I00) for financial support. RLR also acknowledges "Plan Propio of Investigation" of University of Almería, cofunded by CAJAMAR and the Operational Program Funds Europeans of Regional Development of Andalusia (2014–2020) (FEDER), for financial support.*

O15

DISINFECTION WITH SIDE EFFECTS – LEVELS OF CHLORATE AND BROMATE IN DRINKING WATER FROM SOUTH WESTERN GERMANY

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In order to achieve a sound microbiological quality of drinking water, it is often necessary to disinfect the raw water accordingly. In addition to disinfection by UV light, this is often accomplished by applying ozone or chlorine-containing disinfectants such as chlorine gas, chlorine dioxide or hypochlorite solutions (chlorine bleaching lye). Unfortunately, these highly reactive substances also cause undesirable by-products, such as bromate, which is classified as "possibly carcinogenic" and causes kidney and thyroid tumours in laboratory animals when ingested over long periods. [1,2] Another disinfection by-product of health concern is chlorate, which can inhibit iodine uptake and thus negatively affect thyroid function. [3]
A maximum level of 10 µg/L has been set for bromate in the German Drinking Water Ordinance. However, for toxicological reasons, this limit should be much lower. [4] The analysis of 820 drinking water samples from the German federal state Baden-Wuerttemberg in the years 2016 to 2021 showed that the maximum level of 10 µg/L is only exceeded in exceptional cases and that a lower maximum level could be easily complied with. In 99.3% of all samples, the measured bromate amounts were below a level of 4 µg/L. [5]
Regarding chlorate in drinking water, maximum levels in Germany have only been listed in the so-called "§11 list" so far. The "§11 list" specifies requirements for treatment and disinfection processes for drinking water. With regard to chlorate, for example, a maximum level of 70 µg/L is permitted for a permanent dosage of chlorine-containing disinfectants. These maximum levels are also set rather high from a toxicological point of view. [6] Our investigations of 808 drinking water samples from Baden-Wuerttemberg show that most samples were not at all or only marginally contaminated with chlorate. In 91% of the samples, the measured chlorate amounts were below a level of 30 µg/L. However, there were also rare occasions, e.g. in the case of improper chlorination of drinking water from one's own well, in which the permissible chlorate content was considerably exceeded and the drinking water was assessed to be "likely to cause harm to human health." [5]

[1] WHO IARC (1991) Volume 52; Lyon, France
[2] WHO IARC (1999) Volume 73; Lyon, France
[3] EFSA (2015) Scientific Opinion, EFSA Panel on Contaminants in the Food Chain (CONTAM), Parma, Italy, EFSA Journal;13(6):4135-4237
[4] Kämpfe A, El-Athman F, Mahringer D, Röhl C, Chorus I, https://www.trinkwasser-aktuelldigital.de/twa_kz1101.4.
[5] C. Breitling-Utzmann et al., Deutsche Lebensmittelrundschau 2022, in preparation.
[6] Stellungnahme (opinion) Nr. 007/2018, German Federal Institute for Risk Assessment, 15.02.2018

Keywords: *drinking water, chlorate, bromate*

O16

CHANGES OF ORGANOCHLORINE COMPOUNDS DURING SIMULATED VEGETABLE OIL REFINING

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Vegetable oils and fats are an important component of human diet, with varying risk benefit ratios. Especially nowadays, a large percentage of edible oils and fats is subjected to industrial processing. However, many scientific studies have proven, that during food processing, especially oil refining in this case, process-induced contaminants may occur in the final product. The most important processing contaminants of edible fats and oils include (not only) ester-bound chlorinated contaminants. The largest share of the content of chlorinated contaminants in refined oils constitutes of esters of chloropropanediols (MCPD), to a lesser extent, esters of dichloropropanols, and other chlorinated compounds, which are currently very little researched and monitored.
During our work with above mentioned compounds, a hypothesis was started regarding the possible chlorination of double bonds in unsaturated fatty acids bound in acylglycerols to form (bound) chlorinated fatty acids (CFA). Based on available literature, it is considered very likely that under the conditions of fat / oil refining, chemical changes may occur, supposing suitable chlorine donor is present, resulting not only in the formation of known chlorine-containing process contaminants such as MCPD esters, but also other potentially toxic and as yet unmonitored compounds such as CFA.
The aim of this study was, therefore, to document and elucidate changes in the composition of refined vegetable oils when prepared from raw crude oil containing chlorinated paraffins (CP), potential contaminants of this commodity. Laboratory-scale model systems simulating deodoration step were prepared. A high throughput method, utilizing supercritical fluid chromatography coupled to tandem high-resolution mass spectrometry (SFC-HRMS/MS), was developed and optimized for target screening of CFA in vegetable oils and fats. The best chromatographic performance was obtained with 1-aminoanthracene (1-AA) column with methanol as a co-solvent. A nontarget analysis approach, employing ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (U-HPLC-HRMS/MS) and SFC-HRMS/MS methods, was used to tentatively identify markers of CP degradation and lipid species chlorination. Following our results, further efforts will be directed to researching the mechanism of CFA formation in simplified systems of individual acylgycerols.

Keywords: *vegetable oils and fats, processing contaminants, organochlorine compounds, U-HPLC-HRMS/MS, SFC-HRMS/MS*

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O17

HS-SPME-GC-NCD METHOD FOR THE IDENTIFICATION AND DETERMINATION OF VOLATILE N-NITROSAMINES IN MEAT PRODUCTS

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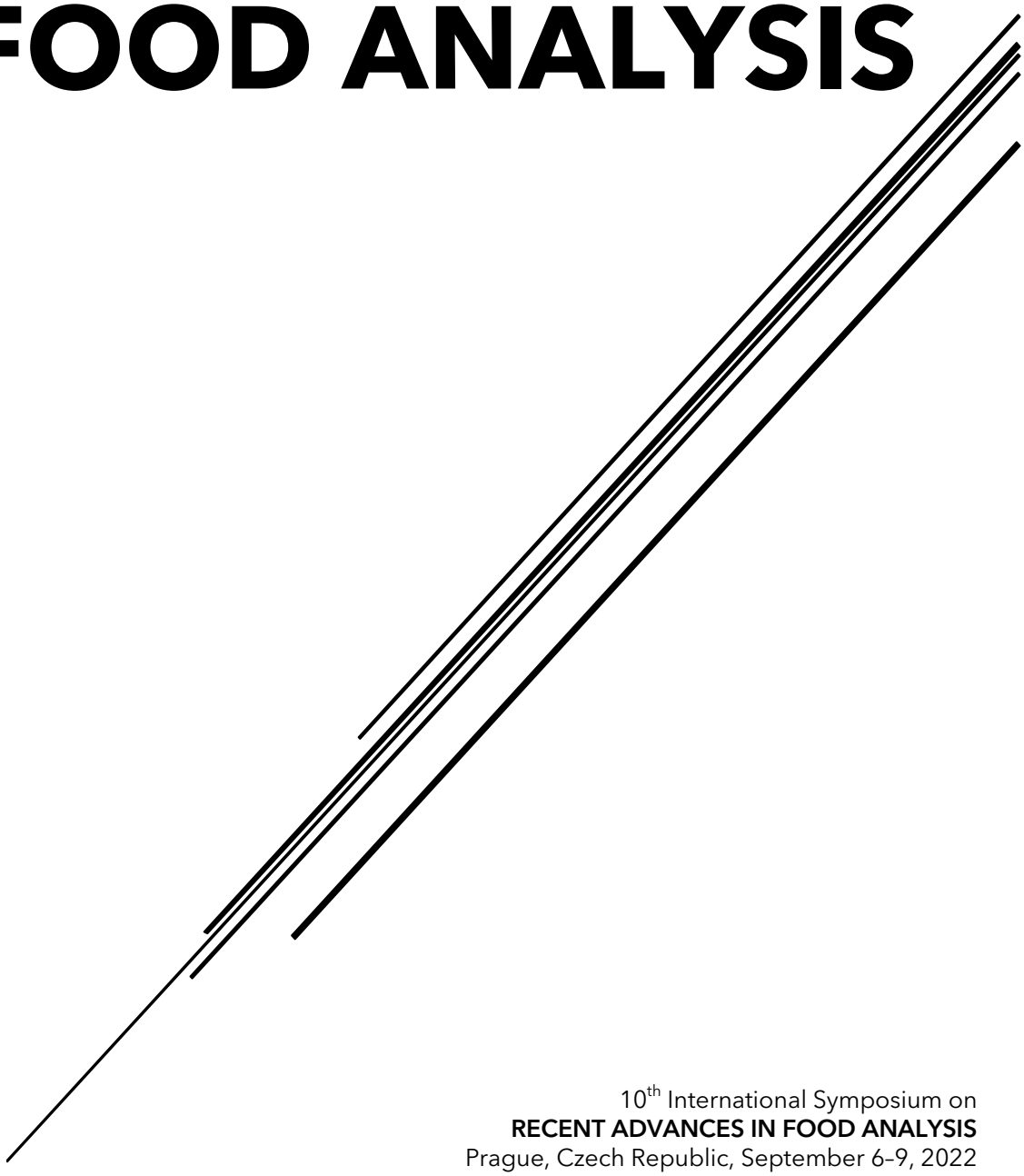
An easy-to-use and environment-friendly method for the identification and determination of five volatile nitrosamines (N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosodipropylamine, N-nitrosodibutylamine and N-nitrosopiperidine) in meat products was developed and validated using headspace sampling by solid phase microextraction and gas chromatography combined with nitrogen chemiluminescence detection (HS-SPME-GC-NCD). A carboxen / polydimethylsiloxane (CAR / PDMS) fiber was used for the extraction of HS-SPME.
The following performance criteria were used to validate this method: linear range, limit of detection (LOD), limit of quantification (LOQ), sensitivity, repeatability and accuracy.
In this study, 144 samples were analyzed: three types of meat preparations (frankfurter, bacon and sausage), both raw and cooked in different ways (boiled, grilled and baked). The presence of NDMA was identified in only one cooked bacon sample.

Keywords: *volatile nitrosamines, meat products, nitrogen chemiluminescence detector, HS-SPME-GC-NCD*

Acknowledgement: *We would like to thank IZS located in Foggia for providing the samples to be analyzed and all those who contributed directly or indirectly to the realization of this study.*

MULTI-OMICS IN FOOD ANALYSIS



10th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, September 6–9, 2022

P1

COMPARISON OF THE METABOLITE PROFILE IN BOVINE RUMEN FLUID, PLASMA, SALIVA AND FECES BY ANION EXCHANGE CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

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It is a common practice to feed grain rich diets to cattle in order to increase their productivity. Such feeding practices lead to an accumulation of short chain fatty acids (SCFAs) and lactate in the rumen, which disrupts the rumen's homeostatic acid-base balance regulation (Zebeli et al., 2012). This results in a number of severe dysfunctions, commonly known as the subacute ruminal acidosis metabolic complex, which has become a prevalent health disorder in dairy and feedlot cattle (Plaizier et al., 2008). In this work, we used metabolomics approaches to determine biomarkers associated with metabolic disorders, and to provide a deeper understanding of bovine rumen-gut health. For this purpose, nine rumen-cannulated non-lactating cows were first fed a forage diet and then gradually transitioned to a grain-rich diet. (Ricci et al., 2022 and Rivera-Chacon et al., 2022). During this experiment, samples of several different matrices were taken once a week. Four of these - rumen fluid, plasma, saliva and feces - were analyzed by a targeted metabolomics approach utilizing anion exchange chromatography coupled to high resolution mass spectrometry. This method is capable of quantifying 89 compounds (mainly carboxylic acids, nucleotides, sugars and sugar phosphates). In feces, concentrations of carboxylic acids, SCFAs and sugars increased noticeably after the switch from forage-rich diet to grain rich diet. In plasma, the change in feed composition led to a slight decline in some analytes, while others increased immediately after the change in feed composition, in part followed by a decline to the original concentration before grain rich diet was fed. In rumen fluid, the concentrations of all sugars increased noticeably, whereas concentrations of some SCFAs and carboxylic acids increased, while no discernible change occurred in others. In saliva, several large outliers containing very high SCFA concentrations were found, likely because these cows ruminated shortly before the saliva sample was taken, thus leading to a contamination of the samples with rumen fluid. Despite different metabolite profiles in the individual matrices, certain common metabolites were identified in every investigated matrix.

Plaizier, J. C., et al. "Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences." Vet J 176.1 (2008): 21-31.
Ricci, S. et al. "Progressive adaptation and plasticity of the bovine rumen and hindgut microbiota in response to a stepwise increase of dietary starch and phytogenic supplementation." Front Microbiol, in press.
Rivera-Chacon R. et al., "Supplementing a Phytogenic Feed Additive Modulates the Risk of Subacute Rumen Acidosis, Rumen Fermentation and Systemic Inflammation in Cattle Fed Acidogenic Diets" Animals 12(9) (2022): 1201.
Zebeli, Q., et al. "Invited review: Role of physically effective fiber and estimation of dietary fiber adequacy in high-producing dairy cattle." J Dairy Sci 95.3 (2012): 1041-1056.

Keywords: *animal metabolomics, anion exchange chromatography, high resolution mass spectrometry, carboxylic acids, targeted metabolomics*

Acknowledgement: *This research was performed in the framework of the Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, funded by the Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development and by BIOMIN Holding GmbH, which is part of DSM.*

P2

OPTIMIZING UNTARGETED METABOLOMICS DATA PROCESSING STRATEGIES FOR ORBITRAP MEASUREMENTS

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Untargeted metabolomics describes the analysis of small molecules in a biological system without prior knowledge about the composition of the sample. Adequate and reliable data processing is needed to handle these complex data; thus, a variety of new software packages have recently been developed. The aim of this project was to develop a workflow for untargeted metabolomics using an anion exchange chromatography system coupled to a Thermo Scientific Q Exactive Orbitrap mass spectrometer.
By measuring a defined standard solution containing carboxylic acids, nucleotides, sugar phosphates and sugars, the different options for data processing were tested and assessed according to the recovery of expected features, as well as the overall quality of features detected. A test study was conducted with bovine saliva extracts spiked with different concentrations of a mixed standard stock and assessed on the correct identification of the expected biomarkers. Compound Discoverer (version 3.3, Thermo Scientific) was used for data processing. The various annotation features integrated within the software were assessed by annotating compounds added to a bovine saliva extract that were unknown to the evaluator.
A workflow capable of detecting all compounds in a standard solution was created. However, applying filters for background signals and for low quality peaks showed a considerable increase in the overall quality of the results. Various parameters showed an effect on the overall number of features detected as well as an influence on the peak rating. Interestingly, the two nodes for peak detection delivered different results. While the *detect compounds* node detected all expected features it consistently overestimated the peak area when the feature showed an elevated baseline. However, this effect was improved when the baseline was removed during the integration process but then, inconsistent integration in blanks and samples were observed. The *detect compounds (legacy)* node showed better estimation of the peak area, while a few features were not detected. However, when the settings and filters were adjusted the loss of features was marginal. The better integration of the peak area was also consistent with the recovery of correct biomarkers during the test study. The *detect compounds (legacy)* node showed better performance, even in lower concentrations, when statistically relevant features were searched.
During the annotation process, most of the compounds could be annotated by at least their elemental formula. Nucleotides could be annotated with high confidence using triggered MS/MS spectra, as they showed distinctive fragmentation. As only database searches were used for annotation, confident annotation according to the structure was rarely achievable. This indicates that an in-house database containing fragmentation spectra and retention times from authentic reference standards is necessary for reliable identification of compounds in an unknown sample.

Keywords: *compound discoverer, high resolution mass spectrometry, Q Exactive orbitrap, untargeted metabolomics, data processing*

Acknowledgement: *This research was performed in the framework of the Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, funded by the Austrian Federal Ministry for Digital and Economic Affairs, the National Foundation for Research, Technology and Development and by BIOMIN Holding GmbH, which is part of DSM.*

P3

NATURAL-STYLE GREEN TABLE OLIVES FROM MANZANILLA CULTIVAR: A MICROBIOTA AND VOLATILOME STUDY

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Table olives are one of the most popular vegetable products in the western countries. In the Natural-style processing, olives are produced by spontaneous fermentation in brine, without any chemical debittering treatment. This natural fermentation process remains empirical and variable since it is strongly influenced by physicochemical parameters, microorganism presence in olive drupes, and olive cultivar. Manzanilla cultivar is the most important cultivar devoted to table olive production in Spain, and it is characterized by relatively high levels of phenolic compounds, which provide desirable health benefits. The microbiota associated with the fermentation process is quite complex and involves the growth of a great diversity of bacteria and yeast species, which determine the final characteristics such as flavor, texture and safety. However, despite extensive research into the microbial ecology of this type of table olive, the roles of the different microbial groups in contributing to the final quality are not fully understood. The profile of volatile compounds (volatilome) is generally related with the food aroma, which is one of the most important characteristics linked to the quality and consumers' preferences. In the present work, we investigated the microbial dynamics and volatilome changes during spontaneous fermentation and post-fermentation stages of Natural-style green table olives from Manzanilla cultivar. The correlations between the major microbial communities at the end of process (after 7 months of brining) and volatile compounds were further explored. Microbial and volatile profiles were identified and quantified by metagenomic and solid-phase microextraction coupled with gas chromatography-mass spectrometric (SPME-GC-MS) approaches, respectively. Lactic acid bacteria (LAB) were not detected throughout the process. At the end, the most abundant microbial genera, including bacteria and fungi, were *Allidiomarina, Halomonas, Saccharomyces, Pichia* and *Nakazawaea*. Alcoholos and esters were the predominant volatiles throughout the process. Strong positive correlations were found between the bacterial and fungal communities mentioned above and numerous volatile compounds, some of them previously reported as aroma-active compounds in table olives, indicating that these communities could contribute to the overall aroma of olives. The results of the present study will contribute to a better understanding of the fermentation process and may help the development of controlled fermentations using starter cultures of bacteria and/or yeasts for the production of high-quality green table olives from Manzanilla cultivar.

Keywords: *green table olives, manzanilla cultivar, spontaneous fermentation, volatilome, microbiota*

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P4

A ROADMAP FOR THE INTEGRATION OF ENVIRONMENTAL MICROBIOMES AS NEW TOOLS FOR RISK ASSESSMENT

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The microbiome refers to communities of microorganisms and their genome in a defined environment. Microbiomes interact with the food system in many ways, i.e. through plant growth, animal health or food spoilage with human pathogens. The study of the microbiomes has accelerated in the last decades. Presently, there is however no universal guidance or methodology proper to account for the structure and dynamics of environmental microbiomes. Even more so, the way environmental microbiomes can be included in risk assessment stays to establish. The Walloon Agricultural Research Center (CRA-W) and the Catholic University of Louvain (UCLouvain) joined their expertise to explore existing data and tools. The purpose of the project, co-financed by EFSA (GP/EFSA/ENCO/2020/02) is to define a roadmap for the potential integration of microbiome considerations under risk assessments, within EFSA's remit.
The first step consists of an extensive review of the existing literature on the subject, which encompasses various ecosystems and wildlife organisms. The information is scrutinized in order to define what a healthy baseline is, if it exists, how it can be described, and what are the beneficial or detrimental impacts on a given microbiome. This is, however, complex as living organisms are dynamic systems and tend to maintain homeostasis. Another aspect relates to the different omics techniques and tools associated with microbiome studies. Data quality, harmonization and correct interpretation are essential for the comparison of results between laboratories and identification of potential risks. At this level, genomic methods based on amplicon high-throughput sequencing are the most advanced for a proposition of guidelines ensuring the quality of results provided by each laboratory. It also includes recommendations as testing the suitability of the targeted regions, their evaluation on mock communities, the determination of the sensitivity of the methods and the use of appropriate database and bioinformatic treatments. It is also needed to integrate the trends going into the direction of longer amplicon sequencing technologies, allowing for more precise taxonomic identification. Such a work requires various expertise and exchange of data between laboratories. This can be realized through capacity building, a mobilization of laboratories towards risk assessment and establishing a network of laboratories to share their expertise in the field.

Keywords: *microbiome, risk assessment, omics, network*

P5

CLASSIFICATION OF POULTRY MEAT CUTS BASED ON APPROACH OF UNTARGETED LIPIDOMIC ANALYSIS AND ADVANCED CHEMOMETRICS

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Meat is an important source of lipids in the human diet and its consumers are increasingly interested in fat composition, since nutritional guidelines recommend reducing total fat intake, especially saturated fat, and increasing polyunsaturated fat (PUFAs) (Li et al. 2022). Chicken meat can be considered important in diets because it contains a higher proportion of polyunsaturated fatty acids, when compared to meat of other species and (Riovanto et al. 2012) which have a known beneficial action in reducing the risk of cardiovascular diseases, inflammatory and immunological disorders (Zhou et al. 2012).It is well-known that humans are unable to synthesize significant amounts of polyunsaturated fatty acids (PUFAs), which therefore must be introduced with the diet. Poultry meat products are in the second place of world consumption of meat among the other species (Camilla *et al.*, 2022). The lipid content of poultry meat is affected by various factors but mainly from raising diet composition (Maragoni et al. 2008). Lipidomics,focuses on the whole lipidome profile of an organism (Wu et al., 2020). HRMS based lipidomics followed by modern chemometric and classification techniques, can offer a valid workflow for the classification of poultry meat cuts. In the present work, meat samples of the two major poultry parts, breast and thigh, were analyzed by RP-UPLC-TIMS-TOF-MS in positive ionization mode. The obtained results were used to build unsupervised and supervised statistical models for the classification of the poultry meat cuts, taking under consideration the lipid content of each part. The mass features with most contribution to the model were extracted. The acquired results, propose that lipidomic profiles of the two poultry meat cuts of this study, vary significantly and can be separated statistically.

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Wu, Z. *et al.* (2020) '"Lipidomics": Mass spectrometric and chemometric analyses of lipids', *Advanced Drug Delivery Reviews*, 159, pp. 294–307. doi: 10.1016/j.addr.2020.06.009.

Keywords: *lipidomics, high resolution mass spectrometry, chemometrics, food authenticity, ion mobility spectrometry*

Acknowledgement: *This research has been co-financed by Greece and the European Union (European Regional Development Fund) within the Operational Program "Competitiveness, Entrepreneurship and Innovation - NSRF 2014-2020". Project Code: Τ1ΕΔΚ-03856. Acronym: GREEN POULTRY MEAT ANTIFREE.*

P6

UNTARGETED 4D LIPIDOMICS COMBINED WITH CHEMOMETRICS, AS A RELIABLE TOOL FOR THE CLASSIFICATION OF PORK MEAT CUTS

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Meat is an essential source of protein, amino acids, lipids and various micronutrients and it is consumed by most of the world's population and cultures (Li *et al.*, 2022). Among the different meat species available, pork meat is the most consumed type of meat worldwide (Camilla *et al.*, 2022). Pork meat is available in different type of cuts, with the most common cuts available in the market consisting of pork belly, steak, shoulder and leg. The lipid content of pork meat is affected by various factors, including animal breed, raising diet composition, as well as meat cut/part and muscle fiber type (Boselli *et al.*, 2008). The main lipid classes present in pork meat are phospholipids (PLs), triglycerides (TAGs) and diglycerides (DAGs), mainly consisting of saturated, mono-unsaturated (MUFAs) and Polyunsaturated (PUFAs) fatty acids [m1] (Pereira and Abreu, 2018). Lipidomics, as a subfield of metabolomics, focuses on the lipidome, that is, the total of lipid molecules of a tissue/organism (Wu *et al.*, 2020). Lipidomic analysis coupled with chemometrics, can provide a reliable and robust tool for the discrimination of pork meat cuts, solely based on their lipid composition. In the present work, meat samples of different pork meat cuts, were analyzed by RP-UPLC-TIMS-TOF-MS[m2] in positive ionization mode. The obtained mass features were annotated and used to construct a Hierarchical Clustering Analysis (HCA) and an Orthogonal Partial Least Squares - Discriminant Analysis (OPLS-DA) [m3] model for the classification of the pork meat cuts based on their lipid profiles. VIPs were extracted from the model, to acquire the lipid molecules that better differentiate the abovementioned meat cuts. The results obtained from this study, strongly suggest that pork meat cuts differ in terms of lipid composition and can be successfully discriminated.

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Li, C. *et al.* (2022) 'Advanced Lipidomics in the Modern Meat Industry: Quality Traceability, Processing Requirement, and Health Concerns', *Frontiers in nutrition*, 9, p. 925846. doi: 10.3389/fnut.2022.925846.
Pereira, A. L. F. and Abreu, V. K. G. (2018) 'Lipid Peroxidation in Meat and Meat Products', in Mansour, M. A. (ed.) *Lipid Peroxidation Research*. Rijeka: IntechOpen. doi: 10.5772/intechopen.81533.
Wu, Z. *et al.* (2020) '"Lipidomics": Mass spectrometric and chemometric analyses of lipids', *Advanced Drug Delivery Reviews*, 159, pp. 294–307. doi: 10.1016/j.addr.2020.06.009

Keywords: *lipidomics, high resolution mass spectrometry, food authenticity, chemometrics, ion mobility spectrometry*

P7

VOLATOLOMICS AS A PROMISING OPTION TO ENHANCE FOOD CHEMICAL SURVEILLANCE

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Food is the main route of human exposure to toxic contaminants, especially environmental micropollutants. To ensure consumer health, analytical techniques must be implemented for the detection and quantification of contaminants in foodstuffs. The current analytical methods for the surveillance of chemical contamination are efficient and sensitive but are hindered by cumbersome implementation, high cost and low throughput. These routine monitoring techniques thus do not allow rapid cost-effective large-scale methods, which are requested for strengthening food safety. To overcome these limitations, alternative approaches based on omics have emerged to detect contaminations. Inspired by research showing the usefulness of the rapid cost-effective analysis of expired volatile organic compound (VOC) markers in clinical diagnosis, VOC-based metabolomics, or volatolomics, was investigated for revealing livestock exposure to chemical contamination. Based on control/test experiments, broiler chickens were experimentally exposed or not to polychlorobyphenyls (PCBs) through their feeding and the volatolome composition of some of their tissues/organs/fluids was analysed by SPME-GC-MS.
The main objectives of the study were (i) to confirm the relevance of liver volatolomics, for strengthening the surveillance of food chemical safety and (ii) to explore the potential of other relevant biological matrices as plasma (information transport role), caeca (gut microbiota activity) or oil from uropygial gland (semiochemical communication role).
The treatment of liver volatolomics data highlighted 22 VOC candidate markers of animal exposure to PCBs, whose biochemical origin was discussed according to literature data. Our study emphasizes that the liver is a relevant biological target in which metabolic reactions are impacted by exposure to PCBs. It confirms the results reported in previous liver volatolomics studies involving livestock (poultry and pork) exposed to other chemical contaminants through spiked-feed (Ratel et al., 2017; Ratel et al. 2022). It provides also a new view on the potential of other biological matrices, never explored to our knowledge, to reveal discriminant information useful for revealing a contamination by PCBs. The investigation by volatolomics of these new matrices is a very promising track since it opens up the possibility of implementing high throughput large scale monitoring based on non-invasive sampling, directly on-farm and not only at the slaughterhouse.

Ratel et al. (2017) *Chemosphere*, 189: 634-642.
Ratel et al. (2022) *Food Chemistry*, 374: 131504.

Keywords: *volatolomics, food safety, PCBs, livestock, SPME-GC-MS*

Acknowledgement: *The research work was funded by the French Agence Nationale pour la Recherche (ANR), contract n° ANR-19-0011 "SENTINEL: High-throughput screening tools for a reinforced chemical safety surveillance of food" https://sentinel.ifip.asso.fr/.*

P8

VOLATILOMICS-BASED MICROBIOME EVALUATION OF FERMENTED DAIRY BY PROTOTYPIC HEADSPACE-GAS CHROMATOGRAPHY-HIGH TEMPERATURE ION MOBILITY SPECTROMETRY (HS-GC-HTIMS) AND NON-NEGATIVE MATRIX FACTORIZATION (NNMF)

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Fermentation of food is an ancient practise for the preservation of food through the production of acids and possibly antimicrobial compounds [1]. Natural fermentation processes involve the symbiotic fermentations of, for example, lactic acid or acetic acid bacteria and yeasts [1]. Since each microorganism produces a versatile spectrum of flavour compounds, the microbiome influences the sensory properties of the product.
Key compounds of fermented dairy are for example ethanol, acetic acid, ethyl acetate and diacetyl. Preliminary analysis found overlapping peaks in both GC-MS and GC-IMS analysis. By raising the drift tube temperature in an optimized prototypic HS-GC-IMS, this problem was overcome. This HS-GC-HTIMS was further used for volatilomic profiling of 33 traditional kefir, 13 commercial kefir and 15 commercial yogurt samples.
Principal component analysis (PCA) is a commonly used pattern recognition technique, which decomposes matrices into independent (orthogonal) principal components (PCs). These PCs, however, lack interpretability, wherefore in this work NNMF was explored for data analysis. NNMF decomposes samples as sums of their parts whereby components are easily interpretable. PCA and NNMF analysis, in combination with non-targeted screening, revealed distinct differences between traditional and commercial kefir, while showing strong similarities between commercial kefir and yogurt. Classification between fermented dairy samples into commercial yogurt, commercial kefir, traditional kefir mild and traditional kefir tangy was also possible for both, PCA and NNMF based models, obtaining CV error rates of 0 % for PCA-LDA, PCA-kNN (k=5) and NNMF-kNN (k=5) and 3.3 % for PCA-SVM and NNMF-LDA. Through back projection of NNMF-loadings, characteristic substances were identified, indicating a mild flavour composition of commercial samples, with high concentrations of buttery flavoured diacetyl. In contrast, traditional kefir showed a diverse volatile profile with high amounts of flavourful alcohols (including ethanol and methyl-1-butanol), esters (including ethyl acetate and 3-methylbutyl acetate) and aldehydes. For validation of results and deeper understanding, qPCR sequencing was used to evaluate the microbial consortia, confirming the microbial associations between commercial kefir and commercial yogurt, and reinforcing the differences between traditional and commercial kefir. The diverse flavour profile of traditional kefir primarily results from the yeast consortium, while commercial kefir and yogurt is primarily, but not exclusively, produced through bacterial fermentation. The flavour profile of fermented dairy products may be used to directly evaluate the microbial consortium using HS-GC-HTIMS analysis.

Keywords: *GC-IMS, metabolomics, dairy, machine learning*

P9

EFFICIENT PEPTIDE DESALTING ON NOVEL C18 STAGETIPS WITH A BROAD CAPACITY RANGE FOR LC-MS/MS PROTEOMIC STUDIES IN FOOD MATRICES

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In recent years, there has been a growing interest in the analysis and identification of proteins in food matrices such as meat, fish or milk. These proteomic studies very often involve bottom-up approaches, with the LC-MS/MS analysis of peptides resulting from an enzymatic hydrolysis of the proteins. However, the salts present in the digestion buffers can generate ion-suppression that can limit the detection of peptides and greatly impacts proteins identification. Therefore, peptide desalting is an indispensable step before the LC-MS/MS analysis.
This desalting step can be very challenging since salts must be efficiently removed while hydrophilic and hydrophobic peptides must be retained to preserve all useful information about the sample.
Among a large diversity of C18 powders, a C18 with a wide spectrum of interactions was selected to interact with the broadest range of peptides. Small particles embedded in a monolithic disk were used to combine high capacity and small dead volume. These disks were tested into home-made SPE tips, called StageTips, to evaluate performances in desalting HeLa digest before peptides analysis by nanoLC-MS/MS.
In a first set of experiments, three different StageTips, with different capacities, were tested for the desalting of 100ng of HeLa digest. Five replicates of each assay were carried out and the MS analyses of peptides were compared to those obtained after direct injection of a non-purified loading solution.
This comparison showed that 97% of proteins could be identified after desalting of the digest on all SPE tips, regardless of their capacity. These excellent results emphasized the efficient desalting of peptides on the whole polarity range, from the most hydrophilic to the most hydrophobic ones, thus demonstrating that all retained peptides are released from the StageTips. Low variability was obtained for intra- and inter-tips assays, highlighting the StageTips reliability.
A second set of experiments was carried in triplicate with 1ng to 10µg of HeLa digest, to evaluate the working range of the lowest capacity StageTips. More than 95% of proteins were identified for digest amounts ranging from 10ng to 10µg, with RSD inferior to 3%. Moreover, no loss of performance was observed for 10µg of digest, thus meaning that the StageTips could also be used for higher quantities of peptides.
The RSD logically increased for 1ng of peptides due to the handling of a very small amount of material, but the number of proteins identified remained very close to the reference sample, thus demonstrating that the SPE tips could potentially be used for single cell like analysis.
Therefore, the home-made C18 StageTips showed very interesting properties of efficient desalting with no loss of peptides, high capacity and broad range of use from single cell to high quantity, in addition to a simplicity of use. This sorbent thus appears as ideal for the purification of digests before proteomics analysis in food matrices.

Keywords: *proteomics, peptide, desalting, SPE, sample preparation*

P10

A MULTI-TIER APPROACH TO SOLVING THE EVOLVING ISSUES AROUND HONEY AUTHENTICITY AND QUALITY

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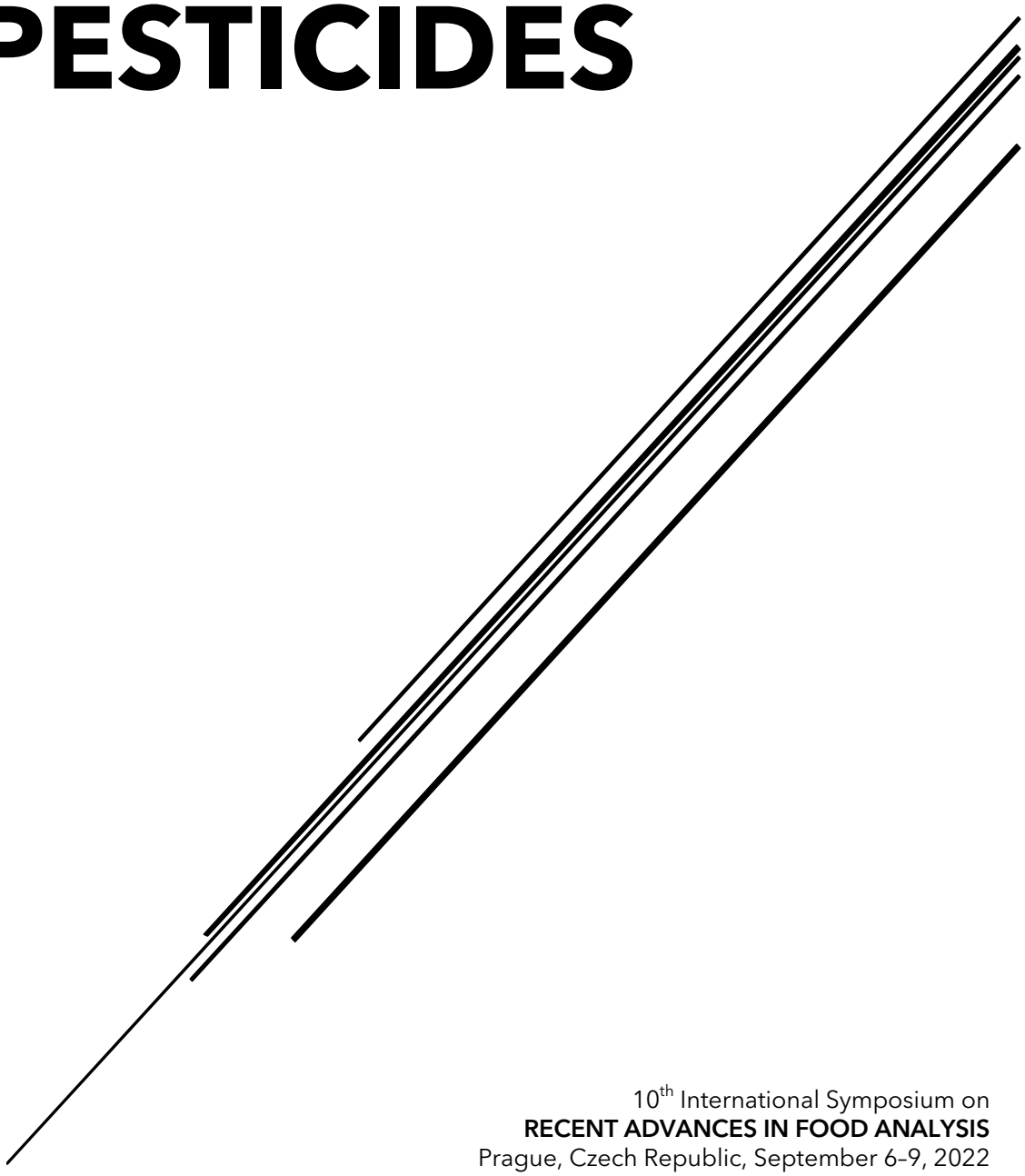
Honey remains a commodity which is always in the headlines due to being very prone to adulteration, mislabelling and often exaggerated health claims. Current testing regimes have not stopped criminals finding loopholes in analytical tests. Many techniques exist in testing honey however as far as the consumer is concerned the testing does not truly reflect the quality of the honey and in some cases the few markers which are tested do not help the consumer in any way. High resolution mass spectrometry has shown promise in solving many of the issues around quality, origin, adulteration and freshness however challenges do exist in making validating and implementing these methods mainly around cost, expertise and lack of evidence on the methods working for the many types of honey.
To validate an untargeted method firstly sufficient samples accounting for as much variation as possible are required to create a reliable fingerprint and more importantly this has to be continuously updated to ensure long term performance of the method.
In this work we propose a multi-tier approach the first uses FTIR spectroscopy as a simple initial screen to detect potential fraud and syrup addition. The second uses UHPLC-QToF using a novel high throughput HILIC separation providing test results in under five minutes and can detect adulteration as well as origin in many types of honey. The third uses GC-HS-SPME-QToF with acts as additional confirmation as well as allowing an orthogonal perspective on the test sample. In total hundreds of samples have been tested including authentic samples from industrial collaborators as well as adulterated samples which were prepared in the laboratory. The samples have been collected over years therefor include samples to account for seasonal variation. The samples in the study form the foundation of truly allowing a potential workflow which could be used worldwide since samples have come from all over the globe and have been selected based on trending issues around honey.

Keywords: *mass spectrometry, spectroscopy, honey, origin, fraud*

RESIDUES – PESTICIDES



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Q1

DETERMINATION OF ACARICIDES IN HONEYS FROM DIFFERENT BOTANICAL ORIGINS. APPLICATION TO EVALUATE MIGRATION FROM STAMPED WAX

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There is great concern about the health status of beehives, attributing bee losses to various factors such as i) weakening of the immune system due to mites and parasites; ii) reduction of natural habitats; iii) environmental pollution; iv) exposure to various agrochemicals used for protection and care of crops. It should be highlighted that the presence of pesticide residues like those from the family of acaricides has become a rather hotly-debated topic considering its dangerousness. These compounds can be found in beehives for several reasons. For example, beekeepers use them to control the damaging effects of the Varroa mite, or they can accumulate in the wax, which is directly in contact with other bee products like honey. The use of stamped wax helps the bees to build honeycombs, however, these wax sheets could contain significant amounts of xenobiotic residues, so it is essential to control their presence. Furthermore, it has been shown that acaricides can migrate from wax to honey, contaminating it and causing a food safety problem. Indeed, food alerts have recently affected honey´s healthy image, as the presence of acaricides could represent a potential risk for consumers. Therefore, efficient, selective, and sensitive methods are needed for determining acaricide residues in honeys. In addition, migration studies are required to assess the potential transfer of pesticides from wax to honey.
In the present study, a new analytical method using gas chromatography-mass spectrometry was proposed and validated to determine seven of the most frequently detected acaricides (atrazine, chlorpyrifos, chlorfenvinphos, endosulfan, bromopropylate, coumaphos, and fluvalinate) in Spanish honeys from three different botanical origins (multifloral, rosemary and heather). Residues of fluvalinate were found in all honeys, although only in rosemary and multifloral honey they could be quantified (5-23 µg/kg). Moreover, a migration study was carried out in an incubator simulating beehive conditions (temperature and agitation). Contaminated wax at different levels was placed in contact with honey, examining over time the acaricide residues present in both matrices. Isolation of the compounds involved the use of solvent extraction for honeys, and the use of special sorbents to remove the lipids present in the wax that causes matrix effects. The amount of pesticide initially present in the wax has been found to substantially influence its transfer to honey. Concentrations in the wax below 40 mg/kg did not migrate significantly into the honey. By contrast, concentrations close to or higher than 400 mg/kg confirmed the transfer of pesticides from the wax to the honey. Hence, we concluded that transfer occurs especially when high concentrations of acaricides are present in stamped waxes.

Keywords: *acaricides, wax, migration, gas chromatography-mass spectrometry, honey*

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Q2

A FAST AND COST-EFFECTIVE METHOD FOR ANALYSING PESTICIDE RESIDUES IN FOODS OF ANIMAL ORIGIN WITH HIGH-FAT CONTENT USING ETHYL ACETATE/ACETONITRILE EXTRACTION AND CLEAN-UP WITH AGILENT CAPTIVA EMR LIPID CARTRIDGES

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Background: Gel permeation chromatography (GPC) is, although an efficient clean-up technique, often a tedious task for the laboratory technician when analysing pesticide residues in foods of animal origin with high-fat content. We thus implemented Agilent´s Captiva EMR lipid cartridges (EMR cartridges) as the clean-up step, after ethyl acetate/acetonitrile extraction, for a faster and more cost-effective analysis.
Materials and methods: 2.5 g of sample was extracted through a double ethyl acetate/acetonitrile (20:80) extraction followed by clean-up using EMR cartridges. The purified samples, with a sample concentration of 0.2 g/mL, were then filtered through a 0.2 µm PTFE filter before analysis by LC-MS/MS and GC-MS/MS. Validation of pesticide residues was carried out according to SANTE/12682/2019 using five-point matrix-matched calibration curves.
Results: We compared the GPC results with the EMR cartridges and found that recoveries were higher overall. Moreover, 113 pesticide residues were validated in bovine fat using the EMR cartridges. 110 achieved an LOQ of 0.01 mg/kg whereas three an LOQ of 0.05 mg/kg.
Discussion: When using the EMR cartridges for analysis of high-fat content in foods of animal origin we managed to reduce the solvent volumes from approximately 5 L using the GPC to only 225 mL while using the cartridges when analysing 20 samples. Furthermore, the time spent for a batch of 20 samples was reduced from two days to one day, mainly because of the redundancy of the GPC and evaporation steps. One drawback is that large analytes as e.g. spinosad and spiroxamine are not able to penetrate the sorbent in the cartridges and thus cannot be quantified using this method.

Keywords: *pesticides, EMR, GPC, ethyl acetate, method development*

Q3

FUTURE-PROOFING CANNABIS ANALYSIS WITH THE SCIEX TRIPLE QUAD™ 7500 LC-MS/MS SYSTEM – QTRAP® READY

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Since 2015, certain states in the USA and some nations have implemented or begun discussions on the legalization of Cannabis. With this comes responsibility for ensuring the safety of consumer products. Compliance requirements around pesticide residues—allowance, detection, and tolerance limits—are varied among regions for which regulations exist. The trend, however, seems to be towards increasingly rigid requirements (more pesticides and lower detection limits). The Cannabis regulatory landscape is continuously evolving. Anticipation of more aggressive analytical requirements necessitates development of pesticide quantification methods which are as sensitive and robust as possible. Cannabis flower as a matrix represents an analytical challenge in complexity. Matrix interference and suppression affect the ability of current methods to deliver the required results. Advancements in analytical technologies represent promising avenues for residue detection in Cannabis in a changing regulatory landscape.
In order to assess the latest advancements in triple quadrupole technology and its potential for residue analysis in Cannabis, the Canadian regulated pesticide list (excluding Kinoprene) was used as a panel for quantitative analysis. The Canadian approach to pesticide regulation in Cannabis is uniquely characterized by a large panel of analytes and very low tolerance limits required in testing.2,3 With the method developed here, very low level detection limits were achieved using very small injection volumes, which illustrates the performance of the SCIEX Triple Quad 7500 LC-MS/MS System — QTRAP Ready.

Keywords: *cannabis, pesticides, matrix, quantification*

Q4

EXPERIENCE OF ANALYSIS OF THIRAM AND ZIRAM BY DIRECT ANALYSIS IN REAL TIME – HIGH RESOLUTION MASS SPECTROMETRY (DART-HRMS)

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Dithiocarbamates (DTCs) are non-systemic fungicides and have been used worldwide in agriculture for many years. DTCs are often grouped into three main sub-classes, (a) dimethyldithiocarbamate (DMD), e.g. ziram, ferbam, thiram, (b) ethlyenebis(dithiocarbamates) (EBD), e.g. maneb, zineb, mancozeb and (c) propylenebis(dithiocarbamates) (PBD), e.g. propineb. Direct analysis of DTCs is challenging because of their low solubility and low stability once in solution. When in contact with acidic plant juices, DTCs can quickly decompose to carbon disulphide (CS_2) and the corresponding amine. The most common approach for quantifying DTCs is by conversion to CS_2 during hot acid digest of the samples. However, many residues below the MRL can incorrectly appear to be above the consumer health risk value when only looking at CS_2 values. It would greatly refine the risk assessment process if an analytical method was able to rule out the most toxic DTCs (e.g. thiram and ziram). Furthermore, specific MRLs for thiram, ziram and propineb have been established in the EU, thus emphasising the need for more specific methods.
"Direct analysis" of DTC is not straight forward and often only involves the detection of the DTC anions. The identity of the anion allows us to determine which sub-group the DTC belongs to (e.g. DMD, EBD and PBD) but not the actual DTC compound itself. Direct analysis typically involves surface washing of the sample (instead of homogenisation) to minimise degradation reactions with sample co-extractives, along with the addition of some form of stabiliser (e.g. TCEP) and a complexing reagent (e.g. EDTA).
Here we report our experiences of determining thiram and ziram as the DMD anion using a simple QuEChERS extraction, followed by analysis with DART-HRMS. Propineb was also investigated but only detectable as the break down product propylene thiourea (PTU) in the sample extracts.

Keywords: *dithiocarbamate, thiram, ziram, DART, HRMS*

Acknowledgement: *Department for Environment Food & Rural Affairs (Defra) and Chemical Regulation Division (CRD) of the Health & Safety Executive (HSE) for funding this work.*

Q5

ETHYLENE OXIDE ANALYSIS IN FOOD

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Ethylene oxide is a low molecular weight, highly volatile compound with ether like odour (1). Ethylene oxide (EO) is mainly used as an intermediate to produce several chemical compounds (e.g., polyethylene glycols). It is also widely used as a fumigant to control pests and a wide range of microorganisms. Several countries including, USA, Canada, and India, permits the use of EO as a food fumigant for grain, spices, nuts, and oilseeds. However, the use of EO as a pesticide is banned in the EU and UK. The EU Maximum Residue Level (MRL) for ethylene oxide is defined as "Sum of ethylene oxide and 2-chloroethanol expressed as ethylene oxide". Where 2-chloroethanol (2-CE) is formed from the reaction of EO with chlorides (e.g., sodium chloride) present in the food matrix. The EU MRLs are set at 0.05, 0.1 and 0.02 mg/kg in oilseed, spices and cereals, respectively.
In 2021, 347 alerts for ethylene oxide in food were reported in the EU Rapid Alert System for Food and Feed portal (RASFF). Over 90% of the alerts were attributed to food products containing sesame seeds originating from India. The alerts reported the findings as “sum of ethylene oxide and 2-chloroethanol, expressed as ethylene oxide” rather than the actual results for the component(s) found.We report here our findings of ethylene oxide analyse in food based on the EURL method. Our method is validated for direct analysis of both EO and 2CE using GCMS/MS in sesame seeds, spices (cumin) and cereals (rice).

1. S. Budavari (Ed.), The Merck Index (12th edition), Merck, Whitehouse Station, NJ (1996).

Keywords: *ethylene oxide, 2 chloroethanol, GCMS/MS*

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Q6

PLANT FIBERS IN COMPARISON WITH OTHER FINING AGENTS FOR THE REDUCTION OF PESTICIDE RESIDUES AND THE EFFECT ON THE VOLATILE PROFILE OF AUSTRIAN WHITE AND RED WINES

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Pesticide residues in Austrian wines have so far been poorly documented. In 250 wines, 33 grape musts and 45 musts in fermentation, no limit values were exceeded, but in some cases high levels (>0.100 mg/L) of single residues were found, meaning that a reduction of these levels before bottling could make sense. In the course of this study, a white and a red wine were spiked with a mix of 23 pesticide residues from the group of fungicides (including botryticides), herbicides and insecticides. The influence of the following treatments on residue concentrations and volatile profiles were investigated: two activated charcoal products, a bentonite clay, two commercial mixed fining agents made of bentonite and charcoal, two yeast cell wall products, and a plant fiber-based novel filter additive. The results of this study show that all the agents tested reduced both residues and volatile compounds in wine, with activated charcoal having the strongest effect and bentonite the weakest. The mixed agents and yeast wall products showed less aroma losses than charcoal products, but also lower residue reduction. Plant fibers showed good reduction of pesticides with moderate aroma damage, but these results need to be confirmed under practical conditions.

Q7

DETERMINATION OF PYRETHROIDS AND MACROCYCLIC LACTONE INSECTICIDES IN SPICES AND TEA

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Introduction: Pyrethroids and macrocyclic lactones are groups of commonly used insecticides in the agriculture and horticulture industries. Macrocyclic lactones are naturally occurring, or semisynthetic compounds produced as fermentation products in soil-dwelling Streptomyces avermitilis. Pyrethroids, on the other hand, are synthetic, and were designed based on the naturally occurring family of pyrethrins, which were originally derived from chrysanthemum flowers. Due to the widespread use of these compounds in the environment, a comprehensive quantitative method is necessary to monitor and control their concentration in final food products destined for human consumption.Here, a method has been developed for the simultaneous identification and quantification of pyrethroid and macrocyclic lactone insecticides at detection levels below the maximum residue level defined by the European Commission under regulation 2018/1514.
Material and Methods: Avermectin, bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin were diluted into mobile phase A for analysis. Calibration curves were constructed at concentrations of 0.02–20 ng/mL.Green tea and paprika powder were milled, homogenized and added to 10 mL of water and 10 mL of acetonitrile. The sample was shaken for 1 min and added to Macherey-Nagel QuEChERS Mix I (ref 730970). The sample was shaken for another 3 min and centrifuged for 5 min. 8 mL of the organicphase was added to Macherey-Nagel QuEChERS Mix III (ref 730648). The sample was shaken for 3 min, centrifuged for 5 min and 7 mL was then taken and acidified with 5% formic acid in acetonitrile . Compounds were spiked into the tea extract at 1 ng/mL and 10 ng/mL. Chromatographic separation was performed using the ExionLC AD system and a Phenomenex Synergi Fusion-RP (4 µm, 50 x 2.1 mm) column. Multiple reaction monitoring (MRM) analysis was performed using a SCIEX 7500 system. The system was operated in positive electrospray ionization (ESI) mode. Data was acquired and processed using SCIEX OS software.
Results: In this study, highly sensitive detection, and quantification of avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ-cyhalothrin, milbemectin A3 and A4 and permethrin from QuEChERS extracts of spices and green tea was performed. High levels of sensitivity were observed, with LLOQ values down to 0.02 ng/mL. Optimization of new parameter Q0D for milbemectin A3 in spices resulted in reduced background and increased signal to noise values. This allowed for lower injection volumes to be used, significantly reducing ion suppression and improving the robustness of the method. The linear range of each insecticide was also assessed, providing r values >0.99 for each compound.

Keywords: *food safety, insecticide testing, MRM, quantification, food contamination*

Q8

COUPLING COST EFFECTIVE SERS SUBSTRATES WITH QUECHERS FOR THE DISCRETE DETECTION OF TRACE CHLORPYRIFOS RESIDUES IN HONEY

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Honey has the propensity to accumulate potentially harmful pesticide residues due to the contamination of bees during pollen and nectar collection or by the direct treatment of their hives. In this study, the cost-effective detection of chlorpyrifos in honey using a planar Surface-enhanced Raman spectroscopy (SERS) surface coupled with a simple extraction method is reported. Initially, Aluminium foil (ALF) and Silicon (Si) were evaluated and simulated in buffer conditions to determine their potential as a SERS surface. ALF coupled with untreated gold nanoparticles (AuNPs) was found to be the most effective, increasing the maximum Raman enhancement by over an order of magnitude when compared with Si coupled with AuNPs ($9.35x10^3$ and $5.97x10^2$, respectively). The SERS approach was then coupled with Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction to detect trace levels of chlorpyrifos in honey. Chlorpyrifos spiked into a honey matrix could be detected down to 0.1 ug/L. Finally, principal component analysis (PCA) was developed so that chlorpyrifos could be reliably detected in a honey matrix spiked with multiple pesticides. The results present the successful development of a cost-effective method with the potential to be made portable and the ability to quantitively and selectively detect chlorpyrifos in honey well below the current limit of determination set by the EU (0.05 mg/L). To further highlight the potential of QuEChERS coupled with SERS on solid metallic substrates for rapid pesticide screening of food commodities, further work on different food matrices, different metallic substrates, and using a handheld Raman equipment have been envisioned.

Keywords: *SERS, QuEChERS, pesticide detection, food matrix, multivariate data analysis*

Acknowledgement: *This work is supported by the Northern Ireland Department of Economy and has been undertaken in Queens University Belfast.*

Q9

LARGE VOLUME INJECTION OF PESTICIDES USING LOW-PRESSURE GAS CHROMATOGRAPHY

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Concurrent Solvent Recondensation Large Sample Volume splitless injection (CSR-LVSI, or LVI) is a sampling technique that overcomes the limitation of the maximum sample volume to 1 – 2 µL valid for classical splitless injection. Low-Pressure Gas Chromatography is a novel technique that had been successfully used for pesticide screening and quantification. The LPGC configuration with the restrictor/guard column lends itself to the requirements of the CSR-LVSI and can potentially improve the sensitivity and lower detection limits. Large volume injection of acetonitrile and acetonitrile – toluene samples were evaluated in a range of 1 – 25 µL for peak shapes and the relationship between the peak area and injection volume was established. The large volume injections (>10 µL) resulted in peak splitting when the acetonitrile was used. The later eluting peaks were more affected, furthermore, the effect of increased volume on the area for injecting larger than 10 µL was negligible. The calibration parameters were compared for 1 µL and 5 µL injection. The limits of detection and other parameters were comparable between the two injection volumes. This presentation will describe both CSR-LVSI and LPGC and demonstrate the applicability of combining these techniques using a variety of solvents.

Keywords: *LPGC, large volume injection, pesticides, CSR-LVSI*

Q10

LC-MS/MS ANALYSIS OF ANIONIC POLAR PESTICIDES IN FRUITS AND VEGETABLES USING A VENUSIL HILIC COLUMN

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Many polar pesticides used in conventional agriculture are difficult to retain on standard C18 reversed phase HPLC columns. In addition, polar pesticides are a very diverse group of analytes which is hard to be analyzed using one standard method to identify and quantify all of them. This forces food testing laboratories analyzing samples multiple times using different methods to identify and quantify all the relevant polar pesticides in each food sample.
The purpose of this study was the development of a fast and robust method for the LC-MS/MS determination and quantification of several common anionic polar pesticides in fruits and vegetables after sample preparation using the QuPPe method. As the samples we tested were from plant origin, we followed the QuPPe-PO-Method suggested by the EU Reference Laboratories for Residues of Pesticides–Single Residue Methods (EURL-SRM). In the frame of the study, we evaluated the effect of sample dilution, injection solvent, injection volume, and concentration of formic acid in the mobile phase on the reduction of matrix effects affecting the recovery and quantification of anionic polar pesticides including phosphonic acid, fosetyl, chlorate, perchlorate, Glyphosate, and AMPA. Presented is the resulting method allowing the identification and quantification of a variety of polar anionic pesticides including a separation of phosphonic acid from phosphoric acid in water samples.

Keywords: *polar pesticides, QuPPe, LC-MS/MS*

Acknowledgement: *Pietro Azzione and Marco Loperfido at EuroQualitylab S.r.l., Gioia del Colle, Italy*

Q11

A FAST AND ROBUST GC/MS/MS ANALYSIS OF 203 PESTICIDES IN 10 MINUTES IN SPINACH

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Introduction: Concern about trace-level food and environmental pollutants is driving the demand for more rapid and robust methods for the identification and quantitation of chemical residues. This work focuses on achieving fast GC/Triple Quadrupole MS (GC/TQ) analysis, while maintaining robust system performance in complex food matrices. Two system configurations described in this presentation provide analysis times of 10 minutes while maintaining sufficient chromatographic resolution for the analysis of 203 compounds. Method robustness is achieved using a mid-column backflush configuration. This presentation will discuss two configurations for achieving a 10-min analysis: A conventional 15 m x 15 m (0.25 mm x 0.25 µm) and a narrow bore 10 m x 10 m (0.18 mm x 0.18 µm) column configurations.
Methods: The conventional 20-min retention time-locked method for 203 pesticides was created with an MRM database and used as a benchmark for the optimized fast analyses. To achieve faster analysis, two approaches were taken. First, the same 15m x 15m (0.25mm x 0.25µm) conventional mid-column backflush column configuration was used with a faster oven ramp, yielding the analysis time of 10 min. Second, a narrow bore column 10m x 10m (0.18mm x 0.18µm) mid-column backflush configuration was used enabling 10- and 8-min analysis time. The latter methods were precisely scaled using method translation.
Preliminary data (results): The use of the conventional 15 m x 15 m configuration allows users to typically choose between a 20- min method for higher chromatographic resolution and analyzing up to 400 compounds at once, and a 10-min method that allows for maintaining similar chromatographic resolution while analyzing up to 250 compounds.
Data illustrations below show the benchmark 20-min analysis of 203 pesticides commonly regulated in food. It also demonstrates the chromatogram acquired with the same configuration using a faster oven ramp. Retention index calibration was used to predict the new retention times as the relative elution order for some compounds changed. Further data shows the chromatograms acquired with a 10 m x 10 m narrow bore configuration. The method translation technique allowed for preserving the relative elution order of the compounds, thereby accurately predicting retention times for both 10- and 8-min methods. The use of the narrow bore columns allowed for maintaining excellent chromatographic resolution, this being highlighted in the second data illustration. Among the advantages provided by chromatographic resolution were reduced matrix interference and minimized interference between co-eluting analytes.
Mid-column backflushing used with both column configurations enabled method robustness by decreasing the need for column head trimming and the EI source cleaning.

Keywords: *pesticides, fast analysis, GC-QQQ, backflush, retention time locking*

Q12

PESTICIDE ANALYSIS USING GC×GC-TOFMS & HYDROGEN CARRIER GAS - A PROOF OF CONCEPT STUDY

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Analysis of pesticides in foodstuffs is a routine, yet often complex analytical requirement, particularly in food materials containing significant levels of interfering matrix materials, which can adversely affect the identification and quantification of numerous pesticides with satisfactory confidence. One approach, using comprehensive two-dimensional gas chromatography (GC×GC) coupled to Time-of-Flight mass spectrometry (TOFMS), allows the impact of interferences from complex food matrices to be resolved, by a combination of significantly enhanced separation capacity together with fast acquisition, un-skewed, full mass range data collection. This results in far superior chromatographic resolution of pesticides and allows effective use mass spectral deconvolution, therefore improving the detection and quantification confidence.Currently, due to significant issues with helium supplies, both in terms of availability and increasing costs, much attention is focussed on the use of Hydrogen as an alternative carrier gas, due to the ease of using generators to source it abundantly and at high purity.
In this study, we performed a proof-of-concept evaluation of the analysis of various pesticide chemistries with hydrogen carrier gas, using GC×GC and a unique TOFMS technology design. Comparisons of mass spectral fragmentation, dynamic range, sensitivity, robustness, chromatographic resolution and run times, obtained with both helium and hydrogen, were performed. The results demonstrated both carrier gases gave very similar mass spectral fragmentation and similarity for NIST MS library matching, similar sensitivity and dynamic range and also the possibility to reduce analysis time using hydrogen.

Keywords: *GC-MS, hydrogen, GCxGC, pesticides analysis*

Q13

PESTICIDE RESIDUE REMOVAL FROM TOMATO AND LETTUCE BY NON-THERMAL DECONTAMINATION PROCEDURES

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In the past few years, there has been an increase in the consumption of ready-to-eat vegetables as lifestyle and dietary trends changed. Producers, needing to keep up with demand, tend to use agrochemicals to aid their production systems, therefore, there's also a need for manufacturers to reduce the amount of any potential residue left in the crops to ensure consumer safety. Several non-thermal technologies are being used, either on their own or combined, like ozone, ultraviolet light, and high-power ultrasound baths.
In this work, we focused on the effects of four cleaning solutions, ozone and ultrasound baths on the amount of pesticide residue left on lettuces and tomatoes. Different pesticides, from several families, types, and modes of action, were applied to these crops grown in controlled conditions. The residues were analyzed with an acetate QuEChERS method using an HPLC-MS/MS system (1). Additionally, water used during the cleaning methods was tested for pesticides, using an SPE-based method and analyzed by HPLC-MS/MS, and LC-QTOF-MS/MS to check whether the pesticides were degraded or just removed from the vegetables.
The QuEChERS method presented, quantitation limits as low as 10 ppb in Chlorsulfuron and Pendimethalin, for example, as this is the lowest limit shown in the Maximum Residue Limits allowed in the European Union (2). Relative standard deviation during validation was below 20% for all compounds, and the detection limit was 5 ppb for pesticides like Hexythiazox Methyl and Fenhexamid, among others.
Regarding the decontamination procedures, in lettuce (3), all the tested methods resulted in a diminution of residues without significant differences among them while for tomatoes significant differences were obtained (Tukey test, alpha=0.05).
This work shows how effective the use of non-thermal treatments can be on pesticide decontamination, and their true power of them, particularly when used in vegetables.

[1]European Commission DG-SANTE. Document No. SANTE 11312/2021. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.
[2]European Commission Database https://ec.europa.eu/food/plants/pesticides/maximum-residue-levels_en
[3]Alonzo, N.; do Carmo, H.; Paullier, A.P.; Santos, I.; de Mattos, B.; Irazoqui, M.; Pareja, L. Effects of Cleaning Procedures on the Concentration of Pesticide Residues on Crisp Fresh-Cut Lettuce (cv. Vera). Biol. Life Sci. Forum 2021, 6, 53. https://doi.org/10.3390/ Foods2021-11023

Keywords: *pesticide removal, QuEChERS, lettuce, tomato, non-thermal decontamination procedures*

Q14

MULTI-RESIDUE ANALYSIS OF PESTICIDES REGULATED BY THE COLORADO STATE IN HEMP PLANT MATERIAL

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Pesticides are used to protect hemp crops from pests and diseases and improve growth yield. Contamination of plants with pesticides may occur not only due to non-optimal direct application, but also due to uptake of pesticides from soil. Modern pesticides are relatively safe and degrade rapidly; however contaminated soil may contain highly toxic persistent compounds use of which has been banned many years ago. Monitoring of pesticide residues in hemp and related products using multi-residue methods with large analyte scope is crucial to ensure consumer safety and compliance applicable regulations. In the United States, pesticides in hemp are regulated at the state level with target lists and action limits differing largely between states. The most stringent regulation in hemp with action limits for certain analytes as low as 0.01 mg/kg has been issued by Colorado. In this study, a QuEChERS-based procedure using both LC-MS/MS and GC-MS/MS techniques was developed and validated for the analysis of 102 pesticides regulated by Colorado state in hemp plant material. The validation experiments evaluated selectivity, accuracy, precision and limits of quantification (LOQ). The determined LOQs were at or below the Colorado action limits with spike recoveries within 70-120% and respective relative standard deviations below 20% for the majority of target analytes.

Keywords: *hemp plant, multi-residue method, LC-MS/MS, GC-MS/MS*

Q15

VALIDATION OF A PESTICIDE RESIDUE METHOD FOR ANALYSIS OF FOOD OILS USING MICRO SPE CLEAN-UP

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According to Euro Stat, the world production of vegetable oils is almost 400 million tons per year. The oils originates from oilseeds, palm, soya beans, rapeseed and sunflower. A major part of the oils is used for cooking, both in private household and in the food industry. As pesticides are used during the production of the oilseeds, it is necessary to check the Maximum Residue Levels in oils. It is well known that fatty matrices are difficult to analyse, as documented in EUPT-CF15 on rape seed cake, where the results showed high standard deviations of 25-38% [1)]. Methods to ensure robust results with a minimum of manual labour is of high importance.
The National Reference Laboratory in Denmark has developed a method for food oils and validated it on three different matrices, rapeseed oil, soya oil and sunflower oil using acetonitrile extraction and µSPE clean-up. A pilot study evaluated the amount of sample to be extracted (1 or 2 g), and whether addition of water to the samples would improve extraction and optimise the clean-up. The final method was based on one gram of sample added 2 ml of water with an extraction using 10 ml ACN. The extract was cleaned-up using a stand-alone Thermo Scientific™ TriPlus™ RSH™ multi-purpose autosampler and commercially available µSPE cartridges containing magnesium sulphate, primary-secondary amine, C18, and graphitized carbon X. The extracts were analysed on GC-MSMS and LC-MSMS.
The validation was performed according to SANTE/11312/2021 at three spike levels, 0.01 mg/kg, 0.05 mg/kg and 0.1 mg/kg with standard mixtures containing >400 compounds. To obtain satisfactory sensitivity on the GC-MSMS 5µl was injected. The calibration standards were prepared using the same stand-alone system mentioned above. The results showed that >250 compounds were validated by GC-MSMS and >230 on LC-MSMS with a LOQ of 0.01 mg/kg. [1)] Proficiency Test on pesticide residues in rapeseed cake. National Food Institute, Technical University of Denmark, Lyngby, Denmark. ISBN: 978-87-93565-82-1

Keywords: *pesticide residues, method development, µSPE, clean-up, LC-MSMS, GC-MSMS, method validation*

Q16

A MONOLITHIC CAPSULE PHASE MICROEXTRACTION PROTOCOL COMBINED WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-DIODE ARRAY DETECTION FOR THE MONITORING OF BENZOYL UREA INSECTICIDES IN APPLE JUICE SAMPLES

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Fruit juices may contain harmful residues of pesticides that are associated with ecological and health risks. Thus, it is critical to develop sensitive and accurate methods for the monitoring of pesticides in these edible products. Undoubtedly, the development of environmentally friendly sample preparation protocols is at the forefront of research in analytical chemistry. In this work, a monolithic sol-gel polycaprolactone-polydimethylsiloxane-polycaprolactone (PCAP-PDMS-PCAP) sorbent platform was synthesized and used for the capsule phase microextraction (CPME) of pesticides from juices samples. Here, we propose a simple and sensitive CPME protocol combined with high-performance liquid chromatography-diode array detection (HPLC-DAD) for the monitoring of benzoyl urea pesticides in apple juice samples. Different monolithic sol-gel sorbent encapsulated CPME media were prepared and evaluated for their performance. Among them, sol-gel PCAP-PDMS-PCAP capsules showed the highest extraction efficiency towards the target analytes. The proposed method was optimized and validated in terms of accuracy, precision, limits of detection (LODs), limits of quantification and linearity. Under optimum conditions, the LODs for all analytes were 0.15-0.30 ng mL^{-1}. The relative recoveries of the method ranged between 89.8% and 108.2%, showing good method accuracy. The precision of the CPME-HPLC-DAD method (expressed as relative standard deviation) was better than 8.0% for intra-day study and better than 9.1% for inter-day study. At a final step, the proposed method was successfully used for the monitoring of the target analytes in commercially available apple juice samples.

Keywords: *pesticides, microextraction, fruit juice, HPLC, residue analysis*

Q17

DEVELOPMENT AND VALIDATION OF A RELIABLE METHOD FOR THE ANALYSIS OF GLYCOSIDES OF ACIDIC HERBICIDES

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The aim of the present research is the development and validation of a reliable method for the deconjugation of acidic herbicides' glycoside followed by a QuEChERS extraction, and UHPLC-MS/MS quantitative analysis. Glycosides are the main secondary plant metabolites. Nowadays, the glycosidic metabolites of a number of acidic herbicides are part of the residue definition, meaning that they should be included in the analytical methods as well. Inclusion of the glycoside metabolites as such is only possible for a limited number of compounds for which the analytical reference standards are available. In other cases, only indirect determination is possible after deconjugation and determination of total free acid. For the latter, various options for deconjugation were exploited, chemical (acidic and basic hydrolysis) and nine enzymatic deconjugation options. One specific enzyme proved to be practical and repeatable at different concentration ranges, leading to the complete deconjugation of the compounds of interest. Additionally, a quantitative method for the evaluation of the intact glycosides content was also optimized and validated for the four acidic herbicide glycosides for which reference compounds are commercially available. Both method validations were performed in compliance with the SANTE/11312/2021 document. Several quali-quantitative figures of merit, such as linearity, matrix effect, limit of quantification, recovery, repeatability, and ion ratio were carefully evaluated. The recovery samples, spiked at three different levels, were assessed in two relevant matrices, linseeds and wheat. In both methods the average recoveries were within 70-120% and RSDr <20%. The LOQs achieved during the validation were 0.0025 mg Kg^{-1} and 0.01 mg Kg^{-1} for the free acids and the intact glycosides, respectively. Finally, the LOQs achieved for the free acids are all lower than the MRLs established in the two matrices.

Keywords: *herbicides, conjugates, LC-MS/MS, deconjugation*

Q18

PESTICIDE RESIDUE ANALYSIS IN CANNABIS: OPTIMIZATION OF CLEAN-UP STRATEGY AND COMPARISON OF GC-MS/MS AND GCXGC-MS TECHNIQUES POTENTIAL

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Determination of pesticide residues in such a complex matrix as *Cannabis* is a challenging task, as the plant contains a number of components including phytocannabinoids, terpenes, and phenolic substances, that are co-extracted and impair accurate analysis. Due to a high ´chemical noise´, achieving low LOQs and other performance characteristics for some pesticides is rather difficult. In this study, we focused on the selection of the most effective clean-up strategy for purification of QuEChERS extract obtained by extraction of matrix spiked by 288 GC-amenable pesticides (x mg/kg). Following approaches were tested (i) dispersive solid phase extraction (dSPE) by primary - secondary amine (PSA), (ii) freezing out followed by dSPE using PSA, (iii) dSPE using Chlorofiltr sorbent (from UCT), (iv) dSPE using Supel QuE-Verde (Supelco), (v) dSPE using a mixture of PSA, Bondesil-C18 and EnviCarb, (vi) solid phase extraction by PSA cartridge (from UCT, 1 and 3 mL of extract), (vii) SPE (PSA cartridge) followed by dSPE by PSA. In the first phase GC-MS/MS (Agilent 7890B gas chromatograph coupled with Agilent 7010 GC-TQ mass spectrometer was used). Obtained data were critically assessed against criteria defined in SANTE/11312/2021 document. The best results were obtained by Supel Que-Verde sorbent clean-up with the highest number of pesticide residues, which performance characteristics met the required parameters. In the second part of the study, GCxGC-MS technique (employing Agilent 7890B gas chromatograph coupled with LECO BT 4D TOF mass spectrometer) was used for analyses in order to separate, at least partly co-extracts still remaining in purified extract and improve detectability. When comparing the investigated techniques, the results showed that a higher number of pesticides (85 %) was detected by GC-MS/MS compared to GCxGC-MS (55 %), on the other hand, the latter technique enabled to detect some of the pesticides e.g. carboxin or desmedipham, problematic in GC-MS/MS.

Keywords: *pesticide residues, cannabis plant, GC-MS/MS, GC×GC-MS*

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Q19

SIMULTANEOUS TARGET AND NON-TARGET ANALYSIS OF PESTICIDES AND AFLATOXINS RESIDUES USING UHPLC-Q-ORBITRAP-MS BASED ON QUECHERS EXTRACTION IN BRAZILIAN BABY FOODS

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The aim of this study was the determination of pesticides and aflatoxins in in baby food marketed in Brazil. For that purpose, ultra-high-performance liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry (UHPLC-Q-Orbitrap-MS) was applied. In relation to sample preparation, our main aim was the application of a generic and simple extraction method. Thus, two different extraction procedures based on the literature, WAHSPE (water, acetonitrile, and n-heptane as solvents in combination with solid-phase extraction-based method) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) were tested. QuEChERS methodology combined with dispersive solid-phase extraction (d-SPE) clean-up was applied and primary secondary amine (PSA), octadecylsilane (C18) and C18 with silica coated with zirconium dioxide (Z-Sep+) was selected because it simplifies the extraction of analytes without adversely affecting their recovery. Suitable performance criteria, set by the SANTE/2020/12830 guidelines, were achieved, and therefore, all targeted analytes were successfully validated. The method was applied to the analysis of 50 baby food samples, and 68% of the samples were contaminated with at least one targeted pesticide. Cypermethrin was detected at 10.3 μg kg^{-1} (above maximum residue level (MRL) established by the European Committee (EC)) in a baby sample composed of yam, banana, and strawberry. Furthermore, suspect screening analysis was performed using a homemade database containing 2424 compounds such as pesticides, mycotoxins, hormones, veterinary drugs and their metabolites. Finally, 10 pesticides and one metabolite were detected, including 5 insecticides, 3 fungicides, one growth regulator, one synergist. Additionally, one aldicarb metabolite was presented, demonstrating the suitability of the proposed approach.

Keywords: *contaminants residues, suspect screening, LC-Q-Orbitrap-MS, QuEChERS, baby food*

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Q20

DETERMINATION OF QUATERNARY AMINE POLAR PESTICIDES USING IMPROVED CATION-EXCHANGE SEPARATION TECHNOLOGY COMBINED WITH SUPPRESSED CONDUCTIVITY AND TANDEM MASS SPECTROMETRY DETECTION

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The analytical determination of quaternary amine pesticides is challenging due to their chemical similarity and strong interaction with the sample matrix and cation-exchange columns. Here, we demonstrate direct determination of chlormequat, mepiquat, diquat and paraquat in food products using a high-performance cation-exchange column designed to chromatographically resolve quaternary amine pesticides and inorganic cations. The pesticides were separated using an electrolytically generated methanesulfonic acid gradient from 3 to 25 mM at 0.3 mL/min and 40 °C. After passing through an electrolytic suppressor, which replaces the acid anion with hydroxide to form water, the pesticides were quantified using a Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system coupled to Thermo Scientific™ TSQ ALTIS™ triple quadrupole mass spectrometer, which was operated in the positive mode and with selected reaction monitoring. Oat cereal samples were extracted using the modified Quick Polar Pesticides extraction (QuPPe) method. Acidification of the extracts with formic acid and with hydrochloric acid were both evaluated. For optimum results, chlormequat and mepiquat were extracted with formic acid, whereas paraquat and diquat were extracted with hydrochloric acid. Each injection of extracts of HCL was followed by injection of mobile phase to remove residual matrix co-extractives in order to maintain optimum column performance. The four quaternary amine polar pesticides exhibited good peak shape with peak asymmetries from 1.0 to 1.1 and eluted from the column, baseline resolved and within 20 min. The oat cereal samples used as blank material did not contain paraquat, diquat, mepiquat, or chlormequat. Recoveries of pesticides spiked into the samples were >80% with the limits of determination at the single digit µg/kg levels.

Keywords: *quaternary amine pesticides, cation-exchange, tandem mass spectrometry, suppressed conductivity, polar pesticides*

Q21

HIGH RESOLUTION MASS SPECTROMETRY FOR THE TARGET AND SUSPECT SCREENING OF OVER 600 PESTICIDES IN DIFFERENT FOODSTUFFS: ADHERENCE TO SANTE REQUIREMENTS FOR IDENTIFICATION

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UHPLC coupled with High Resolution Mass Spectrometry is increasingly being used in Target and Suspect Screening for pesticides in food. With increasing numbers of pesticides required to be monitored and, increasingly, the addition of their metabolites, conventional LC- triple quad (LTQ) methods are challenged to provide a single analytical method capable of monitoring > ca. 600 pesticides. Even with sub-msec dwell times, there is an eventual cycle-time limit on how many compounds (MRMs) can be monitored, in unit time, while still providing sufficient data points across the chromatographic peak to provide for sufficient reproducibility of peak areas. While LTQ acquisition frequently provides the highest sensitivities, recent improvements in Q/TOF technologies provide sufficient sensitivity to address the MRLs associated with residue analysis in food. Additionally, since HRMS is essentially a non-target data acquisition, the number of pesticide residues that can be screened for is unlimited with no impact on cycle time by adding additional compounds. Also, the data can be interrogated retrospectively when required.
While Target Screening and quantitation routinely requires the use of a residue reference standard, Suspect Screening requires only that the residue's retention time has been determined, using the screening method employed, and that qualifying fragments have been previously curated, although any 'suspect' hit should still be confirmed using the relevant reference standard. By employing data-independent MSMS acquisition i.e. *All Ions MSMS*, pesticides can be detected and qualified using fragment ion (s) data, as per the SANTE guidelines for compound identification (1). This present study shows the application of a generic UHPLC/QTOF method to high compound-number screening of pesticides and metabolites in a variety of foodstuffs. Using a Target/Suspect Screening method in MassHunter Quantitative software, >700 pesticides and metabolites were simultaneously monitored while complying with required SANTE identification requirements for accurate mass and overlay of the detected ions and analyte peaks respectively. Additionally, supporting data including retention time and isotope patterns were used in providing further confidence in compound identification.

1. Main changes introduced in Document N° SANTE/11312/2021 with respect to the previous version (Document N° SANTE 12682/2019)

Keywords: *pesticides, QTOF, SANTE, screening*

Q22

SUB 1 µG/KG DETECTION OF GLYPHOSATE AND OTHER ANIONIC POLAR PESTICIDES USING QUPPE EXTRACTION AND DETECTION BY LC-MS/MS

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The area of anionic polar pesticide analysis has been evolving over the past 10 years with the adoption of generic extraction methods, such as the QuPPe[1] method, enabling laboratories to take a multi-residue approach for the analysis of these challenging analytes. The extraction performance of the QuPPe method is well documented and is a common approach for the extraction of polar pesticides from various food matrices. With the recent developments in MS detector technology, lower limits of detection and quantification can be achieved for this analysis by utilizing the enhanced negative ion sensitivity of the Xevo™ TQ Absolute Mass Spectrometer. This poster presents results from the performance of the LC-MS/MS method where limits of quantification of 0.5 µg/kg and 2 ug/kg in cereal samples are achievable. The trueness of the LC-MS/MS method was assessed over 10 injections at 1 and 10 µg/kg in cucumber matrix standards and at 10 and 50 µg/kg wheat flour matrix standards. Trueness in cucumber was between 91 to 117% with RSDs between 0.6 to 8.7% and between 96 to 104% in wheat flour with RSDs between 0.5 to 9.2%.

1. Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC - or IC-MS/MS Measurement - I. Food of Plant Origin (QuPPe-PO-Method) –Version 12 (published on EURL-SRM website on July 23, 2021); M. Anastassiades; A.-K. Wachtler; D. I. Kolberg; E. Eichhorn; H. Marks; A. Benkenstein; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; I. Sigalov; S.Görlich; D.Dörk and G. Cerchia.URL: https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

Keywords: *polar pesticides, anionic, food contaminants, glyphosate*

Q23

DETERMINATION OF PESTICIDE RESIDUES IN RICE-BASED BABY FOOD USING ATMOSPHERIC PRESSURE GAS CHROMATOGRAPHY WITH MS/MS DETECTION AFTER EXTRACTION AND CLEAN UP USING QUECHERS

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Reliable analytical methods are needed for detection, quantification, and identification of hundreds of pesticide residues in many different commodities. This poster describes the development and validation of a comprehensive method based on GC–MS/MS for the determination of 166 pesticides in rice-based baby food. Extracts were prepared using a published version of QuEChERS for cereals followed by determination with GC-MS/MS. The use of GC-MS/MS utilizing atmospheric pressure ionization has been shown to offer significant improvements in performance over electron ionization (EI) for pesticide residue analysis, in terms of selectivity, specificity, and speed of analysis. The extremely high sensitivity of the APGC™ Xevo™ TQ-XS System was demonstrated with reliable detection for almost all analytes at concentrations as low as 0.0003 mg/kg, with an injection volume of 1µL. The method was successfully validated in rice-based baby food using the SANTE guidelines document. The results from analysis of the spikes at both concentrations showed that 91 % and 98 % of the analytes were within the required tolerances for recovery and repeatability, respectively. The method is considered sensitive, specific, accurate, and suitable for the determination of residues of a wide range of GC-amenable pesticides for checking compliance with the specific MRLs set for food intended for infants and young children and has the potential for determination at much lower concentrations.

Keywords: *APGC, pesticides, infant food, QuEChERS*

Q24

A NOVEL WORKFLOW TO DETERMINE OVER 1000 PESTICIDE RESIDUES IN COMPLIANCE WITH SANTE 11312/2021 GUIDELINES IN VARIOUS FOOD MATRICES

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Pesticides play an import role in agriculture and food industry to improve the crop and food production. Residues of pesticides remaining in or on commodities such as fruits, vegetables or cereals can cause adverse health effects as well as environmental concerns.
Regulatory agencies have set maximum residue levels (MRLs) for hundreds of pesticides and their metabolites. Most MRLs are set at low ppb levels, which poses significant challenges especially if hundreds of analytes are screened and quantified in complex food matrices simultaneously.
In Europe, pesticide testing laboratories adhere to the SANTE/ 11312/2021 Guideline. This Guideline ensures a consistent approach controlling MRLs legally permitted in food or animal feed.
Due to the huge number of pesticides, the analysis is very elaborate. Very often multiple analytical approaches and laboratory intensive workflows are involved. Both lead to high operating costs and slow turnaround times.
We present a comprehensive, joint LC-MS and GC-MS workflow for the simultaneous quantitation of >1000 pesticide residues in fruits and cereals of varying water content.
Workflow performance was verified according to SANTE/ 11312/2021.
During initial validation, parameters like limit of detection (LOD) and limit of quantification (LOQ), linearity, recovery and precision were evaluated using the method performance criteria described in this Guideline.
Details of sample preparation procedures and instrumentation set up will be discussed in conjunction with the data analysis parameters enabling the quantification and confirmation of pesticide residues.

Keywords: *pesticide analysis, joint LC-MS and GC-MS workflow, simultaneous quantitation of >1000 pesticide, SANTE/ 11312/2021 guideline*

Q25

ANALYSIS OF EUROPEAN PHARMACOPEIA PESTICIDE RESIDUES IN DRY CANNABIS FLOWER USING A DUAL LC-MS/MS AND GC-MS/MS APPROACH

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Since California became the first state in 1996 to legalize medicinal cannabis and its use for medical purposes, legalization in Europe evolved over the last decade, associated with increasing advancements in analytical testing of cannabis flowers. The importance of testing raw material, as well as cannabis and hemp-based products to ensure high quality products for consumers also plays a key role in the food marked. Cannabis and hemp infused food and beverage products, where mostly edible oil as an ingredient with health promoting effects is added, considerably flood the market nowadays.
Besides the determination of cannabinoids (potency), terpenes, mycotoxins, residual solvents and heavy metals, pesticides are one of the major regulated compound groups.Pesticide testing in cannabis and hemp presents many challenges: A large number of regulated compounds needs to be covered, very low method detection limits have to be reached, many different regulations between geographies are existing and high matrix diversity needs to be covered, correspondent to the natural complexity of the different flowers and sorts.
In this work, we present conditions for an analytical GC-MS/MS and LC-MS/MS method under which the pesticides listed in section 2.8.13 of the European Pharmacopeia (EP) are analyzed in dry cannabis flower matrix. A dual LC-MS/MS and GC-MS/MS approach is used for optimal pesticide coverage and accurate testing. A single sample preparation procedure is used for both platforms, ensuring robust operations without sacrificing sample throughput. Sensitivity, linearity, recovery and robustness data will be presented, as well as strategies and recommendations for the best equilibrium between high-quality results and instrument uptime.

Q26

IMPROVING SENSITIVITY WITH ROBUST, FULLY AUTOMATED SAMPLING AND ANALYSIS OF FUMIGANT RESIDUES FROM FOODSTUFFS

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Fumigants – biocidal mists or gases – are applied to various agricultural products prior to storage or transport to control pests. However, this may leave residues that later negatively affect consumer health. Testing for such substances has seen increased scrutiny by regulatory bodies, with the European Union (EU) stipulating maximum residue limits (MRLs) for commonly used fumigants. For example, hundreds of products across the EU have been recalled over the past 2 years due to alarming levels of ethylene oxide (EtO), and its reaction product 2-chloroethanol (2-CE or ECH), being detected above the EU MRL of 0.05 mg/kg in imported consignments. As such, there is a need for a robust, efficient and high-throughput method to extract and analyse fumigants from foodstuffs. Traditionally, analysis of fumigant residues has relied on liquid extraction methods, followed by gas chromatography – mass spectrometry (GC–MS) analysis. Yet, these extractions are time-consuming with many manual steps, often generating large volumes of environmentally damaging solvent waste. The process also extracts non-target components and leading to introduction of a 'dirty' extract to the GC, causing contamination in the liner and subsequent analyses.

Here we present how recent advances for traditional headspace (HS) have enabled development of a sensitive, robust and fully automated sampling and analysis method for fumigant residues from foodstuffs. The Centri platform combines automated syringe-based HS with cryogen-free trap focusing (HS–trap), allowing large HS volumes (up to 5 mL) to be extracted and preconcentrated prior to GC injection. The process of extraction and preconcentration can be repeated several times prior to GC injection to enrich a sample, effectively increasing the amount of each analyte extracted for detection and therefore, improving the sensitivity achieved. This advancement is known as Multi-Step Enrichment (MSE).

We assessed MSE–HS–trap to detect a range of common fumigants from various foodstuffs (e.g., grains, spices, tea). Three-5 mL extractions were taken from the same sample vial and the analytes enriched on the trap, providing an augmented sample extract for analysis. Next the trap was rapidly heated to transfer the analytes to the GC–MS in a narrow band, enhancing chromatography and thus, improving sensitivity further. We found MSE–HS–trap dramatically improved extraction efficiency over traditional static HS, while full automation ensured a reliably robust, repeatable and quantitative method with results indicating excellent linearity and reproducibility. High-throughput was assured by the prep-ahead feature where the next sample begins extraction while the previous sample injection is analysed by the GC, further allowing for unattended analysis of approximately 48 samples a day. As validation we applied our technique to sesame seeds known to be contaminated with EtO, finding high levels of this toxic fumigant present.

Keywords: *ethylene oxide, automation, fumigants, preconcentration*

Q27

PESTICIDE RESIDUES IN APPLES IN THE CZECH REPUBLIC: DO THEY COMPLY WITH ´LOW RESIDUE PRODUCTION´ LABEL?

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Apples represent an important item in the Czech consumers´ basket, nevertheless, a relatively frequent occurrence of pesticide residues is of concern. Czech producers have been focusing on minimizing residues through ´low residue production´, the practice based, among other factors, on the use of pesticides that rapidly decompose after their application. At present, some retail chains apply for the assessment of fruits / vegetables contamination so called ´action thresholds´, the highest acceptable concentration of pesticide residues in respective commodity not exceeding certain percentage of MRL, e.g., 25%. To obtain up-to-date information about the presence of pesticide residues in locally grown and imported apples and fulfilling MRLs for respective pesticides, we monitored residue profiles in a set of various authentic apple cultivares using both LC-MS / MS and GC-MS / MS methods during the years 2019-2021. 400+ compounds were monitored in this study using optimized method enabling to achieve very low limits of quantification (LOQ) with good repeatability. 265 samples of apples harvested in the Czech Republic were analysed (43.4% of total number of samples) and in other countries 56.6% of samples. Pesticide residues were found in 260 of them (98.10% of tested set). In total, 70 different pesticides were quantified. In general, contamination of apples was relatively low. A positive trend was observed when monitoring action threshold 25% MRL – it was not exceeded in 99.7% of domestic apple samples and in 99.3% of imported ones throughout the observed period. A positive trend was also observed when monitoring action threshold 1%MRL - for domestic apples 51.9% of samples in 2019, 60.7% in 2020 and 62.2% in 2021, respectively and for imported apples 39.7% of samples in 2019, 48.6% in 2020 and 57.3% in 2021). While acetamiprid, captan, methoxyfenozide, pyraclostrobin and pyrimethanil were the most frequently occurring in Czech apples, acetamiprid, boscalid, captan, fludioxonil and pirimicarb sum predominated in imported ones.

Keywords: *apples, pesticide residues, comparison of locally grown and imported apples*

Acknowledgement: *This work was supported from the grant of Specific university research – grants No A1_FPBT_2022_005, project "Research of metabolomic methods for laboratory authentication of apples geographicity", supported by QK - Applied Research Program of the Ministry of Agriculture 2017-2025, CZECH REPUBLIC (QK1910104) and METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, which we are gratefully acknowledged.*

Q28

A FACILE AND RAPID DETECTION OF CHLORPYRIFOS EMPLOYING GOLD NANOPARTICLES FOR SURFACE-ENHANCED RAMAN SCATTERING (SERS)

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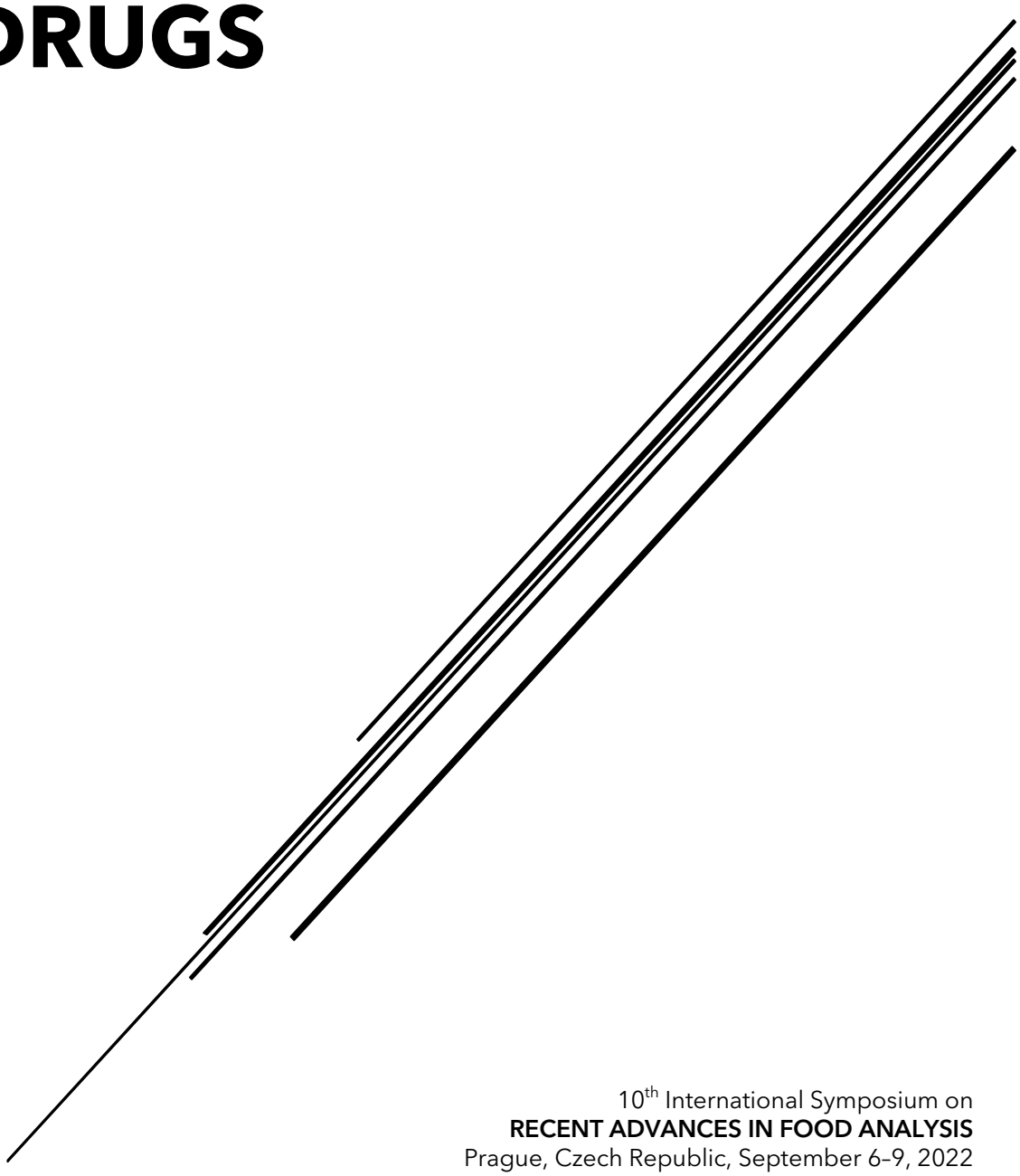
Chlorpyrifos (CPY) is a ubiquitous insecticide that in excess concentration has been shown to cause detrimental effects on the environment and human health. Therefore, developing a highly sensitive, economic, and rapid screening test for determining trace amounts of CPY is important. In this study, the effect that Sodium chloride (NaCl) induced charge-based gold nanoparticle (AuNPs) aggregation has on the detection limit of CPY when combined with surface-enhanced Raman spectroscopy (SERS) is investigated. A cost-effective and chemical stably AuNPs were synthesized using the Turkevich method as a SERS substrate to detect CPY. It was observed that high concentrations of CPY (≤15 mg/kg) induced significant AuNPs aggregation, owing to the presence of amine and phosphorothioate functional groups, improving SERS performance. Nevertheless, at lower CPY concentrations (≥10 mg/kg) AuNPs aggregation wasn't as pronounced, and the CPY could no longer be detected. Therefore, NaCl was added to further induce aggregation and improve the limit of detection. Due to the synergistic aggregation of AuNPs between CPY and NaCl, 0.1 mg/kg of CPY could be detected. In conclusion, the results show that the addition of NaCl can reproducibly improve the SERS detection limit of chemical contaminants in a simple solution using gold nanoparticles. The next step will be to apply SERS coupled with NaCl to the detection of CPY in different food matrices to assess its viability as a screening test in the future.

Keywords: *chlorpyrifos, SERS, gold nanoparticles, sodium chloride, food matrices*

RESIDUES - VETERINARY DRUGS



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R1

DEVELOPING AN UNTARGETED SCREENING METHOD FOR CONTAMINANTS IN ANIMAL FEED

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Zilpaterol was first discovered in the Irish equine industry in 2020. This compound is a beta agonist and legally used in the United States to promote weight gain in livestock that are near slaughter, whereas in the EU and most of the rest of the world, it is an illegal substance. The discovery of zilpaterol in Ireland is of great concern to animal feed and livestock producers, and coupled with the war in Ukraine that forced changes in the global feed supply network, there is a strong impetus to protect these businesses and humans from contaminated products. In response to these issues, an untargeted workflow has been developed to combat feed contaminants.
The workflow developed uses dilute and shoot techniques coupled with an Agilent 6546 LC-QToF. Dilute and shoot was chosen for sample preparation, because it employs a minimal amount of analytical steps so as to retain as many compounds as possible in the extract. Samples are extracted using both neutral and acidified solvent solutions, centrifuged, and filtered into 2-mL autosampler vials before LC-QToF analysis. After an analytical run is completed, the data is then analysed using Agilent MassHunter Quant software coupled with a number of compiled Agilent databases including forensic toxicology, mycotoxins, pesticides, veterinary drugs, and water contaminants. A stringent set of parameters has been set for the database searches to discourage false positive results, including overall score, retention time, number of fragments, and presence of qualifying ions. This workflow is designed to be used commercially in the analytical space just before LC/MS/MS confirmation and quantitation, because it will allow the analyst to add the positively detected compounds to their existing quantification method.

Keywords: *dilute and shoot, untargeted workflow, feed contaminants*

Acknowledgement: *Agilent Corporation, Agri-Food Quest. Food Fortress*

R2

ULTRA-HIGH SENSITIVITY QUANTIFICATION OF VETERINARY DRUG RESIDUES IN ANIMAL BY-PRODUCTS

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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. Several compounds found in these by-products have a maximum residue limit (MRL) to minimize their use, and some compounds are prohibited due to their inherent toxicity. To limit these compounds within the food industry, it is important to achieve LOQ values that are as low as is reasonably possible.
Here, we present a method for analyzing over 180 compounds used in the veterinary industry that can achieve LOQ values as low as 0.005 ng/mL.The pork, milk or chicken (5 g) was weighed before adding EDTA-McIlvaine buffer. All food samples were homogenized, and acetonitrile and ammonium sulfate were added. Samples were mixed and centrifuged. The upper layer was partially transferred into an evaporative vial and DMSO was added. The sample was evaporated, resuspended in water, vortexed, centrifuged and filtered. Post-spike sample preparation was then performed. Chromatographic separation was performed using a Phemomenex Kinetex Polar C18 (2.6 µm, 100 x 2.1 mm). A SCIEX Triple Quad 7500 system was operated in scheduled multiple reaction monitoring (sMRM) mode using electrospray ionization (ESI) with fast positive and negative switching.
When spiked into matrices, LOQs of 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk were achieved. This high level of sensitivity allows routine laboratories to further dilute their samples to minimize any matrix effects observed. The linear range of each compound analyzed has been assessed, with ranges spanning up to 4 orders of magnitude and r values >0.99. 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. In addition, chromatographic separation is important to minimize the number of compounds analyzed at any one time. This allows for a balance between the cycle time of the mass spectrometer and the dwell time for each analyte so that accurate quantification can be performed for each compound. In addition to MRLs, minimum method performance requirements (MMPRs) are recommended by the EU for some prohibited compounds, which are summarized here. In these instances, the sensitivity of the analysis is paramount to ensure the MMPR is achieved or exceeded. In this method the MMPR has been met or improved upon, showing that even for the most difficult to analyze compounds, this method can achieve or improve on the recommended levels of sensitivity.

R3

LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) METHOD FOR DETECTION OF CHLORAMPHENICOL AND NITROFURANS RESIDUE IN FOOD AND FEED

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A simple and sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method to determine chloramphenicol, nitrofurans and nitrofurans metabolites in food and feed is presented. The sample preparation procedure included acid hydrolysis and derivatization steps to aid the detection of the nitrofurans. After simple purification the analytes were analysed in LC-MS/MS. The proposed method has been validated under European Commission Regulation 2021/808. The validation procedure includes determination of specificity, decision limit and precision. The specificity was verified by analysing each considered matrix (muscle tissue, honey and feed). Precision (CV) ranged from 12.2 to 16.1% for muscle, from 11.2 to 15.6% for honey and from 10.3 to 16.3% for feed. Linearity for the examined nitrofurans and chloramphenicol was verified from 0.2 to 2.0 μg kg^{-1}. The decision limits were lower than 0.5 μg kg^{-1} for nitrofurans and lower than 0.1 μg kg^{-1} for chloramphenicol. The accuracy of the method expressed in terms of % CV was less than 19%, for all analysed drugs and for all validated matrices. Under the Official Control Plan activity, several samples, including those from non-European regions, were positive for nitrofuran residues, such as SEM,AMOZ, AOZ, and CAP. The method is used for confirmatory testing of nitrofurans and CAP residues in food and feed and is therefore suitable for laboratories involved in official controls.

Keywords: *nitrofurans, chloramphenicol, LC-MS/MS*

R4

HIGH THROUGHPUT IDENTIFICATION OF ANABOLIC STEROID ESTERS BY COMPACT ATMOSPHERIC SOLID ANALYSIS PROBE MASS SPECTROMETRY SYSTEM

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In food control laboratories, the analytical methodologies usually include sampling, transportation and sample preparation procedures before the subsequent determination of target compounds using chromatographic separation techniques with different detection systems. However, this workflow includes many steps and therefore is time-consuming, hinders the analysis throughput and requires significant amounts of solvents and reagents. Therefore, there is a great interest in developing direct and fast methods for screening food samples, especially during food safety emergencies.
Under this scenario, ambient ionization mass spectrometry (AIMS) techniques permit the analysis of samples in their native environment with minimal or no sample treatment. Additionally, AIMS permits the on-site analysis with miniaturized MS systems, reducing transportation cost and further the analysis time, improving the laboratories throughput. Today, few AIMS techniques, such as desorption electrospray ionization (DESI), direct analysis in real-time (DART), laser ablation electrospray ionization (LAESI), and atmospheric solids analysis probe (ASAP), are commercially available. Still, they are mainly used for research purposes, and their potential for on-site analysis is unclear [1]. For this reason, research and development of new methods with AIMS and (trans)portable mass spectrometers are necessary for future on-site chemical analysis in food samples.
This work, evaluated the feasibility of a compact atmospheric solids analysis probe (ASAP) - single quadrupole mass analyzer system for the screening and rapid identification of anabolic steroid esters [2]. For this purpose, the most critical operational parameters such as sample introduction, applied scan time, and source temperature were optimized. Besides, to improve the techniques selectivity, in-source fragmentation of the selected seventeen steroid esters, commonly found in illicit samples, were determined by applying different cone voltages (12, 20, 30, and 40 V). In addition, a spectral library, based on the four stages of in-source fragmentation spectra, was created for these steroid esters. Finally, the applicability of this method was demonstrated for the rapid identification of steroid esters in oily injection solutions, providing test results in less than 2 min.

[1] Arrizabalaga-Larrañaga A, Ayala-Cabrera JF, Seró R, Santos JF, Moyano E. Chapter 9 - Ambient ionization mass spectrometry in food analysis. In: Galanakis CM, editor. Food Toxicology and Forensics. Academic Press; 2021. p. 271-312.
[2] Arrizabalaga-Larrañaga A, Zoontjes PW, Lasaroms JJP, Nielen MWF, Blokland MH. Simplified screening approach of anabolic steroid esters using a compact atmospheric solid analysis probe mass spectrometric system. Analytical and Bioanalytical Chemistry. 2022;414(11):3459-70.

Keywords: *ambient ionization, mass spectrometry, on-site testing, atmospheric solid analysis probe, transportable MS*

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R5

EVALUATION OF A RECENTLY INTRODUCED GC-ORBITRAP

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The number of compounds that must be analyzed in food safety analysis is increasing each year. Also, questions from competent authorities change; besides knowing which (new) compound is used, they also want to know when this new compounds appeared for the first time in samples, so-called retrospective analysis. Current analyses are mainly based on triple-quad mass spectrometers, which are primarily used for targeted analysis. High-resolution mass spectrometry (HRMS) is much more flexible since the analyzed compounds are not defined on beforehand. With HRMS, compounds are typically selected during data analysis, and new compounds can be searched for retrospectively in the data.
Of course, it is essential to have sensitive and specific HRMS equipment. In the last 20 years, HRMS equipment has improved tremendously in these two aspects and can often compete with triple quadrupole analyzers. In this study, a recently introduced HRMS system, the GC-Exploris, was evaluated for the applicability of the identification and quantification of steroids in urine matrix. Several aspects were assessed during this evaluation e.g., chromatographic characteristics such as S/N ratio, retention time, peak width, resolution (Retention factor (K), plate number (N), and selectivity factor (α) were determined using factorial design), and HRMS related parameters such as C-Trap Offset and Resolution (60000 vs. 120000 vs. 240000). Results from this evaluation are presented and discussed.

Keywords: *Orbitrap, retrospective analysis, steroids*

Acknowledgement: *This project was financially supported by the Dutch Ministry of Agriculture, Nature and Food Quality (project KB-37–002-037).*

R6

DYES RESIDUES IN FISH PRODUCT BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY, VALIDATION STUDY ACCORDING TO EUROPEAN COMMISSION REGULATION 2021/808

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A specific, sensitive and rapid liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to determine malachite green (MG), leucomalachite green (LMG), crystal violet (CV), leucocrystal violet (LCV), methylene blue (MB) and brilliant green (BG) in fish muscle is presented. Extraction of drug residues was performed with acetonitrile, followed by a clean-up with neutral alumina and PSA solid-phase extraction. Dye analysis was performed using liquid chromatography-electrospray ionisation-tandem mass spectrometry (LC-ESI-MS/MS). The method is the first validated in accordance with the requirements set by European Commission Regulation 2021/808. Method's recoveries were calculated adding 0.25, 0.5, 0.75, and 1.0 µg Kg^{-1} to a blank sample and spread from 82 to 105 % for fish and 79 to 106 % for shrimp. Intra-day precision (RSD) ranged from 8.1 to 15.0% for fish and from 9.1 to 16.3% for shrimps, while inter-day precision values extended from 10.2 to 17.6% and from 10.6 to 18.4% respectively for fish and shrimps. Linearity for all analytes was verified from 0.25 to 5.0 µg kg^{-1}. The decision limits (CCα) ranged from 0.3 to 0.4 µg kg^{-1} and from 0.3 to 0.5 µg kg^{-1} for fish and shrimps, respectively. The developed method is currently in use for the confirmation of official control analysis of fish products.

Keywords: *dyes, LC-MS/MS, residue*

R7

FACTORIAL DESIGN-BASED VALIDATION OF A CONFIRMATORY METHOD FOR THE DETERMINATION OF BETA-AGONISTS IN URINE IN ACCORDANCE WITH CIR 2021/808

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In 2020, the EURLs active in the field of veterinary drug residue analysis updated their recommendations for the control of prohibited veterinary drugs in several matrices by introducing the so-called minimum method performance requirements (MMPRs). These recently proposed concentration levels are mostly lower than the "recommended concentrations" previously in effect. Furthermore, Commission Implementing Regulation (EU) 2021/808 revising the performance criteria for the analysis of veterinary drug residues in food and feed entered into force in 2021. As a consequence of the adapted requirements, the BVL's confirmatory multi-residue method for the determination of beta-agonists in urine needed to be updated and revalidated.
The newly developed method consists in principle of the following steps: First, the samples are spiked with internal standard. After an equilibration period, the urine samples are hydrolysed overnight with glucoronidase/sulfatase solution. The following day, phosphate buffer is added and the sample solutions are centrifuged before adding methanol to the supernatant. In order to further reduce matrix interferences, an SPE clean-up using ScreenDau cartridges is conducted. After conditioning and sample loading, the SPE cartridges are washed with acetic acid before eluting the cleaned extracts with a freshly prepared mixture of ethyl acetate and ammonia. Finally, the eluates are evaporated to dryness, reconstituted in LC eluent A, centrifuged using filtration tubes and analysed by UPLC-MS/MS. Quantification using a matrix calibration proved to yield reliable results.
For the validation of the method, the alternative validation concept was applied. To prove the robustness of the method, systematic variations of the operator (routine, occasional), matrix properties (bovine and porcine urine, lyophilised and fresh), storage of the final extract (no storage, storage for 2-3 days at +4 °C), as well as the variation of the injection volume (5 µL, 10 µL) were investigated in a total of eight analytical series. The method was successfully validated for 28 beta-agonists and two separate UPLC-MS/MS systems in accordance with the requirements prescribed by CIR 2021/808 and the MMPRs. The suitability of the method was assessed by the analysis of incurred sample material and reference material.

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

R8

VALIDATION OF A MULTI-RESIDUE METHOD FOR THE DETERMINATION OF 31 COCCIDIOSTATS, 13 NITROIMIDAZOLES AND 5 DUAL-USE SUBSTANCES IN LIVER BY ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TANDEM MASS SPECTROMETRY

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A confirmatory LC-MS/MS multi-residue method was developed and successfully validated in accordance with Commission Implementing Regulation (EU) 2021/808 for the simultaneous determination of 6 ionophores, 25 chemical coccidiostats, 13 nitroimidazoles and 5 dual-use substances in liver. Due to the different MRLs and/or MLs / MMPRs of the individual substances in different animal species, the method was first validated in poultry. In a second step, the validation was extended to all remaining species (bovine, ovine, porcine and rabbit) for which anticoccidials are authorised or potentially used.
The method is suited to control a number of veterinary drugs including authorised substances with established MRLs or MLs and non-authorised or banned substances (as e.g. nitroimidazoles). For the validation, the alternative approach according to Commission Implementing Regulation (CIR) 2021/808 was applied and the method proved to be rugged against changes in the operator, in animal species, in matrix conditions, in HPLC columns and in the storage of the final extract. By applying a factor-comprehensive in-house validation concept, merely eight runs at different concentration levels – if analytically possible, starting at 0.1 x MRL or ML – were analysed in order to gain comprehensive knowledge about the reliability, robustness and performance of the method. The quantitation of the analytes was performed by way of linear matrix calibration curves. The reported validation parameters CCα, the recovery, the relative repeatability standard deviation and the relative within-laboratory reproducibility fulfil the method performance criteria set in Commission Implementing Regulation (EU) 2021/808.

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

R9

DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC MS/MS) METHOD FOR THE DETERMINATION OF FIVE NITROFURAN METABOLITES IN MILK IN ACCORDANCE WITH CIR (EU) 2021/808

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Nitrofurans and their metabolites are banned within the European Union due to mutagenic properties. As such, they are listed in Table II of the annex of the Commission Regulation (EU) No 37/2010.
Commission Regulation (EU) 2019/1871 set reference points for action (RPA) at 0.5 µg/kg for nitrofuran metabolites, effective from 28th November 2022. So far, available methods within the German official control laboratory network do not cover milk as a matrix. Furthermore, dinitrosalicylic acid hydrazide (DNSH), the marker metabolite of nifursol, needs to be measured in negative mode and the other nitrofuran metabolites are analyzed in positive ionization mode. Therefore, two analytic runs are required on older instruments that are not capable of fast polarity switching. In order to fulfil the requirements and speed up the analytic procedure, the hereby presented method was developed and validated.
The method is based on a method originally developed and provided to the European network of official control laboratories by the European reference laboratory in Fougères (France) for the analysis of nitrofuran metabolites in meat. The method consists of a combined hydrolysis and derivatization step (NBA/HCl) followed by neutralization (NaOH) and extraction with ethyl acetate. After the evaporation of the extractant and reconstitution in the HPLC solvent, the sample is analyzed via LC-MS/MS.
Validation was performed in accordance with CIR (EU) 2021/808 using the factorial validation approach. Validation factors included operator (experienced vs. inexperienced), derivatization time (overnight vs. two hours), reconstitution (vortex vs. ultrasonic bath), and two individual instrumental setups of the same make and model (Sciex 6500+ with Agilent 1290 HPLC).
Achieved CCα were between 0.289 µg/kg and 0.365 µg/kg for all five analytes. Recoveries at CCα were found to be at 99.2 - 103.3% and the within laboratory-reproducibility at CCα was at 11.6 - 15.5%. As required by CIR (EU) 2021/808, the matrix effect did not exceed 20% across 20 independent measurements.

Keywords: *nitrofurans, LCMS, veterinary medicine, residue analysis, milk*

R10

MULTIPLEXED DNA DIRECTED IMMUNOARRAY FOR THE DETECTION OF VETERINARY RESIDUES IN COW'S MILK

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Background: Cow's milk containing traces of antibiotic residues poses high risks for consumers, dairy industries, and the environment. Therefore, fast and affordable screening tools to identify these compounds in a reliable manner are essential. In this study, DNA directed Immobilization (DDI) is used to develop a multiplexed microarray platform for screening three classes of antibiotic residues in milk samples. Throughout DDI approach, the hybridization of complementary strands is exploited to direct specific molecules conjugated to oligonucleotides (Oligo-$_{Nup}$) over their counterparts immobilized onto glass slides (Oligo-$_{Ndown}$) in fluorescent microarray format. The application of biosensing platforms in the real field is one of the main challenges along the development of novel technologies and further development is desirable.
Methods: In first place, three different Oligos-N_{down} containing a 5'-NH_2 end were covalently immobilized over epoxy derivatized slides using a piezo-dispense arrayer. Then the complementary strands, that were previously conjugated by chemical means to the haptens (structural analogues of antibiotic) are incubated and guided through DDI over the respective spots. After an incubation, the pool of three monoclonal antibodies (MAb's) for tylosins, sulphonamides and fluoroquinolones were mixed in milk samples and then added over the array. Following an indirect competitive format, then a secondary labelled antibody is utilized for subsequent quantification by fluorescent readout. Matrix effects studies were performed to define optimum conditions for the assay.
Results: Among the most commonly found antibiotics, three target analytes were selected as references for this study, including Tylosin A (TYLA), Sulfathiazole (STZ) and Ciprofloxacin (CIP). For each analyte, the limits of detection archived with this platform were 7.15 µg/kg (TYLA), 8.35 µg/kg (STZ) and 4.45µg/kg (CIP) in direct matrix. According to EU guidelines Maximum Residue Limits (MRL) are set at 50 ug/kg for Tylosins and 100ug/kg for Sulfonamides and Fluoroquinolones in milk showing the potential as screening tool, to undergo a confirmatory analysis if required. On the other hand, studies of cross reactivity with other antibiotics were performed as well as shared reactivity between the same family of compounds showing no interference when the antibodies are employed in a "cocktail" format.
Pre-Validation: Our system allows the multiplexed processing of 22 samples in parallel over a single glass slide, demonstrating the high-throughput capacity of this approach. Furthermore, a pre-validation study spiking milk samples above and below the MRL for the selected antibiotics was performed over 20 randomly distributed samples discriminating between 18 antibiotics belonging to the family of selected residues with high accuracy.

Keywords: *veterinary residues, multiplexation, DNA-directed immobilization, microarray, screening*

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R11

DEVELOPMENT AND VALIDATION OF AN HPLC-MS / MS MULTI-CLASS METHOD FOR THE ANALYSIS OF DIFFERENT CLASSES OF VETERINARY DRUG RESIDUES IN MILK AND POULTRY FEED

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Constant use of veterinary drugs in livestock leads to issues such as residue monitoring, cumulative risk assessment, antimicrobial resistance, and environmental contamination. As a consequence, it is important to set regulatory limits and maintain them with the help of reliable analytical methods. However, the development of a comprehensive analytical method for different compound classes that saves time but retains sensitivity and robustness is still an ongoing task globally. This study aimed to develop and validate an HPLC-MS/MS multi-class method for the analysis of different types of veterinary drug residues in milk and poultry feed. The sample preparation protocol based on the "dilute and shoot" approach previously used for multi-mycotoxin detection (Malachová et al. (2014)) was followed and further optimized in this study. Method validation for >150 analytes was conducted according to the SANTE validation guideline. Method performance characteristics such as linearity, limits of detection, limits of quantification, precision, accuracy, and repeatability were examined. Achieved limits of detection were lower than maximal residual limits (MRLs) for veterinary drug residues in milk for the vast majority of analytes. Limits of quantification for >80% of the analytes in milk were between 10 and 50 µg/kg and lower, while for polutry feed regulations of MRL limits are still not available. Intermediate precision complied with the SANTE criterion of RSD <20% for almost 90% of the analytes. Milk samples were affected by matrix effects with a signal enhancement for 25% of analytes above 120% compared to solvent standards. In contrast, strong signal suppression was observed in polutry feed, a much more complex matrix, with 40% of analytes detected below 70% when compared to the solvent standards. The validation results show that a majority of analytes (80-90%) comply with the SANTE criteria for accuracy with a recovery of the extraction of 70-120%.

Keywords: *veterinary drug residues, HPLC-MS/MS, poultry, feed, milk, validation*

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R12

FEASIBILITY STUDY ON THE JOINT EXTRACTION OF 105 VETERINARY DRUGS FROM MEAT AND DETECTION BY LC-HRMS

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A feasibility study for the simultaneous determination of 105 veterinary drugs or respective metabolites in meat is presented. The aim was to determine analytes, some of which differ greatly in their chemical properties, with one method. The analytes came from the substance classes benzimidazoles, flukicides, benzoylphenylureas, fluoro- and other quinolones, macrolides and lincosamides, pleuromutilines, sedatives, sulfonamides and diaminopyrimidines. The classes were selected according to two criteria. Firstly, classes were selected that are frequently requested together in meat samples for industrial self-control. Secondly, only analytes for which the respective minimum method performance requirements (MMPRs) [1] or enforcement of (cascade) maximum residue limits (MRLs) [2] can be met with a short sample preparation without an evaporation step were included.
For sample preparation, a simple QuEChERS-adapted extraction without clean-up step was used. The influences of different factors relating to extraction solvents, acid concentration, as well as various salt types and amounts for phase separation were investigated. The selection of salts was a crucial step to find the best compromise in terms of good recovery for all substance classes. Stability tests were performed to find the best composition of the final measurement solutions. Hence, standards could be used in the autosampler for at least one working week. A liquid chromatographic method was developed using two precolumns with orthogonal selectivity and one analytical column. In this way, adequate retention and good peak shapes for >100 of the analytes was achieved. Co-elution of analytes was reduced, so that sufficient fragmentation of all substances was assured. Furthermore, all analytes with identical monoisotopic mass were baseline separated. The parameters of the HESI source and of the high-resolution mass spectrometer (Thermo Scientific QExactive™) were optimized for all analytes to obtain the most intense and interference-free signals. For a first method and matrix, the feasibility to determine 105 veterinary drug analytes in meat was demonstrated. All analytes were detectable in chicken muscle when spiked at low levels. Currently, the method is being optimized, especially with regard to salting out and the choice of internal standards. Further matrix tests are planned. We are confident that after this fine-tuning, the method will meet all performance criteria of the Commission Implementing Regulation (EU) 2021/808 [3] and can be validated according to it.

[1] EURL (2022) EURL Guidance on MMPRs; https://eurl-residues.eu/wp-content/uploads/2022/06/EURL_MMPR_guidance_endorsed.pdf
[2] EU Commission (2022) Current consolidated version of Commission Regulation (EU) 37/2010; EUR-Lex, Document 02010R0037-20220509; http://data.europa.eu/eli/reg/2010/37(1)/2022-05-09
[3] EU Commission (2021) Commission Implementing Regulation (EU) 2021/808; Official Journal of the European Union, L 180/84

Keywords: *veterinary drugs, LC-HRMS, multi-class method, salting out*

R13

DEVELOPMENT OF AN ELISA FOR THE DETECTION OF NIFURSOL METABOLITE DNSH IN MEAT AND SEAFOOD AND VALIDATION IN ACCORDANCE WITH COMMISSION IMPLEMENTING REGULATION 2021/808

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Nifursol is a nitrofuran antibiotic banned as a feed additive in the European Union and other countries. Nifursol is metabolised to 3,5-dinitrosalicyclic acid hydrazide (DNSH) in living organisms and it is a marker for the detection of illegal use of nifursol in animal husbandry. The detection of DNSH by LC-MS/MS requires derivatisation of this metabolite with 2-nitrobenzaldehyde to NPDNSH, similar to other nitrofuran metabolites such as SEM, AHD, AMOZ and AOZ. In accordance with Commission Regulation (EU) 2019/1871 a reference point of action of 0.5 µg/kg for DNSH and other nitrofuran metabolites applies from 28 November 2022.
Due to an increasing demand for monitoring for the presence of DNSH in meat and seafood an ELISA method for the specific detection of DNSH was developed and validated. A polyclonal antibody able to recognise DNSH directly, without derivatisation with 2-nitrobenzaldehyde, was produced in rabbits. The competitive ELISA method with sequential incubation of the reagents with a total time of 60 min was developed. A new sample preparation method was optimised and included sample hydrolysis to release bound metabolites and salting-out assisted liquid-liquid extraction. The developed method is simple and does not require any evaporation step for analyte concentration. The sample is analysed directly after acetonitrile phase dilution in assay buffer.
DNSH ELISA was optimised and validated with incurred samples obtained in a feeding study in chicken. The incurred samples were analysed by DNSH ELISA and the results were compared with the results obtained by UPLC-MS/MS method. A good correlation between the two method was found. DNSH ELISA was further validated in accordance with Commission Implementing Regulation 2021/808. The detection capability was found to be 0.25 µg/kg in meat and seafood which corresponds to the half of the refrence point of action (RPA). The method will be transformed into a first commercial ELISA kit for the specific and sensitive detection of nitrofuran metabolite DNSH.

Keywords: *DNSH, nifursol, ELISA, screening, food analysis*

R14

NEW SIMPLE AND EFFICIENT UPLC-MS/MS METHOD FOR THE DETERMINATION OF TOTAL RESIDUES OF NIFURSOL MARKER METABOLITE IN POULTRY MUSCLE TISSUES: DEVELOPMENT, VALIDATION AND APPROVAL ON INCURRED SAMPLES

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Nifursol (nitrofuran, the last authorised antibacterial for histomoniasis prevention in poultry) was prohibited by Regulation 1756/2002/EC. Ban for food-producing animals treatment with nitrofurans within EU and other countries is due to potential carcinogenic and mutagenic properties of their metabolites. Nifursol, as well, rapidly metabolizes *in vivo* and forms stable protein-bound metabolite 3,5-dinitrosalicylic acid hydrazide (DNSH), marker residue of parent drug. According to (EU) 2019/1871, the reference point for action (RPA) for DNSH in food of animal origin is 0.5 µg/kg.
Critical issue of confirmatory determination of nitrofuran metabolites bound residues in animal tissues is complicated and time consuming preliminary washing step by organic solvents to remove free metabolites and part of matrix components. Information about metabolites total residues may be valuable when concluding samples compliance, so the aim of our work was to develop an effective method for DNSH total residues assay in animal tissues and to test its performance on incurred samples.
As a result of developing of new sample preparation protocol and optimisation of chromatographic separation, we have managed to elaborate simple, rapid, sensitive, efficient and robust UPLC-MS/MS technique for DNSH residues determination in meat and liver of broiler chicken, using matrix fortified calibration and isotope-labelled internal standard (LOD~0.05 µg/kg; LOQ~0.1 µg/kg. Main steps of sample preparation are: homogenization, ultrasonic-assisted short time hydrolysis and derivatization by 2-NBA in 0.2 M HCl medium in protein precipitating agent presence, neutralization to pH 7.5 by PBS, extraction by ethyl acetate and non-polar co-extractant, extract evaporation, defatting and reconstitution in mobile phase. UPLC-MS/MS analysis was performed using Waters ACQUITY UPLC H-Class LC system with MS detector Xevo TQ-S Micro equipped with column ACQUITY UPLC BEH C18 50 mm. Mobile phases – methanol and 0.15% $(NH_4)_2CO_3$ in water; flow rate – 0.4 ml/min; separation mode – gradient; time of analysis – 6 min; DNSH retention time – 2.12 min. NP-DNSH acquisition was carried out using MRM of ion transitions 374>182 and 374>226 (ES-). Method for muscles was fully validated as confirmatory according to (EU) 2021/808; decision limit CCα is 0.04 µg/kg, being considerably lower than set RPA.
UPLC-MS/MS method was successfully tested on incurred broiler chicken muscle tissues, obtained during the experiment on chicken feeding with nifursol additive, performed to get incurred samples with target DNSH concentrations, close to RPA, for full validation and approval of new developed DNSH ELISA test kit. For that purpose, preliminary UPLC-MS/MS analysis was carried out to define DNSH level, and then samples with high content were homogenized with proper amounts of blank chicken muscle. Obtained incurred samples considered to be sufficiently homogeneous, according to the approach described in ISO 13528.

Keywords: *nifursol metabolite, UPLC-MS/MS, animal tissues, incurred samples, food safety*

R15

CROSS-CONTAMINATION OF FEEDINGSTUFFS BY ANTIBIOTICS DURING FEED PRODUCTION: RISK OF TRANSFER TO FOOD OF ANIMAL ORIGIN

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In 2017 and 2018, French monitoring plans on feed for food producing animals (pigs, poultry and rabbit) based on 100 samples per year highlighted the presence of antibiotics at relatively high frequencies. Indeed, 15% of the samples were above a concentration of 1 mg/kg and between 32 and 34 % were above 0,125 mg/kg, the limit of quantification of the method. This issue is related to the use of shared production lines for both medicated and non-medicated feedingstuffs but also to the transport or storage of the feeds.
A daily oral exposure of animals to low concentrations of antibiotics raises questions about the risk of transferring antibiotic residues to foodstuffs in addition to the risk of emergence or increase of antibiotic resistance in the gut microbiota. One of the aim of this project was to assess the risk of transfer of residues of oxytetracycline, amoxicillin and a combination sulfadimethoxine/trimethoprim, i.e. the antibiotics most frequently found in pork related food during French monitoring plans
After the development and validation of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods to quantify the 4 targeted antibiotics in the different animal matrices (plasma, muscle, kidney, liver and faeces), a first experimental pilot study with a few animals was conducted in order to obtain a first approach of the kinetic behavior of each antibiotic. The study was conducted on pigs, to which low concentrations of antibiotic were administered in the feed. The administrated concentrations were based on the cross-contamination rates allowed by good manufacturing practices (2%). The results obtained during this study enabled to draw first conclusions: sulfadimethoxine was well absorbed and accumulated in muscle, kidney and liver, where detected concentrations were higher than the Maximum Residue Limits of 100 µg/kg. On the other hand, the amount of oxytetracycline found in faeces was very high, while the concentrations detected in muscle and liver were below the MRL. Amoxicillin concentrations in plasma and tissues were below the limit of quantification of the methods.
A second principal experimental study conducted on eight animals to which the same concentration of sulfadimethoxine close to cross contamination rates for 12 days (with a break of 2 days for kinetics purposes), confirmed the results obtained during the first experimental study. The concentrations in tissue were above the MRL levels. The investigation of sulfadimethoxine metabolites in liver was performed using an LC-HRMS system with two approaches: targeted and non-targeted. Even at these low levels of exposure, some known metabolites of sulfadimethoxine were detected in liver. Plasma concentrations of sulfadimethoxine were estimated and will allow to built a predictive model of tissue concentrations as a function of cross-contamination levels in feed.

Keywords: *cross-contamination, transfer, antibiotic residues, feed, food*

Acknowledgement: *We thank Jérôme Henri and Charlotte Valentin for their help and support.*

R16

RESULTS OF EU PROFICIENCY TESTING FOR THE ANALYSIS OF CHLORAMPHENICOL RESIDUES IN TURKEY MUSCLE

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The organization of proficiency tests (PT) to the attention of National Reference Laboratories (NRLs) is one of the duties of the European Union Reference Laboratory (EU-RL) according to the Directive no (EC) 96/23. The participation in these PT schemes allows the networks of EU-NRLs and of official field laboratories to assess their competence and to prove the reliability of their results.
The aim of this particular PT was to assess the ability of the participants to detect, identify and quantify chloramphenicol residues in naturally incurred turkey muscle samples and to take any decision regarding these samples according to the criteria of the European Decision 2002/657/EC. Participants were asked to implement their routine method to analyse each blind sample to determine the possible content in chloramphenicol residues.
Since 2003 a Minimum Required Performance Limit (MRPL) was set at 0.3 µg/kg in European Union for the presence of residues of chloramphenicol in food products (Commission Decision 2003/181/EC. Recently, according to the Regulation (EC) 2019/1871 which will come into force after the 22 November 2022 the RPA has been reviewed and set at 0.15 µg/kg. This means that analytical methods used to control chloramphenicol residues will have to detect and confirm the compounds at this concentration.
Twenty nine NRLs agreed to participate and received samples prepared at the EU-RL facilities. The materials were batches of mixed turkey muscle spiked with chloramphenicol. Homogeneity and stability have been demonstrated for each final material.
The organization and the statistical analysis of the participants' results were performed through a fully accredited in-house quality management system according to ISO/IEC17043, to national accreditation document LABCIL ref02 rev2 and to local official procedures.
Participants' results and performances will be shown.

Keywords: *proficiency testing, chloramphenicol residues*

R17

DEVELOPMENT AND VALIDATION OF THE METHOD FOR THE DETERMINATION OF AMINOGLYCOSIDE IN FOODS USING LC-MS/MS WITH A ZWITTERIONIC HILIC STATIONARY PHASE

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Aminoglycosides (AMGs) are broad-spectrum antibiotics that have bactericidal activity against aerobic bacterial infection and are commonly used as veterinary drugs on food-producing animals and in human medicine. Thus, it is important to monitor residues in food to control AMG use. Many countries have established maximum residue limits (MRL) for aminoglycosides approved for use on animals. AMGs are currently analysed in honey, eggs, milk, tissues, and fluids of food-producing animals for control and monitoring purposes. AMGs are highly polar compounds and show little to no retention in reversed phase columns. Although ion-pairing reagents have been utilised successfully to chromatograph AMGs on C18 columns, when used with liquid chromatography-tandem mass spectrometry (LC-MS/MS) this approach suffers from ion suppression and contamination of the LC and MS/MS systems. The introduction of hydrophilic interaction chromatography (HILIC) provided a more MS-compatible option for the analysis of polar compounds. Here we show the results from the successful evaluation of the new Atlantis™ Premier BEH™ Z-HILIC column, which has a sulfobetaine zwitterionic chemistry, for the determination of AMGs. The method incorporating the Z-HILIC column for AMG residues was validated in milk, eggs, and honey.

R18

TARGET ANALYSIS AND RETROSPECTIVE SCREENING OF CONTAMINANTS IN COOKED HAM SAMPLES THROUGH UHPLC Q-EXACTIVE ORBITRAP HRMS

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Cooked ham represents the most Italian commonly consumed processed meat, with a percentage equal to 24.8% and an annual production of 271.1 tons in 2020. Based on the latest data reported by FAO, Italian daily per capita pork ham consumption is 8 g for adults and 3 g for infants 1-3 years. Among the variety of cured meat, cooked ham represents the cured meat amply consumed from the first months to all of life. However, it may also be a vehicle for food contaminants, such as mycotoxins and pharmacologically active substances as a result of improper agriculture practices or use of these drugs.
Veterinary drugs (VDs) are extensively administered with a therapeutic purpose for the prevention and treatment of animal disease. However, their improper use may involve their occurrence in the final products intended for human consumption. Veterinary drugs are considered a type of "emerging contaminants", that represent one of the substantial concerns for food animal-derived products (milk, cheese, eggs, meat, and honey), including cured meats as well as for human health. When are not respected the withdrawal times or when drugs are excessively used, residues of VDs can be found in foods. Nowadays, food safety risk assessment has improved thanks to the amelioration of the global food safety regulatory system. There is plenty of attention to the occurrence of antibiotic residues and hormones in animal-food products. Therefore, the European authorities have set maximum residue limits (MRLs) for these drugs to ensure food safety.
On the other hand, feed contaminated mycotoxins could result in the presence of mycotoxins and related metabolites in animal-derived products as evidenced in literature and recently reviewed. Hence, the mycotoxin carry-over into animal-derived products and the ingestion of edible animal products, including dry-cured meat such as cooked ham, contaminated with mycotoxins constitute a public health concern due to the adverse effect upon human health that they may produce.
Based on the above, a method for the investigation of target (n=17) and non-target (n=23) veterinary drugs in cooked ham by ultra-high performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC Q-Exactive-Orbitrap HRMS) was developed. Retrospective screening of mycotoxins and other metabolites was carried out. The extraction was performed based on the QuEChERS approach and validated in accordance with the European Regulations in force. The application of the in-house validated method to cooked ham showed the occurrence of three veterinary drug residues (colchicine, meloxicam, benzylpenicillin-procaine). From the retrospective analysis of the data, up to nine non-target veterinary drug residues were putatively identified.
Contaminants in animal-derived products could exert a health concern on the consumer. It is, therefore, necessary to also inspect the possible content of residual drugs and mycotoxins in food to guarantee consumers' safety.

Keywords: *cooked ham, veterinary drug residues, mycotoxins, UHPLC Q-Orbitrap HRMS*

R19

NITROFURAZONE – DIRECT DETECTION OF UNAUTHORIZED USE OF THE NITROFURANE IN MILK AND MILK PRODUCTS

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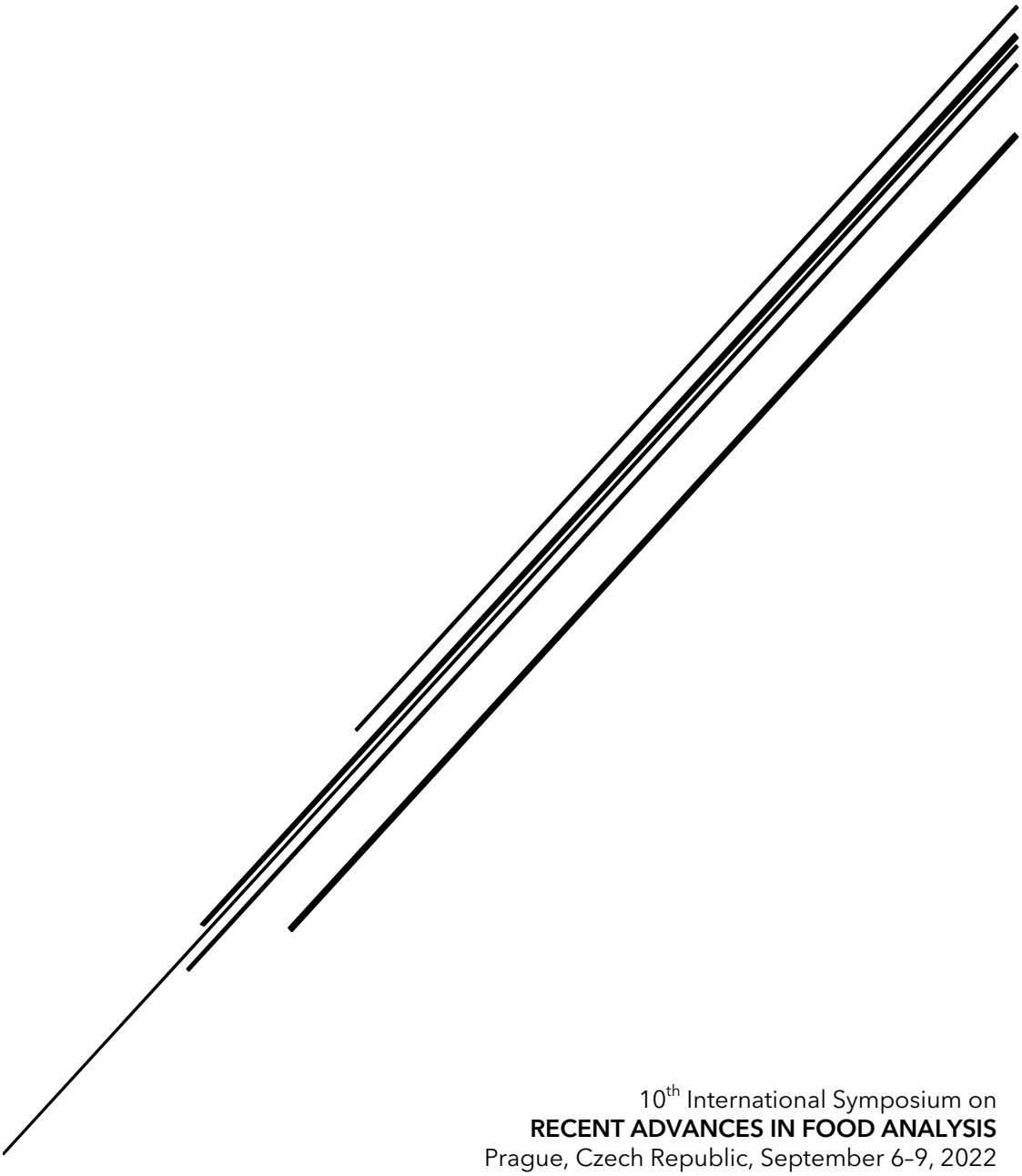
The antibiotic nitrofurazone belongs to the nitrofurans. Due to its mutagenic and carcinogenic effects, it has been officially banned in the EU. In the organism, nitrofurazone can be metabolised rapidly or can be bound on proteins. Therefore, the metabolite SEM is usually analysed in meat and animal products. SEM is regarded as an indicator of illegal use of nitrofurazone (EFSA Opinion 2015 [1]).
However, if the cow is treated with antibiotics, the parent substance nitrofurazone also passes into the milk and can be analysed using highly sensitive methods.
Furthermore, unlike the metabolites of the other nitrofurans, SEM is not only a metabolite of nitrofurazone, but can also occur naturally in products (e.g. carrageenan). In addition, it has been detected more and more frequently in products in which there is evidence that no nitrofurazone had previously been applied. An increasing number of mainly protein-rich, dried products are known in which SEM can be formed during the production process. Furthermore, entry into the product can occur through PVC seals in screw caps. Since the detection of SEM no longer clearly indicates the illegal use of nitrofurazone, it is necessary to analyse nitrofurazone directly. The International Dairy Foundation recommend this for milk and dairy products [2].
As basis for the determination of nitrofurazone in milk and milk products, the standard method of the International Dairy Foundation ISO 22186:2020 [IDF 245:2020] "Milk and milk products - Determination of nitrofurazone" was used. Slight modification in terms of final composition of the measurement solution and an adapted gradient led to improved chromatography. In preparation for the lowering of the reference points for action for nitrofurans and their metabolites to 0.5 µg/kg from November 2022 [3], a lower limit of quantification of 0.3 µg/kg for milk and milk powder has already been established.
With the new, sensitive method, the banned antibiotic can be analysed directly in the matrices milk and dairy products. This complements the previous indirect analysis via the metabolite semicarbazide (SEM) and can confirm whether illegal antibiotic use actually occurred.

[1] EFSA Journal 2015;13(6):4140: Scientific Opinion on nitrofurans and their metabolites in food
[2] International Dairy Fondation (IDF): IDF-Factsheet Juli 2015 – Why semicarbazide is not a suitable marker for nitrofurazone in dairy products
[3] COMMISSION REGULATION (EU) 2019/1871

Keywords: *nitrofurazone, nitrofurane, milk, milk products*

SMART SENSORS



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S1

EVALUATION OF SPECTRAL HANDHELD DEVICES FOR FRESHNESS ASSESSMENT OF CARP AND TROUT FILLETS IN RELATION TO STANDARD METHODS AND NON-TARGETED METABOLOMICS

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Fish is a highly perishable food commodity due to its biological composition. Under normal refrigerated storage conditions, the shelf life of fish is limited by enzymatic and microbiological spoilage. Therefore, freshness is a key element in the assessment of fish quality. To date, there are many different tools available to assess freshness of fish such as sensory, physical, chemical and microbiological methods. However, these methods have certain drawbacks as they are time-consuming, retrospective and expensive. These limitations have initiated interest in near infrared (NIR) spectroscopy as a rapid and low-cost tool for in-the-field assessment of fish freshness and quality.
The current study investigated the potential of three handheld NIR spectrometers (TellSpec, SCiO, MicroNIR) to monitor freshness of organic carp and lake trout fillets after 1, 3, 6 and 9 days stored at +4 °C. In addition, all samples were analyzed with common standard methods (for biogenic amine content, microbiological parameters, total volatile basic nitrogen content, fatty acid profiles, color and texture change) and with dynamic headspace - gas chromatography - time-of-flight mass spectrometry (DHS/GC-TOFMS) applying a non-targeted analytical workflow. Discriminant and class modelling approaches (OPLS-DA and DD-SIMCA) were used in order to evaluate the ability of the NIR handhelds to distinguish between fresh (day 1, day 3) and spoiled fish (day 6 and 9). The data obtained with standard methods and DHS/GC-TOFMS were correlated with the NIR data in order to identify characteristic wavelengths revealing fish spoilage pattern which can improve the chemometric models for fish freshness assessment.
Using TellSpec, carp fillets could be discriminated according to their freshness (fresh vs spoiled) with high accuracy (93-100 % for OPLS-DA), and high sensitivity and specificity (100%, 60-100% for DD-SIMCA). For discrimination of trout, TellSpec and MicroNIR were outperformed by the SCiO (76–100 % accuracy for OPLS-DA, sensitivity of 100 % for all models and 53-100 % specificity for DD-SIMCA). Both classification methods led to lower sensitivity and specificity for trout samples analyzed with all three devices compared to carp. Moreover, wavelengths reduction was performed where only those correlated to the specific parameters (analyzed by standard methods and DHS/GC-TOFMS) were used. The accuracy, sensitivity and specificity of chemometric models for trout (TellSpec and MicroNIR) were improved, while the models for carp samples (MircoNIR and SCiO) did not improve.
The results showed that the NIR portable devices yield different classification accuracies and precision which were depending on the investigated fish species. It can be concluded that NIR handheld devices can be used as a tool for fast screening of fish freshness in the field. However, for a precise determination of fish freshness, confirmatory analysis is required.

Keywords: *NIR, handheld spectrometer, DHS/GC-TOFMS, fish freshness, multivariate analysis*

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S2

DETECTION OF SALMONELLA TYPHIMURIUM WITH ANTIBODY IMMOBILIZED ON QUARTZ CRYSTAL AND GOLD NANOPARTICLES FOR SIGNAL IMPROVEMENT

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Recent trends in bacteria detection methods focus not only on the selectivity of the target bacteria but also on the portability and simplicity. Quartz crystal microbalance (QCM) has demonstrated its effectiveness as mass-based detection methods that measure the frequency shifts due to the mass change of bacteria on the quartz crystal surface. Furthermore, QCM has a high potential to be integrated with a smartphone, transforming into a portable device that can be used on-site. In this study, benchtop version of QCM biosensor was designed and fabricated for detection of *Salmonella* Typhimurium, before advancing on a smartphone based QCM sensor. When *Salmonella* Typhimurium were detected on the quartz crystal surface, the resonant frequency of a quartz crystal was shifted caused by the change in mass. In order to recognize *Salmonella* O antigen, polyclonal antibody was immobilized via self-assembled monolayers (SAM). For antibody immobilization, 11-Mercaptoundecanoic acid (11-MUDA), EDC-NHS and bovine serum albumin (BSA) were applied to the gold surface of the quartz crystal. QCM biosensor was designed with a peristaltic pump that injected a series of chemical solutions to the quartz surface. All frequencies were acquired in real-time while average frequency before and after detecting *Salmonella* was recorded. For *Salmonella* Typhimurium, the largest and smallest frequency shift was -26.91 Hz and -3.65 Hz for 2 x 10^9 CFU/mL and 2 x 10^3 CFU/mL. In order to increase the frequency response to *Salmonella*, 100 nm gold nanoparticles were utilized via biotin-avidin reaction. The average frequency shifts for 2 x 10^9 CFU/mL and 2 x 10^3 CFU/mL were improved which were -52.72 Hz and -28.04 Hz, respectively. Finally, the gold nanoparticles resulted in the increase in 123.74 % and 573.19 % for 2 x 10^9 CFU/mL and 2 x 10^3 CFU/mL.

Keywords: *quartz crystal microbalance, gold nanoparticles, Salmonella Typhimurium, frequency shifts, mass based biosensor*

Acknowledgement: *This research was supported by the Center for Food Safety Engineering at Purdue University, funded by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No. 59-8072-1-002. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.*

S3

EVALUATION OF BENCHTOP VERSUS PORTABLE NEAR-INFRARED SPECTROSCOPIC DEVICES FOR BREED IDENTIFICATION IN IBERIAN HAM

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In recent years, near-infrared spectroscopy (NIRS) has evolved from its exclusive use in laboratories to the development of miniaturized devices that connect via USB or have their own built-in battery and work with an application installed on a smartphone. In the near future these devices could be an alternative to benchtop equipment as they offer applicability to a wide range of products and sample types. One of the fields where it is having an outstanding application is in the inspection and control of meat and meat products. NIRS technology offers a high speed of operation, which makes it a suitable technique for on-site application for these types of products. For decades, the Iberian pig sector has been operating with this technology aimed at detecting fraud in raw materials and final products. In recent years, the progress observed in miniaturised equipment has encouraged research into the possibility of replacing laboratory equipment and to implement this technology online for greater sample control.
In this context, the racial factor is of great interest for Iberian ham industry and market, as there are different labels depending on whether the product is 100% Iberian breed or 50% Iberian-Duroc crossbreed, and the commercial category strongly affects the price of the product. The aim of this work was to study the feasibility of classifying Iberian ham samples according to racial purity (100% Iberian vs 50% crossbreed) using NIRS benchtop and portable devices. A total of 60 pieces of controlled breed Iberian ham were analysed: 24 pieces from the 100% Iberian breed group and 36 pieces from the 50% Iberian breed group. A FOSS 5000 benchtop device with a spectral range of 1100-2000 nm (Foss Instruments) and three portable devices microPHAZIR (Thermo Fisher Scientific) with a spectral range of 1500-2335 nm, MicroNIR Pro ES 1700 (VIAVI) with a spectral range between 908-1676 nm and SciO (Consumer Physics) with a spectral range of 740-1070 nm.
In relation to the portable equipment, when the spectra obtained on the slice with the microPHAZIR were used, 100% of the samples were correctly classified in calibration according to the breed, in the case of the microNIR the percentage was 90% and for the SciO the percentage was 99%. On the other hand, the percentages of samples correctly classified in the validation step were 64% if the spectrum was recorded with microPHAZIR, 64% in the case of microNIR and more than 90% for SciO. These results point to the good discrimination capability of SciO, but these preliminary conclusions need to be confirmed by analysing a larger number of samples, given the large differences in the calibration results compared to the validation results for some of the equipment analysed.

Keywords: *Iberian ham, breed, miniaturized NIR Spectroscopy, classification*

Acknowledgement: *Provincial Council of Salamanca (Spain) and the Carrasco Ibéricos Company (Guijuelo, Spain) for the concession of the project 18VEUH 463AC06, Carrasco Ibéricos Company (Guijuelo, Spain). Hernández-Jimenez, M. thanks the Predoctoral Contract Grants of the University of Salamanca co-funded by Banco Santander and E-COST- GRANT - CA19145 European Network for assuring food integrity using non-destructive spectral sensors.*

S4

MOBILE, MULTIANALYTE BIOSENSING FOR DECISIVE RESULTS AT POINT-OF-NEED

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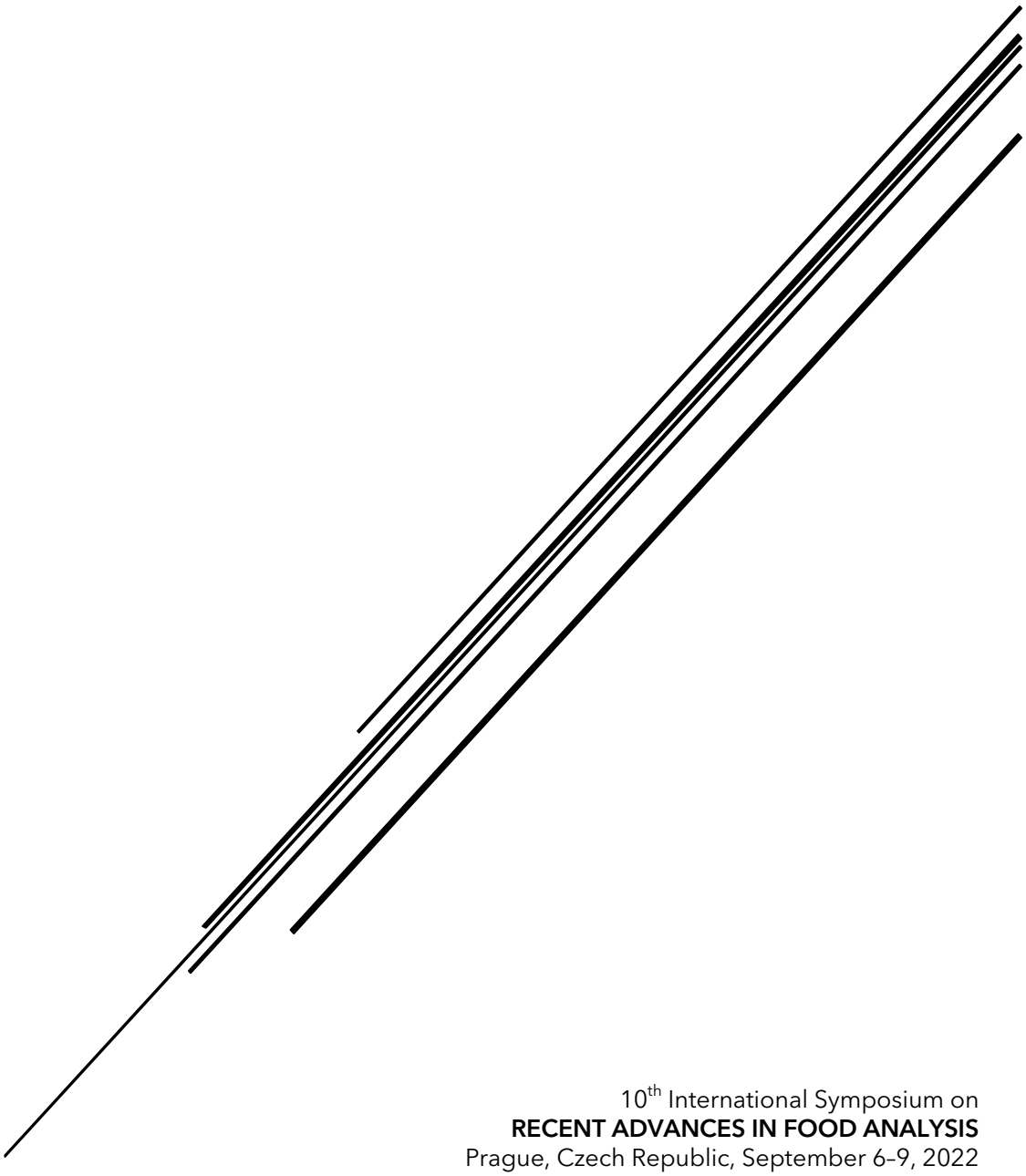
Rapid detection of harmful or regulated substances is important for fast corrective action on site. Early detection of pathogens, for example, allows for fast tracking and preventing actions, for example spoilage detection early in food production chain prevents mixing of spoiled samples with a large production pool.
We present proprietary mass-sensitive microarray (MSMA) biochip, which allows label-free, real-time measurement of biomolecules from small amount of liquid. MSMA consists of the mass sensitive transducer based on Solidly Mounted Resonance (SMR) technology integrated with Application Specific Integrated Circuit (ASIC) [1]. This unique approach allows compact design, high level of multiplexing and an individual control of each sensor on a chip. MSMA consist of 64 individual microbalances capable of detecting different analytes, depending on the biofunctionalization on its surface. As the transduction is based on resonance frequency change upon biomolecule binding on the sensor surface, the detection occurs in real time without need for any labeling. Further advantages of our technology include digital results, which can be transferred into cloud at site.
We are currently developing a portable reader and lab-on-a-chip system, where all components needed for the measurement are stored in a disposable cartridge. The user is required to load the sample into a cartridge and push it into the reader, which then runs the assay. Results are calculated with our data analysis algorithm and displayed on screen and can be transferred on site into cloud and used when and where-ever needed. We have demonstrated our technology for detection of drugs-of-abuse from saliva, antibiotic residues in milk, mycotoxin detection [2-3] and early detection of SARS-CoV-2 virus. We are currently preparing a piloting study focusing on food safety.

1. Nirschl, M. et al. Sensors 10 (2010) 4180-4193.
2. Nolan,P. et al. Food additives & Contaminants (2019): Part A, 36 (5) 800-814
3. Nolan, P. et al. Talanta 222 (2021) 121521

Keywords: *mass-sensitive microarray, multiplexing, real-time, on-site measurement, label-free detection*

LAST MINUTE



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X1

NEAR-INFRARED SPECTROSCOPY AND CHEMOMETRICS TO DETECT RICE FRAUD: THE ITALIAN RICE CASE STUDY

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With 866 products accredited by the EU, of which 305 are PDO, Italy is one of the leaders in premium food items. Among Italian PDOs, there's only one rice called "Riso di Baraggia Biellese e Vercellese", which comprehends seven varieties: Carnaroli, S. Andrea, Baldo, Arborio, Balilla, Loto, e Gladio. Notably, according to "Ente Nazionale Risi", Italy is also the biggest producer of rice in Europe, with a production, of 1.513.057 tons in 2020. Despite the high quality and the fame of Italian rice, to the best of the author's knowledge, there are no studies on Italian rice for what concerns the traceability and the detection of frauds with the use of analytical methods and chemometrics.
In this study, the first in this direction, NIR technology and chemometrics were used to build classification models to discriminate between Arborio rice, the premium quality, and other rice cultivars which could be possible adulterants. The study was conducted using: 60 samples of Arborio, 38 of Originario, 25 of Ribe risotto and 9 of Selenio. Principal Component Analysis (PCA) was used for a first preliminary data classification: the combination between PC1 and PC2 resulted in a good separation among the classes. The accuracy of the K-NN algorithm in discriminating between arborio samples and not arborio samples varied from 80% to 90% according to different values of k.
Moving from unsupervised to supervised analysis, SIMCA software was used to try different PLS-DA and OPLS-DA models. After several considerations, M4 was founded to be the best performing model: its accuracy was 83% for the authentic arborio and 100% for its adulterants, with a cut-off of 0,6. The value given by the internal validation are R2X=0,913, R2Y=0,86 and Q2=0,808.
Considering all the classification models, the results obtained are promising, It could be suggested that even more accurate models could be built by excluding the organic rice from the arborio samples or decreasing the number of variables by using fewer rice cultivars.

Keywords: *fraud, chemometrics, NIR, rice, authenticity*

X2

DETERMINATION OF AUTHENTICITY AND QUALITY OF CHOCOLATE USING REAL-TIME PCR

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Chocolate is among the most popular food and flavor types worldwide, with cacao (*Theobroma cacao*) seeds consumption dating back as far as 3800 years ago. Therefore, quality authentication and safety of ingredients and products for human consumption are of major interest for the cocoa industry. Chocolate-based food products globally have been subjected to outbreaks of foodborne diseases with the main pathogen being *Salmonella* spp. Conventional *Salmonella* detection is usually performed using a culture-based method, which is time-consuming and unsuitable for high-throughput analysis.

With the emergence of molecular methods and advances in biotechnology, faster methods with a stronger fit-for purpose potential are replacing earlier techniques, among them real-time PCR has demonstrated clear advantages. It is highly sensitive and time-effective, thus ideal for pathogen detection. In addition, other applications such as the detection of allergens, which can pose major health risks to consumers, or tracing animal DNA e.g., in vegan food products are possible using real-time PCR. This is especially important as product traceability for consumers with an allergen-free, vegetarian or vegan lifestyle is a key aspect in their dietary needs and selection. Thus, producers of special diet food and ingredients rely on analytical methods, such as real-time PCR, to back up their food-safety and marketing claims. In addition, the uprise in the allergen-free and plant-based diet demand has exponentially increased and so has the necessity of a rapid yet sensitive method to detect any potential process or origin contamination.

In the following study, a combined method for rapid allergen, bacterial and vegan claim is demonstrated. Here, DNA from several kinds of chocolate was extracted using the IST InnuScreen "PME Food DNA Kit". The "SureFast® Salmonella ONE" assay from R-Biopharm's was used to identify *Salmonella* spp. For the detection of potential allergens in chocolate, the "SureFood® ALLERGEN" assay for various nut types was applied. Furthermore, the vegan claim of the chocolate was determined using IST InnuScreen "innuDETECT Mammal & Bird Assay". For best results, the different assays were combined with the high precision real-time PCR technology of the qTOWER³ from Analytik Jena.

Altogether this work demonstrates the suitability of real-time PCR an excellent tool for ensuring food safety and quality due to its reliability, high accuracy, reduced detection time and targeted detection outcome.

X3

METROFOOD-IT - THE ITALIAN RESEARCH INFRASTRUCTURE FOR METROLOGY AND OPEN ACCESS DATA IN SUPPORT TO THE AGRIFOOD

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In the frame of Mission 4 "Education and Research" of the Italian National Recovery & Resilience Plan (NRRP) a new project aimed at strengthening the Italian Research Infrastructure for Metrology and Open Access Data in support of the Agrifood - METROFOOD-IT has been approved for funding. It is related to the Italian Node of the European Infrastructure METROFOOD-RI – Infrastructure for promoting metrology in food and nutrition (www.metrofood.eu - ESFRI Roadmap, domain Health and Food).
The project focuses on the RI's electronic component and its integration with the physical one, to provide services in support of the digitalization of the agrifood system for food quality & safety, traceability, food transparency, sustainability, and resilience of agrifood systems, and circular economy. It aims at developing the organizational and operational framework of the RI and structuring the strategy, procedures, and supporting system for the service provision via TransNational Access and wide Virtual Access, thus making the Italian infrastructure fully implemented, operational and sustainable in the long run. The set of advanced services provided by the RI are addressed to different users categories with access to various physical facilities (laboratories and plants) and e-resources (e.g., apps, software, models) and will allow the RI to act as an interface between research and innovation, industrial players, and consumers, defining and testing different processes and scenarios for the development of sustainable and innovative agrifood systems, food safety, healthy diets, and solutions for a circular bioeconomy. METROFOOD-IT will be characterised by the application of ICT solutions with an integrated supply chain approach. The innovation potential relies on the state-of-the-art services, tools, and concepts deployed, along with FAIR data management, data quality, and open data, crossing the 4th digital evolution applied to the agrifood. Computational modelling and laboratory-based solutions will be integrated via upcoming approaches such as smart and remote sensing systems, IoT, blockchain, and Artificial Intelligence. An open science-based approach will be followed, sharing, and making open data and access to resources. Open communication with the various stakeholders on objectives, results, outcomes, and impacts will allow for the promotion of direct and motivated involvement, through the application of a multi-actor approach. Overall, METROFOOD-IT will contribute to overcoming fragmentation and research compartmentalisation, enabling researchers to access, create, share, connect, analyse, and interpret various and heterogeneous factors and paving the way to ambitious, transnational, transdisciplinary advancements in the agrifood, while significantly reducing duplication of research efforts and thereby providing stimulus for creative thinking to develop innovative practices, products, and services to advance knowledge.

Keywords: *food traceability, digital food safety, agrifood resilience, circular bioeconomy, integrative research tool*

X4

MONITORING AND RISK ASSESSMENT OF PESTICIDES RESIDUES IN READY-TO-EAT BABY FOOD AMONG DOMESTIC SERBIAN BABY FOOD BRAND

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Pesticides have an important role in the development of agriculture. Although we have faced the problem of the impact of pesticides on human health, especially for vulnerable individual groups in society, the application of pesticides is so far irreplaceable. Pesticides used in the treatment of fruits and vegetables may be passed to the ready-to-eat baby food product in any processing stage. A crucial problem regarding the nutrition of infants has been reflected in the high food intake/body weight ratio. This may represent a risk to infant health.
In this paper two techniques were used, gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC–MS/MS) to analyze the content of pesticide residues in fruit and vegetable-based ready-to-eat baby food. The method is based on sample treatment (QuEChERS), which consists of an extraction using acetonitrile, followed by a clean-up step based on dispersive solid-phase extraction with primary secondary amine (PSA).
During the 6 months of monitoring in 2022, 100 samples of domestic branded ready-to-eat baby food were analyzed to present of pesticide residues. It was used in baby food samples for infants aged 4-12 months. In 37% of all samples, the residue of pesticides was below the detection limit. The other 63% of all samples contained detectable residues. About 8% of the sample in which pesticides were detected had residues above MRL allowed in food for infants. The most commonly detected pesticides were acetamiprid and boscalid. The pesticide with the highest concentration is propamocarb (0.3 mg/kg). Exposure to pesticide residues is seen through risk assessment and it was calculated for 4 pesticides: acetamiprid, azoxystrobin, captan and propamocarb. Risk assessment was performed for the mentioned pesticides and was established to remain below the level of concern for acute exposure.

Keywords: *Pesticide residue, QuEChERS, Risk assessment, Baby food, Infant health*

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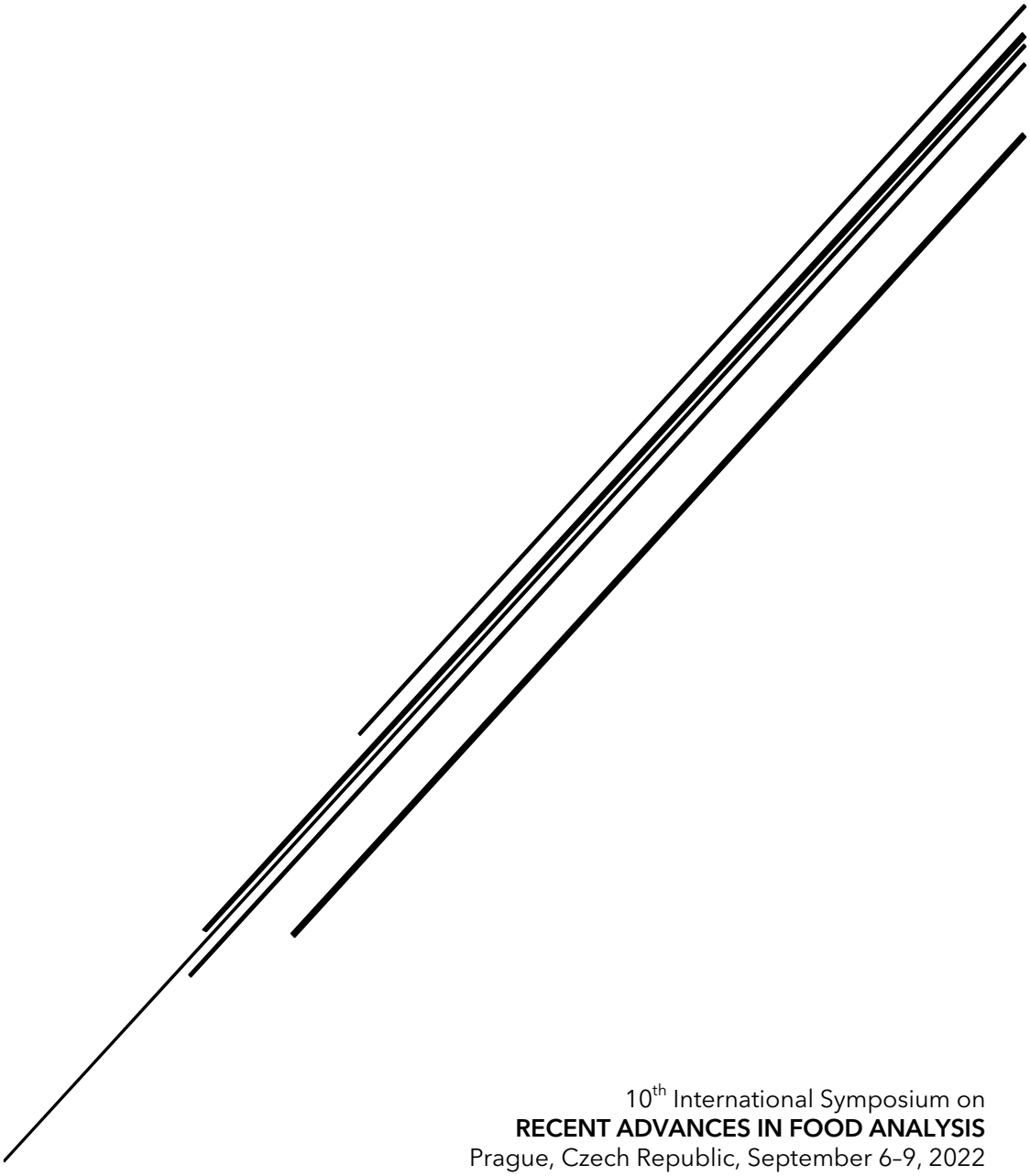

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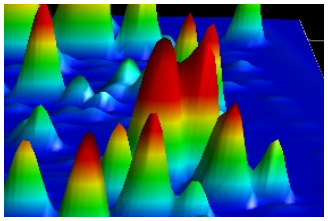

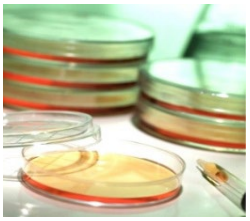


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