BOOK OF ABSTRACTS

8th International Symposium on

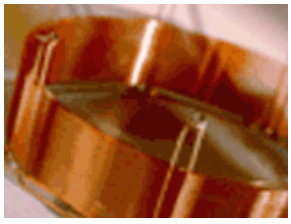
RECENT ADVANCES IN FOOD ANALYSIS

November 7–10, 2017

Prague, Czech Republic

Jana Pulkrabová, Monika Tomaniová, Michel Nielen and Jana Hajšlová
Editors



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

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Published by the University of Chemistry and Technology, Prague
Technická 5
166 28 Praha 6
Czech Republic

Edited by Jana Pulkrabová, Monika Tomaniová, Michel Nielen and Jana Hajšlová

ISBN 978-80-7080-999-0

8th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 7–10, 2017
Prague • Czech Republic

Clarion Congress Hotel Prague

Organized by

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

&

RIKILT
Wageningen University & Research, Wageningen, The Netherlands

MINISTRY OF AGRICULTURE
OF THE CZECH REPUBLIC

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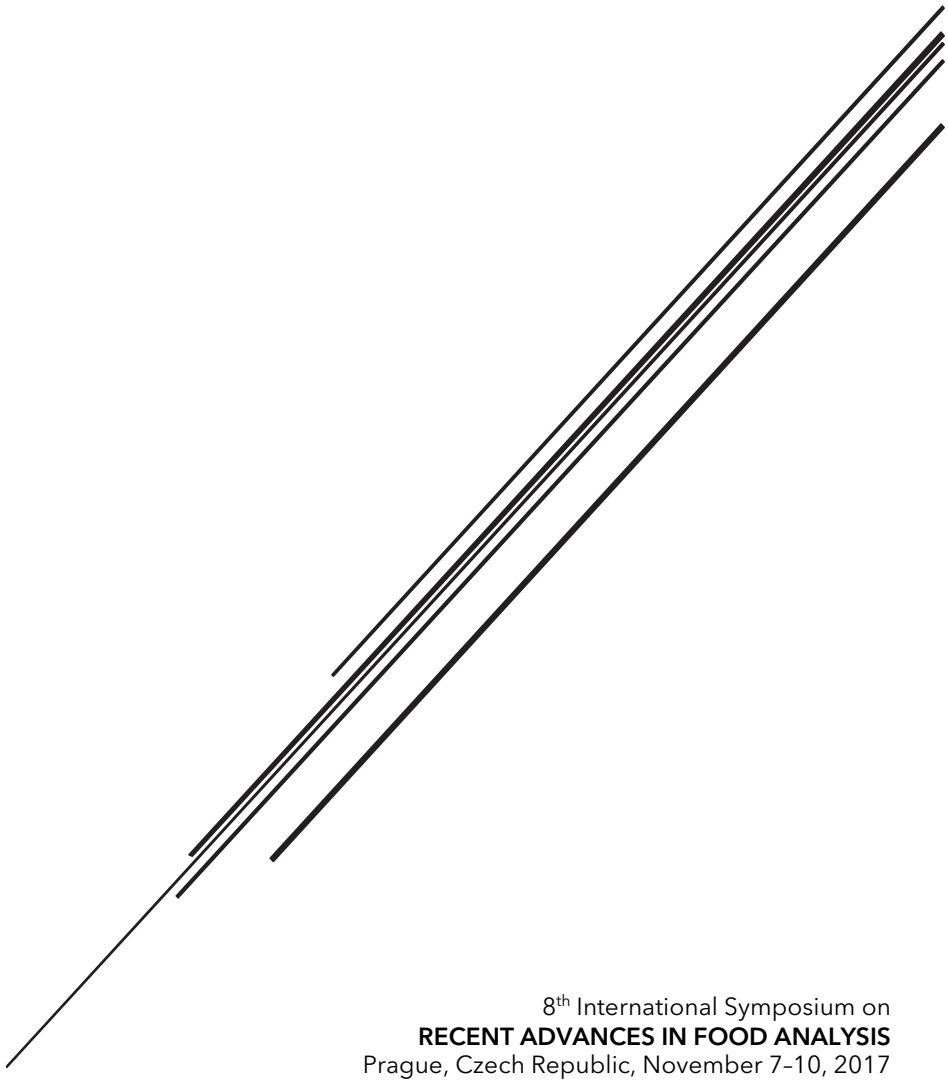

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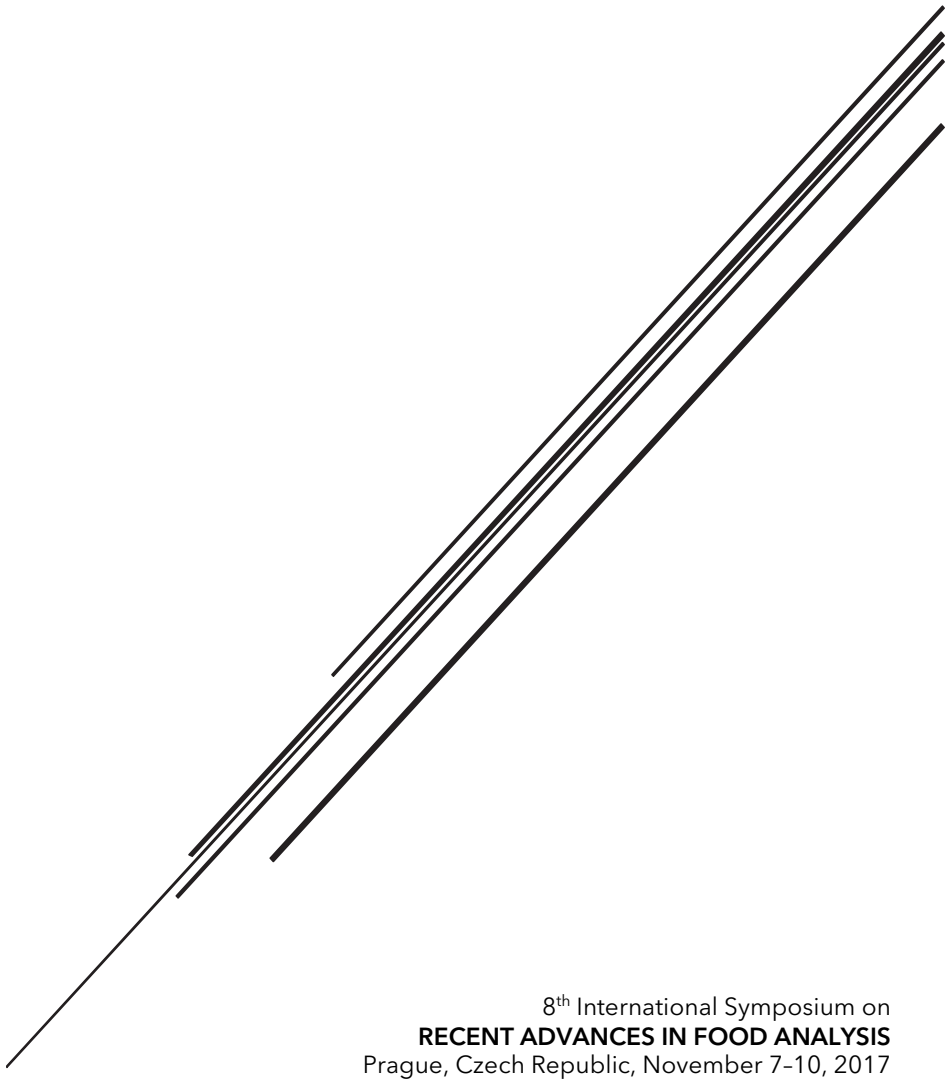

8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

Axel Semrau®

VENDOR SEMINAR:
AUTOMATED SOLUTIONS FOR THE ANALYSIS OF CONTAMINANTS IN FOOD, FOOD CONTACT MATERIAL AND PACKAGING

Automated solutions for the analysis of contaminants in food, food contact material and packaging

Andreas Bruchmann

Axel Semrau GmbH & Co, Stefansbecke 42, 45549 Sprockhövel, Germany

New automated solutions for sample preparation in the MOSH/MOAH analysis

The determination of MOSH and MOAH with online LCGC FID is a common method which meanwhile has become part of international regulations. Analytics require manual sample preparation steps such as aluminum oxide purification and epoxidation. The lecture presents approaches how these sample preparation steps can easily be automated. A further discussion concerning MOSH/MOAH analytics is the use of other methods than FID. Advantages and disadvantages of the usage of mass-spectrometric detection and UV detectors for the MOSH and MOAH analysis will be examined.

imPAHct – a new approach of fast and sensitive analysis of PAH in foodstuff

PAH is one of the most analyzed contaminants in foodstuff. It has a good fat solubility and a low limit of determination. Therefore, analytics are time-consuming and require many sample preparation steps. imPAHct is a complete new approach to solve this analytical problem. It consists of an easy and fast manual sample preparation in combination with a fully automated clean-up with a LCGC and GCMS as detection method. The lecture describes the method and presents experiences and validation data of a routine laboratory.

Automation solution for robust analysis of 2-,3-MCPD and glycidyl esters

Within the last months, analysis of MCPD and glycidyl esters as contamination of fats, oils and other foodstuffs have received high attention. Based on the ALARA principle, producers should try to keep the amount as small as possible. Currently, new thresholds are under consideration. Normally, the esters are determined as free MCPD by an indirect method. But the official methods are extensive and time-consuming. So the need for an efficient automation solution occurred. The lecture presents an MCPD workstation which enables to automate the methods AOCS Cd 29a and c. Special attention is given to the modified AOCS Cd 29c-13 method: DGF Fast and Clean. It enables not only a higher sample throughput. Regarding to the whole system, it also is more robust. Comprehensive validation data and practical experiences of routine laboratories are presented as well.

NOVEMBER 7, 2017 (12:45–13:30)

VENDOR SEMINAR:
FAST, RELIABLE EXTRACTIONS FOR THE ANALYSIS OF MULTIRESIDUE PESTICIDES IN AGRICULTURAL SAMPLES

Fast, Reliable Extractions for the Analysis of Multiresidue Pesticides in Agricultural Samples

Phil Germansderfer, Hamid Shirkhan

FMS – Fluid Management Systems, USA

Introduction

Recent years have seen an increased interest in the analysis of agricultural products, foods and cannabis with regards to pesticides. Many methods have been developed for quick and effective screening of matrices for multiclass and multiresidue analysis (QuEChERS). Automation of such methods can greatly enhance laboratory efficiency, and provided with the right tools, lead to same day sample analysis with greater accuracy and reproducibility.

Method

Pressurized Liquid Extraction (PLE) is an automated technique for extraction of complex matrices. This new technique is a 10-minute extraction and cleanup ready for direct injection. Work done focused on grains, cannabis, plant leaves and roots. Matrices included spices, supplements, and tea. Goal was to develop a simple, rapid, reliable method to analyze between 120-200 pesticides. Matrices were placed in stainless steel extraction cells mixed with MgSO4. Extract clean-up was performed using XtractClean™ layered in the cell. Samples were extracted with acetonitrile; no additional clean-up was done. One extract is used for the Sample analysis that is performed on MS triple quads using both GC and LC interfaces.

Preliminary Data

Comparisons were made between extraction with the clean-up sorbents inside the extraction cell, as well as outside the cell. Excellent recoveries of analytes within the targeted group of 120-200 pesticides. Pressurized Liquid extraction delivers fast, reproducible, quantitateable data

Novel Aspect

PLE - Pressurized Liquid Extraction has shown fast, reproducible, results for the multi-residue pesticide analysis of various food matrices. The same extract is used for both GC/MS and LC/MS analysis. It reduces solvent consumption. The entire process is completed in 15 minutes for up to eight sample extraction and cleanups simultaneously. Up to 192 in an 8 hour day.

SPEX® Europe™

VENDOR SEMINAR: THE IMPORTANCE OF CRMS & SAMPLE PREPARATION IN ENSURING FOOD INTEGRITY & SAFETY

The importance of CRMs & Sample Preparation in Ensuring Food Integrity & Safety

Kshitish Shukla, Mark Tutty

SPEX Europe, Stanmore, United Kingdom

Providing assurance to consumers about the safety, authenticity and quality of food is one of the key drivers within the global Agri-Food economy.

Drawing on data from a number of sources, aspects such as low-level pesticide extraction; food contamination & adulteration; and food origin will be considered. The use of SPEX Europe's CRMs, chemical standards and sample preparation equipment help ensure high quality analytical results are achieved, cost effectively.

Presentation will introduce;

- Certified Reference Materials (CRM), an important Quality control tool for contaminants
- Heavy metals and speciation standards
- Pesticide residues, regulation, New pesticides kits that conform with the latest EU regulations
- Increased pesticide recovery using GG2010 with the QuEChERS method
- Use of cryogenic milling to enable scale-down and increase sample throughput

The use of MiniG in sample preparation for Stable Isotope Ratio Analysis (SIRA), to test the authenticity of food product origin and production method claims.

NOVEMBER 8, 2017 (13:30–14:15)

VENDOR SEMINAR: MAXIMIZING LABORATORY PRODUCTIVITY IN ROUTINE FOOD ANALYSIS (1)

Elemental Profiling of Whiskey, Wine and Beer by Microwave Plasma Atomic Emission Spectroscopy (MP-AES), Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Jenny Nelson [1-3], Helene Hopfer [1-2,4], Greg Gilleland[3], Susan E. Ebeler [1-2]

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[2] *Food Safety & Measurement Facility, University of California, Davis, CA, USA*
[3] *Agilent Technologies, Inc., Santa Clara, CA, USA*
[4] *Department of Food Science, The Pennsylvania State University, University Park, PA, USA*

The elemental composition of beverage products (Whiskey, Wine and Beer) can provide vital information about potential contaminants, product authenticity and origin, quality, and nutritional content. Also, testing throughout the manufacturing and production process can help to identify the source of the elements.

In this study we compare the elemental fingerprints of 68 commercial whiskies for differentiation due to type (Bourbon, Tennessee, Scotch, Irish, Japanese) and region. In this study we used MP-AES, ICP-OES, ICP-MS and used in subsequent statistical analyses by Mass Profiler Professional (MPP).

Authentication of food products of specific geographic origin, such as wine, has garnered interest in consumers, growers and producers, because wine is often associated with qualities only found in certain regions. In this study, possibly the first of its kind, we measured elemental profiles to distinguish mono-varietal pinot noir wines in their region from six different sub-appellations within a single American Viticultural Area (AVA) in Northern California.

Trace metals in beer can also originate from environmental contamination, including fertilizers and pesticides. Also, metals may be introduced by the brewers in the form of salts (e.g., calcium salt, calcium chloride, magnesium sulfate, and zinc sulfate) prior to fermentation. Metal pick-up can come from copper piping and the filtration process through diatomaceous earth. The mineral content in water is a contributor to the overall flavor of beer, which historically led to different beer brewing styles throughout the world.

Application of new, miniaturized triple quadrupole LC/MS system for robust, trace level quantitation of pesticides and mycotoxins in food

Terri Sosienski

LC/MS Applications Scientist, Agilent Technologies, Santa Clara, CA, USA

The new Agilent Ultivo Triple Quadrupole LC/MS is designed to address the challenges and changing needs faced by routine production laboratories, especially those performing high-throughput food safety analysis. In this seminar, we will present, two fast, robust, and precise methods for quantifying trace levels of mycotoxins and pesticide residues in various food matrices developed with Ultivo. Advanced technological innovations within Ultivo allow the instrument to have a dramatically reduced footprint such that it can be housed within our Infinity II HPLC/UHPLC stacks whilst maintaining all the power and sensitivity of much larger triple quadrupole LC/MS instruments. In addition to hardware innovations, smart diagnostics and customizable user interfaces both allow Ultivo to be operated by a novice LC/MS user. MassHunter software simplifies method setup, data analysis and reporting, resulting in the fastest acquisition-to-reporting time possible, increasing lab productivity and confidence in results. This seminar will highlight the innovations of Ultivo, and demonstrate the sustained power of Ultivo for these two common, high-throughput trace level analyses in complex food matrices.

VENDOR SEMINARS

NOVEMBER 8, 2017 (13:30–14:15)

VENDOR SEMINAR: ENSURING THE MERIT OF FOOD

Non-targeted and targeted approaches to determine the authenticity of olive oil using LC-QTOF and GC-APCI-QTOF

Alegría Carrasco Pancorbo

University of Granada, Spain

Non-destructive off-flavor analysis of cork below odor threshold

Gordon van't Slot

Bruker Daltonik GmbH, Bremen

Cork taint is in fact a set of very undesirable aroma and flavor characters that are imparted to bottled wines following contact with their cork. 2,4,6-Trichloroanisole (TCA) is sensorily very potent, it makes a wine smell moldy or musty, like cardboard, damp cement or wet newspapers. At its worst, the wine is undrinkable. Some tasters can detect TCA at 1 ppt to 2 ppt, and a rare few can perceive it at even lower levels. We describe a non-destructive off-flavor method for analysis of cork. We perform automated sample preparation and quantitation of off-flavors using an EVOQ GC-TQ. The method is fast, ultra-sensitive and selective for confident determination of TCA in cork samples at trace levels. For TCA the LODs is at 0.1 ppt and the LOQ at 0.2 ppt.

A validation study of pesticides in food and vegetables according SANTE/11945/2015 using TargetScreener HR 3.0

Carsten Baessmann

Bruker Daltonik GmbH, Bremen

Rapid, comprehensive screening for residues using full scan accurate mass has become a powerful tool in facilitating food safety monitoring. In addition to the high number of possible target compounds, the technique enables unknown screening and retrospective analysis. We describe a validation study according EU AQC Doc. SANTE/11945/2015 for about 300 pesticides in QuEChHERS extracts of orange and lettuce. Required identification criteria are mass accuracy ≤ 5 ppm or < 1 mDa for masses < 200 m/z, RT difference < 0.1 min, the identification of two diagnostic ions and the determination of the ion ratio ≤ 30%. The experiments were performed with TargetScreener HR 3.0, consisting of an Bruker Elute UHPLC interfaced to a Bruker impact II QTOF mass spectrometer. Data acquisition was performed with a 15 minute reverse phase UHPLC gradient in alternating full scan and bbCID fragmentation modes. Automatic data evaluation was performed using TASQ 1.4 processing software. The high quality pesticide database contains more than 820 pesticides. For confident identification we use retention time, precursor accurate mass, isotopic pattern, ion ratio and up to 3 qualifier ions in full scan and 7 qualifier ions in bbCID acquisition. As part of the recovery study we analyzed 3 spike levels of 10, 20 and 50 µg/kg. For the linearity study we were looking at 8 levels in solvent and matrix extracts at 0, 1, 2, 5, 10, 20, 50 and 100 ng/ml. For both studies we did 6 replicates and determined the required information about linearity, recovery, RT, matrix effects and mass accuracy.

NOVEMBER 8, 2017 (13:30–14:15)

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

VENDOR SEMINAR: RECENT ADVANCEMENTS IN AMBIENT IONISATION TECHNOLOGY FOR FOOD AUTHENTICITY AND PROFILING

Advances in Rapid Evaporative Ionisation Mass Spectrometry – a disruptive technology for the food testing industry?

Sara Stead

Waters Corporation, Stamford House, Altrincham Road, Wilmslow, SK9 4AX, UK

The advent of ambient ionization mass spectrometric (AIMS) methods remove most of the constraints associated with sample preparation and have opened new opportunities for point-of-control monitoring for food analysis. The spectral profiles generated from these methods are highly characteristic of the type, origin, age etc. of the sample, which make these approaches excellent for rapid profiling analysis. The MS spectral information can be used as a 'fingerprint' for the identification of critical attributes associated with both the genetic origin and environmental exposure of the sample.

Rapid Evaporative Ionization MS (REIMS) was originally developed as a direct combination of electrosurgery (surgical diathermy) and MS, for the intraoperative identification of cancerous tissue and surgical margin control. However, it has become clear from extensive collaborative studies with the food testing industry that the method can equally be used for the instantaneous characterisation of meat and fish as well as practically any water-containing food commodity and has potential for the development of an automated at-line testing platform.

Join us during the Waters Innovation session to find out more about the advances in instrumentation, software and proof-of-principle applications and making it possible to address various food industry challenges such as; the detection of boar taint in pigs (carcass grading); authenticity of Protected Designation of Origin (PDO)products and detection of milk from mastitis infected cows.

ThermoFisher SCIENTIFIC

VENDOR SEMINAR: USING ADVANCED MASS SPECTROMETRY TECHNOLOGIES TO ADDRESS NEW FOOD SAFETY CHALLENGES

Comprehensive multi-class veterinary medicines workflow solution using a new best-in-class triple quadrupole mass spectrometer

D. Borts[1], Ed George[2]

[1]College of Veterinary Medicine, Iowa State University, U.S.
[2]Thermo Fisher Scientific, San Jose, U.S.
Corresponding author – E-mail: ed.george@thermofisher.com

Thermo Fisher Scientific introduces the Vet Drugs Explorer – an off-the-shelf validated workflow solution for the analysis of over 160 multi-class veterinary medicines in animal matrices by a new highly sensitive and robust LC-triple quadrupole mass spectrometer. This new system incorporates a high capacity ion transfer tube and segmented quadrupoles to increase ion flux, and an improved detector design to increase sensitivity and linear dynamic range. This advanced technology enables quantification and identification of residues at low regulatory levels. The integrated workflow solution includes everything from sample preparation to report using the new system, and is ideal for new and existing residue testing laboratories. Data on the validation of the method in cattle muscle, salmon, and milk matrices, will be presented.

GC-Orbitrap-MS: a new way to simultaneously determine different halogenated POPs?

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The coupling of gas chromatography (GC) to ultra-high resolution mass spectrometry (UHRMS) using the Orbitrap technology opens up a broad spectrum of possible applications in environmental and food/feed analysis. Although known for several decades and widely used as plasticisers or flame retardants [1], short-chain chlorinated paraffins (SCCPs) have only been recently added to Annex A of the Stockholm Convention list of persistent organic pollutants (POPs) [2].

Caused by previous efforts to ban SCCPs, medium-chain CP (MCCP) production increased, often to replace SCCPs [3]. As SCCPs alone consist of several thousand congeners with only four different carbon chain lengths to choose from, quantification of SCCPs and MCCPs in samples is a highly complex problem. In addition to that, other halogenated POPs like polychlorinated biphenyls (PCBs) are known to co-elute and add to the complexity of any analysis.

With this in mind, experiments focused on selectivity were performed using full-scan acquisition and negative chemical ionisation (NCI) at 60,000 and 120,000 resolution (FWHM, m/z 200). In this presentation, the results for mixtures of different CP and PCB standards will be shown as well as a comparison of food samples that were analysed with and without separation of co-eluting POPs during sample clean-up. Our results demonstrate that GC in combination with ultra-high resolution Orbitrap-MS enables the determination of CPs without having to fear mass interferences from both other CP homologues and PCBs, indicating the same for other halogenated compounds.

[1] Tomy GT (2010) *The Handbook of Environmental Chemistry*, Vol. 10, Springer, London.
[2] Stockholm Convention on Persistent Organic Pollutants, UNEP-POPS-COP.8/14, final report not yet published as of 24.07.2017.
[3] Gluege J, Wang Z et al (2016) *Science of the Total Environment*, 573 1132–1146.

Acknowledgement: *We would like to thank the EU Commission for the financial support of the work of the EU-RL for Dioxins and PCBs in Feed and Food and Thermo Fisher Scientific, Runcorn/UK division, for their support with the GC-Orbitrap-MS measurements.*

NOVEMBER 8, 2017 (14:45–15:30)

SHIMADZU
Excellence in Science

VENDOR SEMINAR:
IN FOOD WE TRUST – THE PASSION OF FOOD ANALYSIS

Advanced beer aroma analysis

Erich Leitner

TU Graz, Institute of Analytical Chemistry and Food Chemistry, Graz, Austria

Beer is a complex alcoholic beverage, containing several hundred volatiles and flavor active compounds. The concentrations cover a huge range from picogram to milligram per liter which makes the analysis really challenging. The main constituents of beer are water, ethanol, carbohydrates, proteins and carbon dioxide, but in terms of sensory perception the minor constituents are the relevant ones. They derive from the used raw materials or are formed during the brewing process or storage of beer. The impact of the raw materials (barley, hop) and the processing steps (mashing, fermentation, hop isomerization) and their impact on the final product quality will be discussed in this presentation. Further on analytical strategies from sample preparation procedure to targeted and untargeted analysis of volatile and aroma active compounds using different one and two dimensional gas chromatography based methods (GC-MS, GC-MSMS, GCxGC, MDGC) will be shown. Finally relevant off odor compounds will be discussed in detail in terms of source, formation and analytical determination.

1) C.W. Bamforth, Brewing and brewing research: Past, present and future, Journal of the Science of Food and Agriculture, 80, 9, 2000, 1371-1378
2) L.C.Verhagen, Beer Flavor, Reference Module in Chemistry, Molecular Sciences and Chemical Engineering, Comprehensive Natural Products II, 3, 2010, 967-997
3) M.Meilgaard, Effects on flavor of innovations in brewery equipment and processing: A review, Journal of the Institute of Brewing, 107, 5, 2001, 271-286

Characterization of the Oxygen Heterocyclic Compounds (Coumarins, Psoralens and Polymtehoxylated Flavones) in Food Products

Mariosimone Zoccali[1*], Adriana Arigò[2], Marina Russo[3], Francesca Rigano[1], Paola Dugo[1,2,3] and Luigi Mondello[1,2,3]

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[3] University Campus Bio-Medico of Rome, Italy

Oxygen heterocyclic compounds were identified by using an HPLC-PDA/MS/MS system equipped with an APCI source in positive ionization mode, in a single run in less than 10 minutes. The MS/MS transitions obtained at a defined collision energy have been used to build a library. The use of an MS/MS system allowed to achieve limit of detection and quantification (LOD and LOQ) in the ppb range, thus making the method very useful for food industries. Furthermore, the goal of the present research was to propose an easier to use method for the characterization of the oxygen heterocyclic compunds in food, by using a simpler HPLC-PDA instrument and the internal standard/response factor approach. Psoralens are a class of photoactive compounds found in several plant species and maybe responsible for the observed association between consumption of *citrus* products and the risk of phototoxicity.

How Safe is Safe? Analytical Tools for Tracing Contaminants in Food

Uwe Oppermann, Anja Grüning, Gesa Schad, Carola Schultz

Shimadzu Europa GmbH, Duisburg, Germany

Contaminants like pesticide residues, mycotoxins and heavy metals may occur in our food from a variety of different sources. These are in the focus of European food and safety authorities, and are controlled by national and international regulations. Analysis of relevant chemical contaminants is therefore an essential part of the food safety policy of the European Commission to ensure the highest level of protection of human health. Modern hyphenated analytical techniques such as chromatography (LC-MS), spectroscopy and mass spectrometry can determine these contaminants in complex food matrices with high sensitivity at ultra-low concentration levels in order to keep the food and beverage chain safe.

VENDOR SEMINAR: ANALYTICAL EFFICIENCY IN FOOD ALLERGEN TESTING

Analytical efficiency in food allergen testing

Christine Gutschelhofer, Ronald Niemeijer

R-Biopharm AG, Germany

About 2% - 5% of the adult population suffers from one or more food allergies. Amongst children these numbers seem to be twice as high. Although not a food allergy, celiac disease is often mentioned in the same context. It is estimated around 1% of the population suffers from this gluten triggered auto-immune disease. Extrapolating this number to the number of households, this would mean that the number of people affected by a food allergy (directly or indirectly) is probably 3 – 4 times higher. On top of that if people are asked if they think they have a food allergy up to 25% - 30% of the people answers positively. All in all the number of consumers that need or want to be informed about the presence of allergens in food is significant and this has become an important market segment for the food industry.

To protect allergic consumers and to assure consumers are informed in a correct way about the presence of allergens in their food, labeling legislation is in place in a growing number of countries all around the world. These legislations reflect to some extent the local differences in occurrence of allergens, but are almost all based on the so called "Big 8" of food allergens (Milk, egg, fish, crustacean, tree nuts, peanuts, wheat and soybeans). For gluten (or "gluten-free") separate legislation is in place in many countries.

In order to meet this legislation and customer requirements, food producers should have a solid allergen management in place. One of the pillars of food management is testing – raw materials, finished products but also at the actual production site. Testing for food allergens may however be challenging, many factors influence the quality of the results like sampling, sample preparation, analytical method. Nevertheless, results should be available rapidly, preferably at low costs, thus emphasizing the need for increased analytical efficiency.

This seminar will address solutions to improve the analytical efficiency of food allergen testing from different angles:

- The use of rapid test methods in allergen management
- Automation of analytical methods in food allergen testing
- Multiplexing by PCR for food allergen detection
- Quality assurance in food allergen testing

Trends in food allergen analysis will be discussed as well as the impact they may have.

NOVEMBER 8, 2017 (14:45–15:30)

VENDOR SEMINAR:
POLAR PESTICIDES AND VETERINARY DRUGS FOR ROUTINE LABS: NEW LC-MS/MS METHODOLOGIES PRESENTED BY SCIEX CUSTOMERS

A robust Analysis of Glyphosate and other polar pesticides in food and feed - a challenge no more

Vim Broer

Manager, Science and Development, NofaLab, Netherlands

The direct analyses of Glyphosate and other polar pesticides like Glufosinate, Fosetyl, Erheohon, chlorate and their metabolites with LCMS is well described in the EURL QUPPE-method. But the chromatographic separation is not robust and a lot of maintenance is required. For Glyphosate, AMPA and Glufosinate a known robust method is to derivative these compounds with FMOC.

This method, however, is laborious and still needs a separate method for the other pesticides. At Nofalab a robust method is developed on a SCIEX 6500+ with a Shimadzu UHPLC. Basically the chromatographic separation is done on a polyvinyl alcohol with quarternary ammonium groups' column at a pH of 9. This ionograhic condition puts the glyphosate in the ideal configuration allowing a stable separation whale the other anions are still well separated. In this presentation an overview of the method is shown as long term stability, robustness and validation results.

The developed method meets the Santé requirements regarding the Reproducibility (<20%) and recovery (80-110%). The LOD of the method is below 0.01 mg/kg.

Fast, high-throughput food safety testing in meat production QC

Kirsten Kirkeby

Quality Assurance Supervisor, Danish Crown, Denmark

The Danish Crown Group is a global processing company and among the largest in its field. It is the world's largest pork exporter and Europe's largest pork processor and meat processing company. Danish Crown is committed to ensuring that consumers and customers have confidence in its products.

It is therefore imperative that the raw materials comply with a number of quality requirements, one of which is the testing for veterinary drug residues. The decision was made in 2014 to implement a state-of-the-art LC/MS-MS method as part of our self-monitoring program for these compounds. The Danish Crown Laboratory was, at that time, mainly involved in microbiological testing, so there was a very steep learning curve with a high profile.

A complete Sciex solution based upon a 4500 triple quad was chosen because it included method set-up, training and ongoing support. During 2015, Danish Crown worked together with Sciex to implement and optimize a method based on that from the ANSES reference laboratory in Fougères, France and from the Danish authorities. The final method was developed for the screening for 33 different antibiotics in one single run. For the first time in Europe, a new risk-based surveillance program, developed by the Danish Agricultural and Food Council in corporation with the Danish authorities was implemented at the same time.

VENDOR SEMINARS

NOVEMBER 9, 2017 (7:45–8:30)

VENDOR SEMINAR:
GC-TOF MS – THE IDEAL TOOL FOR NON-TARGET SCREENING OF FOOD MIGRANTS

Food contact materials are all materials and articles intended to come into contact with food, such as packaging and containers, kitchen equipment, cutlery and dishes.[1] The high number of authorized substances (including (semi)volatiles, non-volatiles and complex mixtures) used within the packaging materials' manufacturing together with complex physico-chemical conditions at the contact layer with food matrix can potentially result in the chemical contamination of particular food/beverage by non-intentionally added substances (NIAS).

The packaging material has to be manufactured in compliance with given legislative requirements and good manufacturing practice (GMP). Despite this fact, the migration of compounds from packaging material into food products is a strong concern for manufacturers and consumers, since the transfer of NIAS components via extraction and/or leaching can impact the quality of the product, the integrity of the packaging material, and cause concern related to consumer health and product safety.

The nature of time of flight mass-spectrometry (TOF MS) technology – fast and sensitive acquisition of unskewed full mass spectra – makes GC-TOF MS the technique of choice in non-targeted examination of NIAS components in food samples.

[1] *https://www.efsa.europa.eu/en/topics/topic/food-contact-materials?qt-quicktabs_field_ collection=3#qt-quicktabs_field_collection*

SPME-GC-HR-TOF-MS based search for off-flavor compounds in chocolate advent calendars

Jaromír Hradecky, Eliska Kludska, Jana Hajslova

University of Chemistry and Technology, Prague, Department of Food Analysis and Nutrition, Czech Republic, E-mail: jaromir.hradecky@vscht.cz

Identification of compound(s) responsible for food off-flavor often represents a challenging analytical task, since these compounds might have a very low odor threshold. It is rather difficult to identify them, especially when present in a complex mixture. For this purpose, head-space solid-phase microextraction sampling followed by gas chromatography – mass spectrometry (SPME-GC-MS) employing time of flight (TOF) mass analyzer seems to be the optimal option.

Within our study, both low resolution TOF and high resolution (HR-TOF) MS were employed. Non-target screening in a set of advent calendars was performed with the aim to access aroma quality. Statistical compare – peak alignment tool was used and data matrix was then processed using multivariate statistical analysis. Surprisingly, styrene, a possible human carcinogen, migrating from plastic packaging was discovered at a significant level in one of the chocolate samples, dedicated for children. Unknown off-flavor compounds in chocolate from one of the calendars were identified using HR-TOF MS. Acrylates, responsible for an unpleasant smell of chocolate were identified in green painted parts of wholesale cardboard box. According to obtained results, SPME-GC-(HR)TOF-MS proved to be a reliable tool for non-target screening and unknown identification.

Investigation of Extracted and Leached Analytes from Packaging Materials with GC-TOF MS

Tomáš Kovalczuk[1], Elizabeth M. Humston-Fulmer[2], Joe Binkley[2]

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Migration of analytes from packaging material into food products is a concern for manufacturers and consumers because of the potential to contaminate food and beverages. The extraction and/or leaching can impact the quality of the product, the integrity of the packaging material, and cause concern related to consumer health and product safety. A general extraction of a variety of food packaging products, including sealable plastic bags and plastic food

containers, was performed. Solvent was placed inside each packaging product for an extended period of time and then concentrated through evaporation prior to analysis. General screening of this extract with non-targeted analytical techniques was used to understand what analytes were present and may have the potential to migrate into the food. GC was used for separating analytes from each other and TOFMS provided full mass range data. Nominal mass TOFMS data were acquired and were searched against library databases for tentative identifications. High resolution TOFMS data were also acquired to add confidence to identifications with accurate mass information. Several analytes were determined in the various packaging types and are highlighted here.

VENDOR SEMINARS

NOVEMBER 9, 2017 (7:45–8:30)

VENDOR SEMINAR:
MODERN APPROACHES FOR PFAS/PFC'S ANALYSIS FROM FOOD AND ENVIRONMENTAL MATRICES BY LC-MS/MS

Modern Approaches for PFAS/PFC's Analysis from Food and Environmental Matrices by LC-MS/MS

Scott Krepich

Senior Field Application Scientist, Phenomenex

Per- and poly-fluoroalkyl substances (PFASs) or Perfluorinated chemicals (PFC's) are synthetic organofluorine compounds which are extremely resistant to degradation. Human exposure to PFAS residues has been implicated in the incidence of cancer, obesity, endocrine system disruption, and other adverse health effects. They are present in a variety of products; including textiles, fire-fighting foams and food packaging and have been detected in the environment as well as tissues of animals. Thus, the USEPA has issued health advisory limits for PFASs: PFOA and PFOS. Presented will be a variety of modernized methods for the analysis of PFAS in various matrices such as drinking water, sediment, and food. In addition, various sample preparation techniques were used including, QuEChERS, offline SPE, online SPE as well as direct injection using LC-MS/MS with both HPLC and UHPLC options.

NOVEMBER 9, 2017 (13:30–14:15)

ThermoFisher SCIENTIFIC

VENDOR SEMINAR: ISOTOPE FINGERPRINTS IN AUTHENTICITY AND FOOD FRAUD

Food Integrity with Isotope Fingerprints: unlocking the truth

Christopher Brodie

Thermo Fisher Scientific

Fraud in food and beverage products include misrepresentation or tampering with packaging and labelling; adulteration, normally replacing a higher quality, original material with one of lesser quality one or extending a product by adding an adulterant; and misrepresentation of product origin. Increased complexity in the food and beverage supply chain has provided greater opportunity for economically motivated food and beverage fraud. Consequently, legislation has been enacted globally to protect food and beverage products with respect to production processes and product labelling. The combination of legislation and food fraud practices demand a reliable, high throughput and cost effective analytical technique that can identify food and beverage products that are not what they are claimed to be. Detecting food and beverage fraud can be achieved using stable isotope fingerprints because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. We briefly explore the technology available to detect isotope fingerprints before leading onto examples of how isotope fingerprints really detect food and beverage fraud.

The power of stable isotope fingerprints in authenticity control and fraud prevention

Simon Kelly

International Atomic Energy Agency (IAEA), Section of Food and Environmental Protection

Food and beverage products have unique isotopic fingerprints, which allow such products to be identified with respect to their origin, composition and processing. Isotope Ratio Mass Spectrometry (IRMS) can be used to visualize these fingerprints. Any biochemical or physical reaction in growing and processing of food can result in changes in the isotopic composition of individual compounds or the bulk isotope ratio of a food product. Major targets are the stable isotopes of carbon, nitrogen, sulfur, oxygen and hydrogen, which individually or in combination can give unique insights. The different IRMS tool sets based on coupling with GC, LC, elemental analyzers and other sample preparation technologies allow to uncover stable isotope fingerprints using several individual compounds in a food sample, multiple stable isotopes of N, C, S, O and H in bulk or parts of a sample or combinations of both. The recent improvements in sample throughput and automation allow fast and inexpensive analysis of several ten – thousands of samples per year with a single IRMS system. The presentation will focus on the principles of isotope fingerprints showing examples of applications and highlighting the latest technological improvements in IRMS.

VENDOR SEMINARS

NOVEMBER 9, 2017 (13:30–14:15)

VENDOR SEMINAR: MAXIMIZING LABORATORY PRODUCTIVITY IN ROUTINE FOOD ANALYSIS (2)

Application of a new GC/Q-TOF for the screening of pesticides and other contaminants in food

John Lee

Global Food Marketing Manager, Agilent Technologies, UK

The demand on efficiently screening against a wide scope of food contaminants is rapidly increasing owing to the enforcement of regulatory Maximum Residue Levels (MRLs) and a growing interest in untargeted screening for risk assessment. Recent advances in high resolution GC/Q-TOF mass spectrometry are helping to meet this demand by providing enhanced identification as well as accurate quantitation of GC amenable compounds.

Agilent has created an accurate mass EI spectral library to enable facile identification and verification of 100's of pesticides using the 7250's capability. Additionally, it is possible to expand the investigation scope to other contaminants using external public libraries (e.g. NIST) and assess potential hits through fragment formula prediction based on the molecular formula of a proposed hit. Furthermore, the 7250 GC/QTOF can aid hit-verification by seamlessly switching to a new Low energy EI mode which enhances the possibility to preserve molecular ions, with the added capability to deliver high resolution MS/MS for structural elucidation.

Strategies for Rugged GC-MS and LC-MS Analysis in Routine Food Testing

Katerina Mastovska

Covance Food Solutions, Madison, WI, USA

Food testing laboratories employ GC-MS and LC-MS techniques routinely in many different applications. Modern instruments offer high speed, sensitivity and selectivity, which can be utilized to improve various method performance characteristics and also help deal with challenging food matrices. For instance, increased detection sensitivity can be used to improve analyte limits of detection/quantitation (LOD/LOQ) or to decrease the sample introduction (injection volume) into the analytical system while maintaining the LOD/LOQ levels. The latter option is very attractive in routine food analysis, where matrix can detrimentally affect chromatographic separation, ionization efficiency, method ruggedness and routine maintenance costs. This seminar will discuss the use of high-sensitivity LC and GC triple quadrupole MS/MS systems and their beneficial impact on short- and long-term method performance, especially when combined with other strategies and tools, such as the use of analyte protectants and column backflushing for increased ruggedness in GC-MS or the application of a novel on-line dilution set-up for improved analysis of more polar compounds in LC-MS.

NOVEMBER 9, 2017 (13:30–14:15)

VENDOR SEMINAR:
SOFT SOLUTIONS TO HARD PROBLEMS: SAVING TIME AND MONEY IN THE LABORATORY WITH THE EZGC WEB TOOLS

Soft Solutions to Hard Problems: Saving Time and Money in the Laboratory with the EZGC Web Tools

Hansjoerg Majer, Jonathan Keim, Chris Rattray, Chris English

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Analytical laboratories face continual pressure to increase productivity while decreasing costs. With recent advances in available technology, focus on optimizing analytical methods, and optimization of key instrument parameters, we can find valuable opportunities to save time and money. In this talk, we will share work showing how the *EZ*GC suite of online tools can be used to improve gas chromatographic analyses.

We will show several examples of how the free software can be used for method development. Additionally, these tools can be used to improve your analysis in various ways such as maintaining high quality separations with much shorter columns, the importance of optimizing your splitless valve time, and switching carrier gasses.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

VENDOR SEMINAR: OVERCOMING THE CHALLENGES OF ANALYSING ANIONIC POLAR PESTICIDES

Glyphosate and other anionic pesticides: what makes these pesticides so po(pu)lar?

Euan Ross

Waters Corporation, Stamford House, Altrincham Road, Wilmslow, SK9 4AX, UK

Glyphosate continues to be the most widely used agricultural chemical worldwide. While the toxicity is under debate, maximum residue levels (MRLs) are enforced around the globe, based on crop application in line with good agricultural practices. Therefore, analytical testing of glyphosate, other herbicides and their metabolites is required to ensure consumer safety.

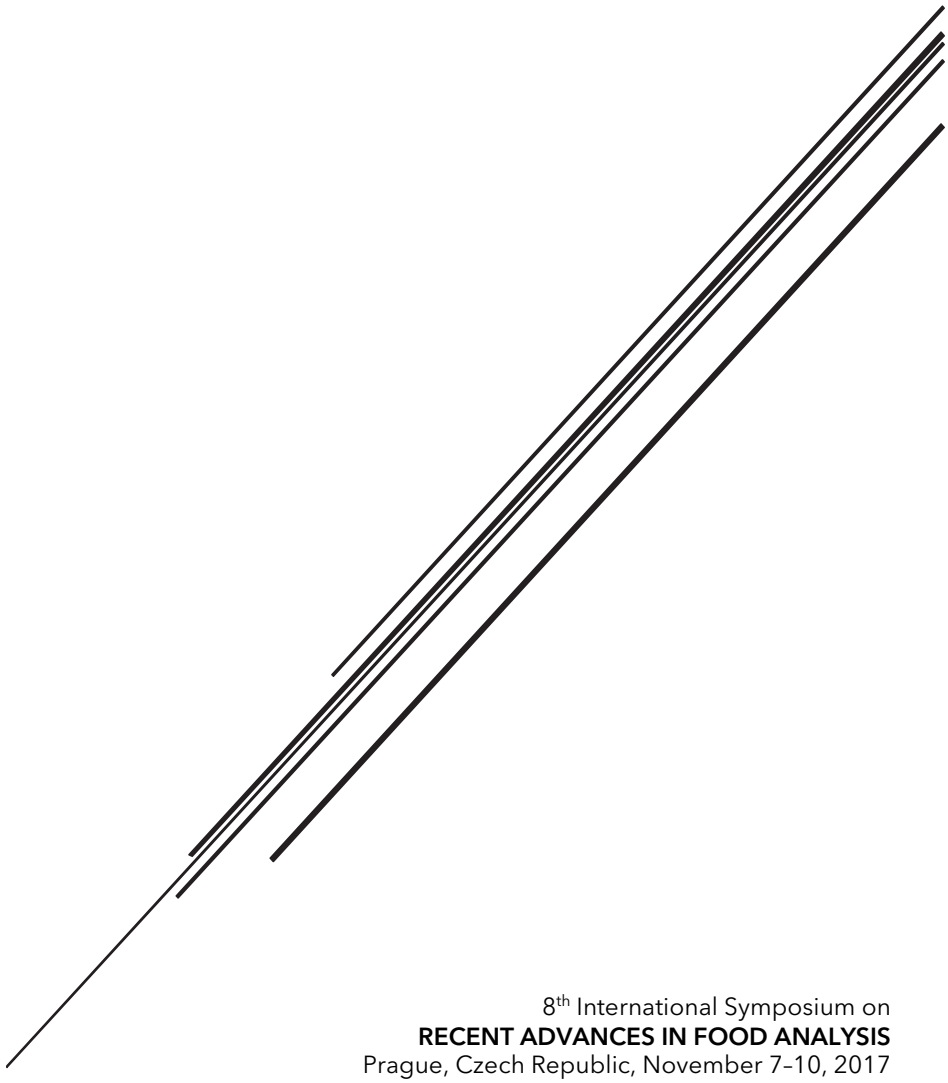
Due to physiochemical properties, glyphosate and other polar pesticides are challenging to analyse. These properties impact on the various stages of the analysis from sample preparation, separation and detection. Whether you're a gardener, environmentalist, formulator of pesticides or analytical chemist, the words glyphosate and anionic pesticides will invoke a reaction!

Join us in Virgo on Thursday, where we will discuss these highly polar anionic compounds and demonstrate:

- Reliable analysis by LC-MS/MS, without the need for derivatisation or specialty ion chromatography instrumentation
- Excellent sensitivity and precision across a variety of relevant food commodities
- Accurate quantitation of incurred residues, in the absence of costly isotopically labelled internal standards
- LC column selection and key method considerations
- Achieving and maintaining a robust LC-MS/MS system

LECTURES

Oral Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

LECTURES

L1
FOOD ANALYSIS FROM THE PERSPECTIVE OF THE RISK ASSESSOR

Robert van Gorcom[*1]

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The needs for high level analysis of food and feed products from the perspective of the risk assessor are not always in line with those of food/feed producers and the food authorities. Whereas most of the analyses are aimed to verify whether the products are compliant to the current legislation, the questions of risk assessors are more related to the preparation of new or re-evaluation of current legislation and/or standards and acute and lifelong effect levels.
New legislation/standards may be related to new products or compounds, possible new risks, new evaluation criteria and/or new scientific knowledge. So the risk assessor is very interested in analytical results for compounds that are not (yet) routinely tested by the service laboratories. Furthermore, risk assessors are interested in the analysis results of randomly selected samples (monitoring) and much less in the more and more risk based selection of samples of the current daily practice.
Also the emerge of combination toxicology and the need to understand the effects of the combined exposure in our complete diet of toxicologically related compounds requests much more data and also data at sub (single residue) MRL levels since their combined exposure might be more of relevance.
I my lecture I will illustrate these topics in more detail and underline the enormous relevance of further advances in food analysis.

Keywords: *risk assessment, combinatorial toxicology, advanced food analysis*

L2
A FOOD CONSUMER'S VIEW ON PERSONALIZED FOOD ANALYSIS

James Lawrence[*1]

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In a perfect world, consumers would be able to test the food they eat for any substances they do not wish to ingest. This may be for a variety of reasons including personal health, religious or ethical beliefs. Health reasons may include the detection of substances that might cause a consumer immediate illness or discomfort such as the well-known allergens and other substances such as lactose and gluten. Consumers may also be concerned about undesirable substances that may not be immediately life threatening. Chemicals such as pesticides, natural toxins, environmental contaminants are a few examples. This presentation will offer a balanced consumer perspective on the current state of personalized testing of foods and the responsibility consumers, the government and the food industry have in ensuring that the food we ingest is acceptable to us.

L3
ANALYTICAL CHALLENGES FOR AN EFFECTIVE EU POLICY ON CONTAMINANTS IN FOOD AND FEED TO ENSURE A HIGH LEVEL OF ANIMAL AND HUMAN HEALTH PROTECTION

Frans Verstraete[*1]

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**Corresponding author - E-mail: Frans.Verstraete@ec.europa.eu*

Directive 2002/32/EC of 7 May 2002 of the European Parliament and of the Council on undesirable substances in animal feed is the framework for the European Union action on undesirable substances in feed.
Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food is the framework for the Union action on contaminants in food.
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 foods shall be presented in particular on the presence of ergot alkaloids, deoxynivalenol and tis modified forms, pyrrolizidine alkaloids, tropane alkaloids, erucic acid, perchlorate, …
The list is not exhaustive!
Particular attention shall be paid to the analytical challenges that are faced for an effective EU policy on contaminants in feed and food such as
- modified forms of mycotoxins
- "multi component" contaminants, such as pyrrolizidine alkaloids, ergot alkaloids, ….
- the need for methods able to analyse low concentrations in certain foods such as glycidyl esters in infant formula,
- speciation of metals

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.
The presentation will bring you fully up to date on what is ongoing and what can be expected in the near future as regards EU policy on contaminants in feed and food, with a particular focus on the related analytical challenges!

Keywords: *contaminants, EU policy, food and feed, analytical challenges*

L4
MULTIMODAL MOLECULAR IMAGING IN HEALTH AND NUTRITION

Ron Heeren[*1]

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State-of-the-Art molecular imaging with Mass Spectrometry now enables high resolution tissue screening that provides direct insight into tissue metabolism. Applications have penetrated various research domains from the visualization of molecular signaling pathways in disease. In this lecture we will demonstrate how mass spectrometry based multimodal molecular imaging can be used to reveal the complexity of nature. We will discuss the development and application of new MS based chemical microscopes that target biomedical tissue analysis in various diseases as well as other chemically complex surfaces.
We will demonstrate how to elucidate the way in which local environments can influence molecular signaling pathways on various scales. We will illustrate how e.g. isotope labeling studies can be employed to trace the local dynamics of metabolic processes. The integration of this pathway information in a surgical setting is imminent, but innovations that push the boundaries of the technology and its application are still needed. The imaging MS community is driving translational molecular imaging research and these needed developments rapidly forward. These new developments will be discussed in the context of food, health and nutrition. The lecture will focus on a survey of novel imaging technologies and their potential impact on food and nutrition studies.

Keywords: *mass spectrometry, molecular imaging, tissue, metabolism, isotope labeling*

L5
NON VOLATILE PRODUCTS OF LIPIDS OXIDATION: WHAT ANALYSIS OF OUR DIET AND PLASMA SAMPLES CAN DISCLOSE

Jana Hajslova*[1], Vit Kosek[1], Vojtech Hrbek[1], Leos Uttl, Kamila Hurkova[1], Jana Pulkrabova[1]

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Strong evidence exists on an important role of diet in the development of major chronic ailments in population from developed countries. The association of these negative trends with various dietary components has been widely investigated. In this context, lipids, specifically their oxidized forms, have become under growing concern. Attention has been paid not only to lipids, peroxidation of which takes place in vivo, but also to oxidized lipids absorbed from the diet. These compounds have been shown to be toxic in cell systems and are known to exert a wide range of other adverse effects. Interestingly, while the mechanism of oxidation processes, regardless they are autocatalytic or enzymatic, are well described, rather limited information is available on the occurrence of individual oxidized lipid species in the Western diet. Similarly, only few studies have been concerned with a comprehensive characterization of a set of oxidized lipids in human tissues and fluids, in most cases, mainly known markers of oxidative stress, such as isoprostanes, have been monitored and their signaling role in various physiological processes and diseases discussed.
The current methods employed under routine conditions can be classified into several groups, based on what they measure, in any case, either the sum of hydroperoxides (as primary oxidation products) or the formation of some secondary oxidation products (originated from hydroperoxide decomposition) are determined
In this study, advanced separation techniques, UHPLC and SFC, coupled with a tandem high resolution mass spectrometry (HRMS/MS), were employed for a non-target screening of a large set of oxidized lipids (including tri-, di- and monoacylglycerols, phospholipids, fatty acids and sterols), both in human diet and plasma. In the latter case, plasma was obtained from the cohort of mothers and their children exposed in a different extent to PAHs, known to induce, besides of other toxic effects, also an oxidative stress. Multidimensional chemometric methods employed for the processing of generated data documented differing patterns of some oxidized lipid species in the examined groups. As regards samples of diet, profiles of individual groups of oxidized lipids were screened and the data correlated with those, obtained conventional approaches used for measurements of food lipids oxidation. The challenges offered by oxidized lipids measurement for a follow up nutrition research will be outlined, the bottlenecks encountered in analytical work will be discussed.

Keywords: *lipidomics, oxidised lipids, dietary exposure, human plasma, processed oils*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). Financial support of Ministry of Health of the Czech Republic (no 15-28745A) is also gratefully acknowledged. This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

L6
NOVEL INFRARED SPECTROSCOPIC AND MASS SPECTROMETRIC TOOLS FOR THE QUANTIFICATION OF (EMERGING) MYCOTOXINS IN CEREALS AND FOODS

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The occurrence of fungal and subsequently, mycotoxin contamination in various crops is of major concern since it has significant implications for food and feed safety, food security and international trade. The European Commission pointed out that 5 – 10% of global crop produce are lost annually due to mycotoxin contamination (European Commission, 2015). Despite huge research investments, the fast and accurate identification and quantification of mycotoxins and other secondary metabolites of plants and fungi continues to be a challenge.
In the meanwhile, mass spectrometry based analytical methods have become the most important means for the determination of more than 600 secondary metabolites of plants and fungi incl. all regulated mycotoxins in cereals and foods and for the investigation of the metabolism of these toxic compounds in body fluids such as serum and urine. Hence, high performance liquid chromatography- tandem mass spectrometric LC-(HR)MS(/MS) methods have also led to unexpected occurrence data on the presence of so-called emerging mycotoxins including novel and conjugated (masked) mycotoxins, as e.g. the glutathione-mediated detoxification products of the *Fusarium* toxin deoxynivalenol and the novel A-trichothecene NX-2. In the last decade, high resolution mass spectrometric based metabolomics has emerged and shows great potential to determine hundreds to thousands of metabolites at once over a wide range of concentrations.
In the area of rapid, non-destructive on-site analytical techniques that can be applied to measure fungal contamination in cereals and food samples, novel infrared spectroscopy has recently been studied [1]. In particular, the advances in microfabrication and miniaturization of sensor components such as tunable quantum cascade lasers, detectors and integrated optics will aid in advancing the applicability of such techniques, particularly in the environment of food and feed analysis.

[1] D. McMullin, B. Mizaikoff, R. Krska. ANAL BIOANAL CHEM. 2015; 407(3): 653-660.

Keywords: *emerging mycotoxins, mass spectrometry, IR-laser spectroscopy*

Acknowledgement: *EU-Project No.678012 Safe Food and Feed through an Integrated Toolbox for Mycotoxin Management; FWF-Project No. F03715 Metabolomics of plant-Fusarium interactions*

L7*
ION MOBILITY FOR FOOD SAFETY: EVALUATION OF ION MOBILITY MASS SPECTROMETRY INSTRUMENTS

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The field of food safety analysis deals with many different biological samples, such as feed, urine, hair, and tissue. These matrices can interfere with the analysis and cause challenges for identification and quantification of regulated compounds. Additionally, with the rise of ambient ionization techniques capable of high-throughput screening, non-chromatographic separation of isomers is becoming more and more significant. A non-chromatographic technique offering possibilities for additional separation is ion mobility mass spectrometry (IM-MS). Several, commercially available, mass spectrometers featuring ion mobility have been evaluated on their ability to separate isomers and reduce matrix interference, but also to strengthen confirmatory analysis by use of the CCS values of molecules.
A broad test set of compounds related to food safety has been comprised. The test set consisted of isomers with relatively low molecular weight (<1000 Da) and is used to test IM-MS instruments from different venders. Separation of the isomers, both as standard solutions and in appropriate sample matrix, was investigated, including the effect of adduct formation on IM separation. Following these results, implementation of IM in existing methods was considered and, when possible, CCS values were calculated and compared with literature and modeled data.
An extensive evaluation was performed of instruments utilizing different IM techniques: differential ion mobility spectrometry (DMS), travelling-wave ion mobility spectrometry (TWIMS), drift tube ion mobility spectrometry (DTIMS), and trapped ion mobility spectrometry (TIMS). In this lecture, the performance of the different IM-MS techniques will be discussed based on isomer separation, reduction of matrix interference, and CCS calculation.
Although tuning possibilities for the IM separation were somewhat limited for some instruments, separation of several isomers could be achieved. When alkali metal adduct formation was promoted, additional separation in the IM dimension could be achieved for various isomers. Next to this, the application of IM in food safety analysis was assessed. To achieve additional confirmation, CCS values were calculated when possible. The resulting data was found to be reproducible when proper calibration was performed, even over extended time periods, and could be compared to modeled data and literature or library data.

Keywords: *ion mobility, mass spectrometry, residues, food safety*

L8*
ANALYSIS OF 3-MCPD ESTERS BY SUPERCRITICAL FLUID CHROMATOGRAPHY

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3- and 2-monochloropropane-1,2-diol (3- and 2-MCPD) esters occurring primarily in refined fats and oils, originate mainly (together with glycidyl esters) during the deodorization step. Bound MCPDs are present also in many other foodstuffs such as bread, fine bakery wares, margarines, smoked meats dairy products etc. The free form, 3-MCPD, that is released *in vivo* by action of gastrointestinal lipases, has been classified as a non-genotoxic, threshold carcinogen. Recently, EFSA CONTAM panel established lowered tolerable daily intake (TDI) of 0.8 ug/kg bw per day for 3-MCPD. Not needed to emphasize, that monitoring of these processing contaminants is necessary.
The determination of 3- and 2-MCPD bound in fatty acid mono- and/or diesters is possible using indirect methods based on transmethylation, nevertheless, compound losses or artefact formation cannot be fully avoided, moreover, the information on a contamination pattern is not available anymore. In this context, direct methods might seem more challenging as individual MCPD esters could be determined. On the other hand, taking into account the number of possible naturally present fatty acids and various positional isomers, the number of analytes might be as high as 100, then it is clear that this is not an easy task. Moreover, sample purification (including e.g., preparative silica gel columns) has to be done prior analysis.
To prevent involvement of a purification step, supercritical fluid chromatography coupled to quadrupole time-of-flight mass spectrometry for detection (SFC-Q HRMS) was used. A number of separation columns was tested. The major advantage provided by this technique was elution of most of target analytes before lipid fraction, interferences could be efficiently eliminated. The method was validated for various food matrices. For more in-depth studies of MCPD esters structure, ion mobility was employed to get a 3rd separation dimension, collision cross sections were calculated to support compounds identification. SFC-Q HRMS has been demonstrated to be a powerful tool for rapid MCPD esters screening.

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). Financial support of Ministry of Health of the Czech Republic (no 15-28745A) is also gratefully acknowledged.*

L9*
TOWARDS AN UNTARGETED FOODOMICS APPROACH FOR FOOD CHEMICAL SAFETY ASSESSMENT

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Chronic exposure to small doses of chemicals is a major today's public health issue, food being known to be an important contributor to the presence of exogenous hazardous substances in body burden. Monitoring food chemical contaminations currently relies on selective targeted approaches. The need for more global untargeted approaches ("foodomics" approaches) has recently emerged, but their development raises many analytical challenges, namely trace concentrations (down to µg/kg), structural diversity of contaminants, chemical complexity of food matrices, and above all, the need for data treatment tools to work "blindly" (without any information on potential contaminants).
In this context, we have developed an untargeted foodomics approach dedicated to food chemical contamination assessment. Our methodology relies on four pillars from analytical chemistry, metabolomics and chemometrics: (i) a broad-range analytical method based on UHPLC-HRMS using a novel reversed-phase offering enhanced retention for polar compounds (C18-PFP-silica) and minimal sample treatment; (ii) feature detection based on XCMS and data cleaning tools; (iii) blind discrimination of sample groups by Independent Component Analysis (ICA); (iv) powerful data exploration strategies for annotation and suspect features highlighting.
Tea has been chosen as a development food matrix since it is the most consumed hot beverage over the world, offering a high diversity and complexity as well. In addition, tea often exhibits a lack of traceability and is regularly the subject of alerts on food safety networks (like the European Rapid Alert System for Food and Feed) mainly due to chemical contamination issues. Samples were spiked with a mix of pesticides, mycotoxins, process-induced toxicants and packaging contaminants in the range of 10 to 100 µg/kg which is relevant regarding European regulations. Efficient data filtration, normalization and scaling strategies are key issues since a discerning choice of tools enables a blind discrimination between control and spiked sample groups at levels as low as 10 µg/kg. Annotation step appears to be the main challenge, but in-house data mining tools are key factors to reduce data complexity and also provide the user a quick feedback about potential identity or property (presence of an isotope or potential adduct) of unexpected compounds. Our foodomics approach has been first developed on green tea, being further applied to other types of tea (respectively black tea and smoked green tea) for its validation.

Keywords: *ICA, time-of-fligth mass spectrometry, data mining, chemical contaminants, tea*

L10
ANALYTICAL FOOD AUTHENTICATION - FROM RESEARCH TO ROUTINE

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Because food fraud is still an important issue and incidents are even increasing there is a need for monitoring and verifying the authenticity of food products by analytical methods. A variety of analytical approaches for authenticity assessment and for detection of fraudulent practices have been developed and which are in use within food control. Besides classical approaches – namely the targeted analysis of marker compounds characteristic for a certain adulteration and the analysis of typical food ingredients and their comparison to reference data – profiling and fingerprinting techniques became more and more important in the recent years. Opportunities and stumbling blocks of targeted and non-targeted methodologies will be discussed. Particular emphasis will be put on the court-proof application, data accessibility, exchangeability and interpretation, validation approaches and standardization needs.

L11
ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY FOR THE DETECTION OF ANIMAL BY-PRODUCTS IN FEED

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In the context of the expansion of the human population, food availability is a big issue. Solutions can be found by increasing the efficiency of food production, finding new food sources and/or reusing wastes.
Animal by-products are an interesting source of feed materials. Indeed, up to 50% of the slaughtered animal weight is not intended for human consumption. These materials are rich in proteins of high nutritional value and have also an economic interest since their non-use or underuse results to a logical loss of potential gains.
After the mad cow crisis, the use of animal by-products was strictly regulated. Since 2013, non-ruminant processed animal proteins (PAPs) are reauthorised in aquafeed. Ruminant PAPs remain forbidden in all types of feed. Official control of constituents of animal origin in feed is based on two methods that can be used in combination: Light microscopy detects the presence of PAP particles but is unable to identify their species origin. PCR gives information on the genetic origin of the DNA present in the feed without any consideration on the source of the signal. But sometimes these methods are unable to distinguish some feed materials. Ruminant blood meal (forbidden) and milk products (authorised) are an example. In such case, the simultaneous identification of the type of protein and the species of origin is therefore crucial to fill in this analytical gap and ensure feed safety.
The objective of our work was to develop a sensitive ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) method for qualitative and specific detection of bovine blood derived products and milk powder in feed. Peptide biomarkers identified in previous studies were used.
The sample preparation and the analytical method were designed to provide a fast, simple and powerful method suitable for routine. Proteins were extracted in a buffer containing 200 mM TRIS-HCl pH 9.2, 2 M urea followed by a trypsin digestion and a purification with SPE tC18 (Waters). Analyses were performed by liquid chromatography (Acquity system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQS, Waters). The acquisition and processing of data was carried out by MassLynx software (Waters). Concatenated labelled peptides were used as standard in order to compare the results independently of the retention time variation due to the matrix effect.
Various commercial aquafeeds artificially adulterated at levels of 0.1% to 1% (w/w) with bovine blood meal, bovine blood products or milk powder were analysed in order to assess the influence of matrix composition and to experimentally evaluate sensitivity and specificity of the method.
The optimised method was able to detect all adulterants at the 0.1% (w/w) level in all contaminated test portions. This level corresponds to the legal LOD imposed by the EC for animal proteins analytical method. This makes this method suitable to be applied in the future in feed control.

Keywords: *processed animal proteins (PAPs), blood, milk, feed safety, tandem mass spectrometry (MS/MS)*

Acknowledgement: *The authors wish to thank the technical teams of the Food and Feed Quality Unit & the Authentication and Traceability Unit (CRA-W) and of the CER Groupe for their technical assistance and valuable collaboration.*

L12*
THE DETECTION OF ECONOMICALLY MOTIVATED ADULTERATION IN THE HERB AND SPICE INDUSTRY

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The global herb and spice industry, valued at approximately US$4 billion, is under threat from criminals dealing in economically motivated adulteration (EMA). Consultation with stakeholders prioritised sage, paprika and garlic, with regard to their risk of adulteration. In this study, rapid screening techniques to detect adulteration in sage, paprika and garlic are being developed using the spectroscopic techniques, Fourier Transform Infra-Red (FTIR), and Near Infra-Red (NIR) combined with chemometric modelling. The raw spectral data of a range of sage samples along with possible known adulterants, olive leaves, myrtle leaves, sumac, hazelnut leaves, cistus, sandalwood, strawberry tree leaves and phlomis have been collected. Spectral data from paprika and the bulking product, spent paprika, have been obtained. Garlic, along with possible white powder adulterants, talc, maltodextrin and corn starch, have also been analysed on spectroscopic techniques. Chemometrics are applied to convert this spectroscopic data into qualitative models using algorithms such as orthogonal partial least squares- discriminant analysis (OPLS-DA) following pre-processing of the data. To date, these methods proved to be capable of determining sage, paprika and garlic from their adulterants. These rapid and inexpensive techniques can be used in the fight against fraud in the herb and spice industry.

Keywords: *spectroscopy, adulteration, herb, spice, chemometrics*

L13
FIGHTING THE FOOD FRAUDS BY MEANS OF LIPID ANALYSIS: PALM OIL ADDITION

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Palm oil (PO) is the most widely consumed vegetable oil on the planet, and it is in most part of the packaged products sold in the supermarket. The demand for PO has increased steeply in last time as substitute of animal fat and as an alternative to hydrogenated vegetable oils. It is produced in tropical areas and its rapid expansion threatens some of the planet's most important and sensitive habitats. It has a high percent of saturated fatty acids that are not favorable to the health and recently the problems concerning food safety have evidenced the dangerous content of 3-MCPD in it (efsa.2016.4426). The consciousness of these facts has led the consumers to request food with no PO as ingredient. Starting from December 2014, all food contain palm oil sold in Europe must state it in the ingredient list (EU Reg 1169/2011).
Today in Italian market many food items are now advertised as PO free but because of its low price and rheological and stability properties it is possible its use without a label declaration. To avoid and prevent this food fraud it is important to have reliable and easy analytical methods to verify this statement.
This presentation investigates different methods to determine the presence of PO in food, choosing between classical analysis with chromatographic separations (GC and HPLC) and the fast Direct Analysis in Real Time approach (DART-MS). Different mixture of sunflower oil and rapeseed oil with PO were used to determine a LOD of PO addition. For GC, HPLC and DART-MS 21 samples were analyzed and for DART-HRMS 63 samples. For the determination of fatty acids and phytosterols, GC-FID was used; for the determination of tocopherols and tocotrienols HPLC with fluorescence detector was used; for TAGs, DAGs and MAGs an HPLC coupled with RI detector was used. Data were elaborated using Unscrambler and Metaboanalyst programs.
Fatty acid composition is quite different from other vegetable oils and phytosterols content is much lower in PO; nevertheless, these two analysis were not useful for our target and statistical analysis of this data could scarcely differentiate products with PO from the others. The tocopherols and tocotrienols analysis allowed to distinguish the PO presence in the samples and could act as confirmatory analysis. The TAGs profile is characteristic for PO so that it was possible recognize presence of this oil in false labelled as PO free food. With DART-ion trap in MS-MS mode (PPP as precursor ion) it was possible to detect PO presence.
Using chemometric evaluation on DART-HRMS and the ratio PPO/OOO data it was possible to define a threshold TAGs ratio for the presence of PO in the most of food products with more than 1% of PO.
It was demonstrated that the use of an DART method allows to detect PO addition in food samples directly on sample with a minimum preparation. Fatty acids, fytosterols and DART-MS could be considered screening methods. Tocopherols and tocotrienols determination is a confirmatory analysis.

Acknowledgement: *This work was supported by the Italian Health Ministry Project n. PRC2014-014.*

L14*
DISCRIMINATION OF PROCESSING GRADES OF OLIVE OIL AND OTHER VEGETABLE OILS BY MONOCHLOROPROPANEDIOL ESTERS AND GLYCIDYL ESTERS COMPOSITIONS

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Introduction: Olive oils are among the most fraud vulnerable products on the market. Especially analytically challenging is the discrimination between extra virgin olive oil (EVOO) and lower grade counterparts (refined olive oils (ROO) and pomace olive oils (POO)). Highly sensitive and reliable markers are desired to reveal the authenticity of EVOO and its adulterations with lower grade olive oils (ROO and POO).
Objective: In this study, the process derived contaminants 2- and 3-monochloropropanediol (2- and 3-MCPD) esters and glycidyl esters (GEs) are evaluated for its functionality as characteristic markers for processed grades olive oils and its applicability as a potential authentication tool.
Material and methods: The 2- and 3-MCPD esters and GEs were analysed in 84 oil samples (30 EVOO samples, 18 ROO samples, 16 POO samples, 8 cold pressed vegetable oil samples, and 12 refined vegetable oil samples) by GC-MS/MS using an official international method from the American Oil Chemists' Society (AOCS Cd29a-13). The obtained concentrations of these three compounds in the olive oils and other vegetable oils were statistically analysed by means of ANOVA and post-hoc tests.
Results: Concentrations of the 2- and 3-MCPD esters and the GEs varied in the ranges of 0 - 6 mg/kg, 0 - 1.5 mg/kg, and 0 - 1 mg/kg oil, respectively. The concentrations of the three compounds in lower grade olive oils were significantly higher ($P<0.001$) than in EVOO. A similar difference was observed for other refined and cold-pressed vegetable oils. The limit of fraud detection of lower grade oils in EVOO was 2% when using 3-MCPD esters, 5% for 2-MCPD esters, and 13-14% for GEs based on calculations of virtual mixtures of the current sample set.
Conclusion: The MCPD esters appear very specific, sensitive, and promising for the detection of lower processing grade oils in EVOO. Especially, 3-MCPD can be adopted as a new authentication tool for the discrimination of processing grades of olive oils.

Keywords: *authenticity, fraud, GC-MS/MS, processing contaminants, 3-MCPD esters*

Acknowledgement: *We thank Hennie van Rossum of RIKILT, Wageningen University & Research, for his technical support with the GC-MS/MS analysis. Special gratitude is dedicated to the producers, traders, and retailers for their sample supply.*

L15
TOP-DOWN FOOD FRAUD DETECTION AND PREVENTION – HOW TO AVOID THE GOLDEN HAMMER

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Detection and prevention of food fraud is currently to a large degree based on a bottom-up approach, where large numbers of individual food items are analyzed using various methods and instruments. One reason for this is the significant pre-existing investments in these methods and instruments, and in the accompanying skills. This is what is called the "golden hammer principle", where availability and knowledge of a given method leads you to think that it is the best tool for all more or less similar purposes. The alternative is a top-down approach where we study the whole problem "from a distance", and gradually examine the constituent parts more in detail. When it comes to detection and prevention of food fraud, the top-down approach seems to be missing, or at least to be underrepresented, and if do not remedy this situation our efforts to fight food fraud will be seriously undermined.
This presentation outlines some examples of more holistic approaches to food fraud detection and prevention. One way to get an overview of food fraud on overall level is the combined automatic and manual detection of media-reported food fraud incidents. The automatic part is done by web-crawling and keyword detection, the manual part by correspondents, and the resulting joint database is made publicly available as part of the Food Authenticity Research Network Hub (FARNHub) which is developed as part of the Authent-Net H2020 project. Another important task when addressing a multi-disciplinary problem is to agree on the exact meaning of the related terms and concepts; this to avoid miscommunication and wasted effort. To facilitate this, the voluntary European standard (CWA) "Authenticity in the feed and food chain - General terms and concepts" is currently under development, and the latest version will be outlined here. The food fraud incident database will help us get an overview of food fraud, and the terms and concepts standard will help us communicate about the issue, but to actually detect and prevent food fraud on overall level we need to study the chains in more detail. Detailed mapping and analysis of the supply chains, with input-output analysis and mass-balance accounting, can help us identify fraud on aggregate level. This presentation will indicate how this methodology works, and show a concrete application of it in supply chains for wine originating in Europe and being exported to China.
The main message in this presentation is that top-down approaches like the ones outlined here should be further developed, and used to supplement the many bottom-up initiatives that exist. It is unlikely that analytical methods alone can solve the increasing challenges related to detecting and preventing food fraud. if we want to fight food fraud, many other scientific disciplines need to be involved, including economists, supply chain analysts, computer scientists, market analysts and legal experts.

Keywords: *food fraud, standard, supply chain, mass-balance accounting*

Acknowledgement: *The EU FP7 project FoodIntegrity, The EU 2020 projects Authent-Net and EU-China-Safe*

L16*
HIGH RESOLUTION MASS SPECTROMETRY BASED METABOLOMIC FINGERPRINTING OF RED AND BLUE BERRIES FOR DETECTION OF FRUIT-BASED PRODUCTS ADULTERATION

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Red and blue berries such as lingonberry, cranberry, bilberry or black currant have been obtaining a high popularity due to their well-documented health-promoting properties. Similarly to other highly prized food commodities, these premium fruits are potential target of adulteration. Typically, the substitution of these high value berries is made by the use of less expensive ingredients; admixtures were reported mainly in case of fruit juices, compotes and purees. Under such conditions, determination of the fruit authenticity, and also its content in respective product, becomes essential for quality control practices, consumer protection, as well as for avoiding unfair competition. Not needed to emphasize, that reliable analytical strategies applicable for authentication of fruit-based products have to be searched and validated.
In this study, metabolomic fingerprinting of red and blue berries (in addition to those mentioned above, also strawberry, swamp cranberry, raspberry, blackberry, red currant, elderberry, and chokeberry), employing ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (U-HPLC-HRMS/MS) has been investigated. Altogether, 90 samples of 11 species were extracted with methanol and then analyzed using the above mentioned analytical platform, in order to record metabolome pattern of individual fruit species. Subsequently, chemometric evaluation was performed to assess the differences between the samples and to identify significant markers, which might be present exclusively or at more significantly higher level in one of the fruit species. In parallel, accurate mass of ions in MS and MS/MS spectra, various software packages and online libraries were employed for tentative marker identification. Regarding data handling, in the first phase, the PCA (Principal Component Analysis) revealed significant differences between metabolome components in prepared extracts of fruit species. Clear clustering was found for both ionization mode polarities. In the next phase, PLS-DA (Partial Least Square Discriminant Analysis) was performed and statistical models were created and validated. Better results were obtained for a negative ESI ionization mode. Finally, characteristic markers contributing the most to the separation of the groups were identified, they belonged primarily to the groups of polyphenols (anthocyanins, flavanols and flavonol-glycosides), phospholipids (phosphatidylcholines, phosphatidylethanolamines and phosphatidic acid) and triterpenes. It is assumed that these distinctive markers will help to improve the authentication process of fruit based food and food supplements under routine laboratory conditions.

Keywords: *berry fruits, authenticity, metabolomic fingerprinting, U-HPLC-HRMS/MS*
Acknowledgement: *This work was supported by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272), by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by specific university research (MSMT No 20-SVV/2017).*

L17*
COMBINATION OF NON-TARGETED AND TARGETED APPROACHES FOR THE DISCRIMINATION OF EXTRA VIRGIN OLIVE OILS FROM DIFFERENT PROTECTED DESIGNATIONS OF ORIGIN AND THE IDENTIFICATION OF POTENTIAL "ORIGIN MARKERS"

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Product labeling has gained considerable attention over the last years, as a means to both provide product-specific information and reduce quality uncertainty faced by consumers in their food-choice decision process. Implementation of Protected Designations of Origin (PDOs) is, indeed, one of the most prominent differentiation strategies used in olive oil market. These PDOs are often perceived as valuable tools that promote specific attributes of the oil linked to its geographical provenance.
Looking for a strategy to authenticate the declared origin of commercial extra virgin olive oil, 126 oil samples from six different Mediterranean PDOs (Meknès and Ouazzane (Morocco), Priego de Córdoba and Baena (Spain), Kalamata (Greece) and Toscana (Italy)) were collected and analysed by means of two different platforms (LC-MS and GC-MS) combined to chemometrics. The sample preparation (unselective 3-steps liquid-liquid extraction) and chromatographic and detection conditions (in both platforms) were optimized to facilitate the determination of a great number of minor compounds (phenolic compounds, triterpenic acids and dialcohols, sterols, tocopherols, free fatty acids, etc.) within a single run.
The extracts were eluted in LC using a C18 (2.1x 100 mm, 1.8 µm) column, with acidified water and acetonitrile as mobile phases and a flow gradient (0.4-0.6 mL/min) at 40 °C (total run time 14 min). The same extracts (after derivatization with BSTFA+TMCS (99:1, v/v)) were separated in GC using a BR-5 column with a T gradient from 150 to 320 °C at a rate of 4°C/min. Both chromatographic systems were coupled to a Compact™ QTOF spectrometer (Bruker Daltonik) using an ESI interface in the case of LC and an APCI ionization source in GC (the latter can preserve the pseudo-molecular ion information, which is a great advantage over the "classical" GC systems). Accurate mass data and isotopic pattern information enabled the tentative identification of possible relevant markers.
The combination of non-targeted and targeted approaches - quite common strategy nowadays- was considered as the best option in this study. The chosen workflow offers maximum coverage of the olive oil metabolome's chemical space in a first step, and the possible validation of the identified markers in further targeted metabolomics experiments. Selection of molecular features, bucketing, filtering, scaling, normalization and application of principal components analysis (PCA) to the LC-MS and GC-MS data were done by using MetaboScape® software. Noticeable discrimination among the six evaluated PDOs was achieved taking into account the data coming from both platforms. The contribution of a few thousand molecular features to the statistic models was evaluated in depth and several compounds such as elenolic acid, acetoxypinoresinol, oleuropein and ligstroside aglycones, and some other tentatively identified substances were pointed out as possible PDOs distinctive markers.

Keywords: *virgin olive oil, origin authentication, minor compounds profiling, mass spectrometry, food metabolomics*

Acknowledgement: *L O-G wants to thank the financial support from the Spanish Government (FPU13/06438 and FPU short stay grant).*

L18*
GC-MS AND HPTLC FINGERPRINT OF POLISH HONEYS WITH DIFFERENT BOTANICAL ORIGIN – AS A TOOL FOR THEIR AUTHENTICATION

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Honey is a complex mixture of a variety of different chemical classes of compounds. The chemical composition of honeys is very variable and significantly dependent on huge number of varied factors, such as botanical origin, geographical area of origin, bee species, honey processing, age of honey and storage method. Alongside with constantly increasing interest of consumers of natural and healthy food products, growing popularity of bee products is also observed, mostly honeys. In addition to flavor and nutritional values, therapeutic effect in one of the most valuable qualities of honey. In Poland, average production of honey in last eight years reached about 19 thousand tons (according to Institute of Agricultural Economics and Food Economy). Despite such a large production, still the largest contribution, on the domestic market (as well as in European market) have cheaper honeys, which are characterized by poor quality. Additionally, large fraction of honeys available on the Polish market is falsified by addition of glucose – fructose syrups or admixes with imported inferior quality honeys. Therefore, nowadays honey quality and authenticity evaluation is an important applied research area with relevant impact on industry and consumer production. Currently, the widely used traditional method for determination of the botanical origin of honeys is melissopalynology. Despite the fact that this method is characterized by high accuracy and precision, it is not sufficient for a clear assessment of the authenticity of the honey origin. Nowadays, there is a strong growth in number of different methods being proposed as more appropriate for determination of honey quality and origin.
The purpose of presented study was evaluation of volatiles chemical content of Polish honeys with different botanical origin, which are characterized by high flavour and nutritional values (e.g. goldenrod, phacelia, yellow sweet clover, dandelion, raspberry or runner bean honeys) and construction of chemical profiles based on the different extraction methods and GC-MS analysis. Moreover, for analysis of volatiles extracts of honeys HPTLC method was apply. This method allows us to construct something like cod-bars useful to differentiate of Polish honeys of different botanical and geographic origin as well as to determine their quality. Additionally, based on the obtained results we could determine and identify the markers, for different unifloral honeys and show that combination of GC-MS and HPTLC analysis create a specific fingerprint of honeys with different botanical origin and that those methods are powerful techniques for differentiation origin of honeys.

Keywords: *volatile compounds, chemical profiles, HPTLC, unifloral honeys*

Acknowledgement: *This study was carried out with financial support from the National Science Centre in Poland project No. 2014/15/B/NZ9/02182 "Chemical markers of unifloral honeys".*

L19
ANALYTICAL CHALLENGES OF ORIGIN DETERMINATION OF PROCESSED AUTHENTIC FOOD ON THE EXAMPLE OF STURGEON CAVIAR AND AQUACULTURED FISH

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In today's global food market the geographic origin of food is often connected with price and quality. However, food labelling is primarily based on paper work, which is vulnerable to fraud. Therefore, analytical methods allowing for unambiguous verification of origin and thus labelling compliance are needed. The strontium $^{87}Sr/^{86}Sr$ isotope ratio is a well-established tracer for origin determination of food. The bioavailable fraction of Sr is taken up and stored by living organisms from soil or water without significant isotopic fractionation representing a direct proxy of the geographic origin. However, the isotopic composition of a primary agricultural product can be significantly altered e.g. by fertilization, feeding or processing. This renders the direct attribution of the sample to its geographical source difficult or impossible. This study addresses the challenge of changed compositions of processed (salted) sturgeon caviar and of otoliths of aquacultured fish by the combination of isotope ratio measurements and isotope pattern deconvolution. For this purpose, the Sr isotopic and elemental composition of raw and salted sturgeon caviar, otoliths, water, fish feed and salt (representing the assumed main contributors to the final isotopic composition of strontium in caviar) was analyzed using (MC) ICP-MS. The contribution of different sources to the final $^{87}Sr/^{86}Sr$ isotopic composition of the samples was determined using multiple-linear regression modeling and linear algebra calculations. The developed procedure was applied to caviar and otolith samples of different origin. Isotope pattern deconvolution revealed that the Sr isotopic composition of raw caviar and otoliths was formed to 79.8 ± 4.3 % by water and 20.2 ± 4.3 % by fish feed (1 *SD*, *n*=5, between sites variation). Deconvolution was possible even when the difference of the $^{87}Sr/^{86}Sr$ isotopic ratio between sources was less than 0.1 ‰. The influence of salting to the Sr isotopic composition of processed caviar accounted up to almost 80 % for samples treated with salt containing high concentrations of Sr. In contrast, salt with low Sr concentrations had no measurable effect on the $^{87}Sr/^{86}Sr$ composition of the caviar. The developed methodology provides the basis for the origin determination of samples by $^{87}Sr/^{86}Sr$ isotopic ratios in cases where the initial natural signature has been modified by known additives.

Keywords: *isotope pattern deconvolution, strontium isotopes, MC ICP-MS, salt, processed food*

LECTURES

L20*
POTENTIAL OF FLUORESCENCE MICROSCOPY TECHNIQUE TO ASSESS LIPID PEROXIDATION STATUS IN SOUS-VIDE COOKED ATLANTIC MACKEREL DURING CHILLED STORAGE

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Sous-vide cooking is a good alternative to conventional heat treatment techniques to provide safe, palatable, ready-to-serve meals with enhanced health benefits and extended shelf life. It involves cooking the raw material under controlled temperature and time inside vacuum pouches followed by the subsequent preservation under chilled conditions (0-4°C) until further processing or consumption.
However, sous-vide cooking and further chilled storage of food products, and particularly seafood, can accelerate lipid peroxidation in the final product, leading to deterioration of its sensory parameters. Oxidation of lipids is an important index to denote quality of sous-vide products during storage, because rancid lipids not only spoil their sensory characteristics, but may also generate detrimental compounds, which negatively contribute to human health. The oxidation of lipids occurs by a free-radical chain mechanism and gives rise to formation of lipid hydroperoxides as primary products. A rapid, sensitive, non-destructive and accurate method for determination of lipid peroxidation rate is needed to evaluate the efficiency of sous-vide cooking regimes and subsequent chilled storage to retard oxidation of lipids and to prevent stored foods from further rancidity.
It has been shown that oxidized lipids and fats emit light in the near UV-region. This specific autofluorescence has mostly been studied spectrophotometrically to estimate the oxidation rate of foods containing polyunsaturated lipids. Nevertheless, to the best of our knowledge, there is no information available on the use of fluorescence microscopy technique to rapidly assess the extent of oxidized lipids in seafood products during storage.
The present study aimed to evaluate the ability of fluorescence microscopy to predict the rate of lipid oxidation in Atlantic mackerel over a 7-day period of chilled storage at 0°C following the sous-vide cooking at 60-75-90°C for 10-15-20. Collected fat samples from sous-vide cooked mackerel were directly visualized with a Zeiss Axio Imager Upright microscope under the 44 FITC filter set. Primary oxidation compounds were determined by two wet-chemical methods: peroxide value analysis via iodometric titration and determination of conjugated dienes through specific extinction at 233 nm, since the formed hydroperoxides of polyunsaturated fatty acids possess strong absorbance at this wavelength. The autofluorescence of mackerel fat gradually increased with increasing sous-vide cooking time and temperature, as well as duration of chilled storage, while being directly correlated with both PV-value (r=0.982) and conjugated diene production (r=0.856).
The study has shown that the proposed fluorescence microscopy method permits rapid and reliable assessment of lipid oxidation status in seafood both during processing and storage and is claimed to have a high degree of criterion validity (validation performed on different types of fish oil).

Keywords: *autofluorescence, lipid oxidation, sous-vide cooking, chilled storage, Atlantic mackerel*

L21
MULTI-ANALYTE METHODS FOR THE ANALYSIS OF UNHEALTHY COMPOUNDS IN FAT-RICH FOODS: OXIDIZED LIPIDS, MCPD- AND GLYCIDOL-ESTERS, AND MOAH

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The classical way of pesticide analysis had much of 'looking to the right and being hit by a car from the left'. Up to about 15 years ago selective methods were used for the different pesticide groups. This approach was understandable from the perspective of the technical options available, but had a high risk of missing pesticides not falling in the group that was monitored. The expanding number of pesticides (residues) that had to be measured made another approach necessary. Technical improvements, mainly in the chromatography and mass spectrometry area, provided the technology to do so. Multi-residue methods are nowadays used that cover many different groups of pesticides. Sample preparation has been made less selective and the MS provides the required selectivity. The most advanced modern multi-residue methods nowadays cover hundreds of pesticides from multiple groups.
The more general field of food safety analysis holds the same risk as classical pesticide analysis. Many natural and transportation- or processing-induced toxic species can be present and specific methods only focus on one group. In the specific methods selectivity is commonly introduced already at the sample introduction stage and all information on other compounds is lost. Following the analogy of multi-residue pesticide analysis our interest is in the opposite: Apply little or no sample preparation and use the mass spectral information for compound identification and ultimately quantification. The work initially focusses on fat-rich foods such as edible oils, margarines and dressings, and covers compound classes like the MCPD- and glycidyl-esters, oxidized lipids and mineral oil contaminants (MOSH/MOAH). MS holds a prominent place in the method, as does normal phase LC. There is still a lot of work to do, but we believe this is the way to go.

Keywords: *food contaminants, fat-rich foods, multi-analyte methods, MCPD- and glycidylesters, lipid oxidation products*

L22
FURAN FORMATION FROM LIPIDS UPON THERMAL FOOD PROCESSING

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The survey of furan in canned and jarred foods that undergo heat treatment published by the US Food and Drug Administration in 2004 [1] initiated many studies dealing with the analysis, occurrence, exposure, toxicity, formation, and mitigation of furan in foods and beverages. A number of reviews describe the results obtained so far [2-5]. There are many precursors and, therefore, multiple routes of furan formation upon thermal treatment, in particular Maillard-type reactions and lipid oxidation. Recent data indicate polyunsaturated fatty acids (PUFAs) and ascorbic acid to be the major sources of furan, followed by carotenes, carbohydrates, and certain amino acids. Furan can be formed from an intact carbon chain or by condensation reactions of carbonyls obtained from different sources. Due to this complexity, it is vital to better understand the role of the individual precursors and formation pathways.
In the current presentation, we will elaborate on the formation of furan from PUFAs with focus on the elucidation of the formation mechanisms in both model systems and food products. Various classical and advanced analytical techniques were used, such as headspace gas chromatography coupled to mass spectrometry, proton transfer reaction mass spectrometry, stable isotope dilution assay, and labelling techniques. The knowledge gained is the base for developing concepts leading to a reduction of furan upon industrial and domestic food processing while maintaining the overall food quality.

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L23
ION MOBILITY SPECTROMETRY: A RAPID TOOL TO ASSESS EGGPRODUCTS FRESHNESS

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In the last decade, the attention on the authenticity and quality of food commodities increased for commercial and safety reasons and that is why, from both industries and research institutes, the demand for the development of rapid methods able to detect frauds increased as well.
Eggs, mostly in the egg products form, are largely used for the creation of different industrial products and their freshness is a crucial step for the production of safe and high quality commodities; however, there are not several rapid ways able to assess if egg products are as fresh as declared. In this sense, our research group developed in the past an artificial olfactory systemsolution [1].
In this study, GC-IMS (Flavourspec® instrument – Gas Dortmund Company - Germany, constituted by a Gas Chromatograph coupled with an Ion Mobility Spectrometer) technique is proposed in view of its ability to record the volatile profile of liquid or solid samples without any relevant pretreatment.
In particular, GC-IMS is presented as a rapid, low cost and low sample demanding way able to assess eggs freshness, thanks to the identification of specific marker spots in the final 2D graph that change their intensity or that can be detected or not, according to the time and storage conditions of the egg products.
An attempt to identify these spots have been performed analyzing the same samples with a GC-MS equipped with a 50/30 mm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber. After the identification of some markers with the mass spectrometry approach, these compounds have been searched in the GC-IMS libraries and, when available, their specific reference standards have been injected in the Flavourspec® system, with the aim to confirm the retentiontime and the drift time of the spot.
The method has been validated; this GC-IMS technique can be implemented in production sites: the total time of the analysis (from sample preparation to report/outcomes generation) is lower than 50 minutes, allowing the acceptance or the rejection of an egg product batch before its introduction in the plant and consequent exploitation in the production process.

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Keywords: *eggproducts, ion mobility spectrometry, freshness, chemical markers*

L24
FOOD IN THE 21ST CENTURY: THE NEED OF MITIGATION STRATEGIES FOR FOOD-BORNE TOXICANTS IN COMBINATION WITH SENSORY PROPERTIES ACCEPTED BY CONSUMERS

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In the past, many studies and efforts have been undertaken to elucidate the key odorants of food and to identify formation pathways of the so-called "food-borne toxicants" with a subsequent mitigation strategy. But, up to now, analytical approaches including the quantitation of desirable aroma-active compounds in combination with undesirable toxicologically relevant substances by sensitive methods are scarcely available. The lecture will present recent studies, which were combining the analysis of important aroma compounds and of selected food-borne toxicants (e.g., acrylamide, acrolein, crotonaldehyde, styrene, etc.) formed during food-processing, e.g., brewing of beer or deep-frying of potato chips and donuts in different edible oils. Odorants were identified by gas chromatography-olfactometry as well as GC-MS and quantitated by stable isotope dilution analysis (SIDA). For the toxicants, new quantitation methods using stable isotopically labeled standards were developed and formation pathways were proven by labeling experiments. In summary, it will be shown that lowering the amounts of undesirable compounds in combination with the maintenance of an overall aroma well accepted by the consumers is a challenging task, but mitigation strategies of the "bad guys" can be advised after getting the knowledge of their formation pathways. Successful mitigation examples performed by industry will be shown.

L25
MULTIVARIATE 1HNMR METHOD MEETS SUB-1% TRANS FAT QUANTIFICATION TARGET

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Extensive scientific evidence points at the consumption of *trans* fatty acids (TFAs) as a major risk factor in the development of coronary heart disease and other cardiovascular health conditions. In the US the "zero *trans* fat" label (0.5 g TFAs per serving, or 1.8 g/100 g of total fat) was adopted already in 2003, but this is does not satisfy the demand of several countries to banning the use of *trans* fat from partially hydrogenated vegetable oils in foodstuff. Currently a global consensus for a "free of TFAs" label is under discussion at CODEX level. In order to meet prospect lower thresholds an analytical range for TFAs quantification below 1.0 g/100 g of fat is mandatory.
Very few analytical methods capable of accurately measuring such levels exist. Among those are the gas-chromatography-based official reference methods, which involve prior sample derivatization and require skillful analysts. Several simpler and more cost-effective methods, mostly based on infrared spectroscopy, have been alternatively developed. However, they fall short in meeting the sensitivity requirements and/or on providing basic performance characteristics, in particular method uncertainty and limit of detection.
A multivariate method for the quantification of total TFAs in oil mixes by proton nuclear magnetic resonance (^{1}HNMR) is presented hereby. The method has been validated according to a sample-specific approach, which takes into account the compositional variability of the matrix in the determination of performance characteristics. The approach was extended to another quality attribute of edible oils, the content of lipophilic vitamin C (in the form of ascorbyl palmitate), for which a maximum concentration of 260 mg/kg is tolerated.
The performance of partial least square regression models based on selected spectral variables was highly satisfactory. Limits of detection in the modelled populations fell in the range of 0.23-0.24 g/100 g and 36-41 mg/kg for total TFAs and ascorbyl palmitate, respectively, both well below targeted limits.

Keywords: *trans fatty acids, nuclear magnetic resonance, partial least square regression, interval limit of detection, ascorbyl palmitate*

L26
ORGANIC ANTHROPOGENIC POLLUTANTS IN FOOD PRODUCTS: LINKING ENVIRONMENTAL TRACE ANALYSIS AND FOOD SAFETY ISSUES

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Many synthesised organic substances are applied in large quantities as important tools for improving modern food production, for product conservation as well as product refinement. Synthetic chemicals are applied as product amendments throughout the production process for most food products. The application of these chemicals is today regulated according to national and international consumer protection laws. The national and international food control authorities are obliged to determine threshold values as well as monitor and control levels and distribution of potential harmful substances in national food products as well as imported food from the international markets.
Especially in large-scale food productions chemical support is important to guarantee the high quality and competitiveness of the respective products. Therefore, continuous pesticide usage is required to minimise the occurrence and spreading of diseases in livestock or commercial production of vegetables on agricultural lands. Also, application of chemicals for veterinary treatment and growth enhancements in husbandry or aquaculture is important to consider. However, many of these chemicals may find their way into the environment and will eventually be detected in environmental sample).
Many examples where organic chemicals, highly valued for food product enhancement, were identified as environmental pollutantsare reported. Already in the early 1960s, para, para´-dichlorodiphenyltrichloroethane (*p,p´*-DDT) was identified as persistent organic pollutant (POPs), more than 30 years after the substance was commercially introduced as potent insecticide on the international market. Currently, the usage of poly- and perfluoroalkyl substances (PFASs) as oil-repellents in food packaging is discussed vividly in the food chemistry community. In addition, usage of antibiotics as growth enhancers or in various veterinary treatments in life stocks may lead to uncontrolled release antibacterial agents into the environmental microbial communities and, thus, increase the risk of multi-bacterial resistance, with obvious consequences for the usage of anti-bacterial agents in future veterinary treatments. Many substances identified and applied as important amendments in food production are also considered as hazardous for the environment. Therefore, a strong collaboration between food production, food safety control and environmental monitoring is today required to provide a sustainable platform for application of chemical amendments in food production.
A variety of linkages are identified where chemical analytical strategies in food safety and environmental monitoring may be combined for the benefit of both national and international regulation. Several examples will be discussed and elucidated during the presentation. Sustainable strategies for the effective combination of food safety and environmental pollutant monitoring will be discussed during the presentation.

Keywords: *environmental pollution, chemical trace analysis, organic contaminants, food safety*

L27
PFOA AND ITS SUBSTITUTE GENX IN THE ENVIRONMENT AROUND A TEFLON PLANT IN THE NETHERLANDS

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Between 1970 and 2012 Chemours in Dordrecht, the Netherlands, founded in 2015 as a spin-off of DuPont, has used perfluorinated octanoic acid (PFOA) as an intermediate in their Teflon production. PFOA is highly persistent, bioaccumulative, toxic, ubiquitously present in the environment and listed by the European Chemical Agency as a substance of very high concern (1). Manufacturers have, therefore, changed their production and moved towards alternative fluorinated compounds for Teflon production (2). One of the, also fluorinated, alternatives is GenX. GenX is the commercial name for the ammonium salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (HFPO-DA). HFPO-DA can therefore be used to monitor the GenX concentrations.
To better understand the emission and the occurrence of GenX in the environment, grass and leaves were collected within a radius of 3 km from the Dordrecht plant. In this pilot study the GenX levels were compared with the PFOA levels measured in the same samples. The extraction method was based on the method of Zafeiraki et al. (3). Analysis was done by LC-MS/MS (Bruker EVOQ TQ).
GenX was found in levels between 1 and 27 ng/g ww in grass and between 4 and 87 ng/g in leaves of various trees. PFOA levels varied between 0.7 and 11 ng/g in grass and between 0.9 and 28 ng/g in leaves. Both GenX and PFOA levels increased nearer to the factory. The results indicate that most likely the factory is a point source for the emission of GenX. The PFOA levels found suggest a strong contamination of the soil and possibly groundwater in this area. Drinking water, produced from surface water from the river Rhine/Merwede, close to the Teflon factory contained GenX levels of 3-5 ng/L. Food items from local gardens are currently being analysed for these compounds.

[1] ANNEX XV PROPOSAL FOR A RESTRICTION - Perf luorooctanoic acid (PFOA), PFOA salts and PFOA-related substances, Version 1.0; ECHA European Chemicals Agency, Helsinki, Finland, 2014.
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L28
MINERAL OIL RESIDUES IN FOOD-HANDLING DIFFERENT MATRICES AND GETTING DETAILED INFORMATION ON THE COMPOSITION

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The problem for food analysis is the fact that the matrix food actually does not exist. It can range from (almost) pure water containing just a few dissolved ions to more complex composition with different concentrations of water, fat, protein, carbohydrates and alcohol. Therefore it is essential for the development of routine methods to carefully take these considerations into account. To pick out one example this presentation will focus on the influence of sample matrix on the determination of mineral oil residues in food products.
The discussion of mineral oil residues in food is well known in the scientific community since the early 1990 but was brought to a broader audience through the last years [1]. In general public it is widely supposed that the source of mineral oil residues derives mainly from food contact material made from recycled materials, but in fact numerous other contamination routes exists.
Although so far no legal limits for the residue concentration of both mineral oil fraction (MOSH mineral oil saturated hydrocarbons and MOAH mineral oil aromatic hydrocarbons) exists a widely accepted method for the determination is used. State of the art is a fully automated combination of online LC-GC with flame ionization detection. A large volume transfer in combination of a rapid temperature program is used to reach limits of quantification.
The systems can efficiently handle large sample numbers per day, but the composition of the sample can have a negative impact on the performance of the analysis. Several real world samples of different food matrices and the treatment for improved results will be discussed.
In addition, there is still a big demand for identification of individual substances or substance groups especially for toxicological risk assessment of the mineral oil fractions, mainly the aromatic fraction which is under suspect to have cancerogenic potential.
The last part of the presentation will discuss the possibilities of advanced multidimensional gas chromatography for the identification of individual chemical groups and substances.

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Keywords: *mineral oil contamination, MOSH, MOAH*

Acknowledgement: *The authors want to express their gratitude to Axel Semrau and Shimadzu for their support and valuable and intensive discussions.*

L29
SWEET ANALYSES IN NON-TARGETED AND TARGETED MODE WITH HIGH RESOLUTION MASS SPECTROMETRY

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The food safety laboratories are not only left with an ever-increasing number of analytes to screen for – they must always be on the lookout for new threats too. Of course, all of this must be done with high turnaround times, and at a competitive cost per sample.
The challenge for food safety laboratories is therefore to find ways of efficiently combining targeted workflows with characterization analysis. In other words, the aim is to facilitate identification of unknown compounds as a routine and convenient approach similar to conventional quantitations.
With a range of polar and non-polar contaminants potentially present in food samples, it is essential that the sample preparation approaches are able to extract a very broad range of analytes. However, it can be challenging to develop methods that are both comprehensive as well as convenient to use.
The main extraction techniques used today are making high-moisture food sample preparation faster and more straightforward. Approaches such as SweEt (Swedish Ethyl acetate), QuEChERS (Quick, Easy, Cheap, Effective, Rugged Safe) and Dutch Mini-Luke methods, are amenable to a wide range of matrices and offer a number of advantages over the traditional sample preparation techniques.
One of the most unique features of the SweEt method, developed at the Swedish National Food Agency, is that it involves a single step ethyl acetate extraction procedure, after which the sample can be injected directly into the instrument for analysis. Such approaches are minimizing sample preparation time and allowing labs to analyze a greater number of samples using fewer resources.
This presentation will describe the workflow for SweEt with around 550 pesticide analytes in targeted mode with triple quadrupole techniques as well as the Q Exactive Focus Orbitrap for LC-amendable analytes.
Examples and workflows how the National Food Agency uses high resolution techniques to solve sudden emerging food risks will be shown, with the userfriendly software Tracefinder as well as Compound Discoverer.

L30
A DECADE OF EURL ACTIVITIES AT THE JRC IN GEEL – ISSUES SOLVED AND FUTURE CHALLENGES

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In 2006 three European Union Reference Laboratories (EURL) were inaugurated at the Joint Rreseach Centre in Geel: for heavy metals, for mycotoxins and for polyaromatic hydrocarbons. The role of these EURLs was to coordinate and collaborate with the network of National Reference Laboratories in the European Union in order to promote and implement harmonisation of analytical methods for the determination of contaminants of concern.
The following actions were undertaken: organisation of proficiency tests; development and ring-trial validation of methods for the determination of multiple contaminants in complex matrices; specific training; and several research projects.
The successful activities of the more than forty EURLs set by DG SANTE triggered the interest worldwide, with several countries outside the EU considered establishing similar networks of reference laboratories (cf. Canada, India, ASEAN countries…) in the field of mycotoxins, PAHs and heavy metals.
The presentation will give an overview of the activities of the EURLs based on selected examples, while concluding with an outlook on possible future trends and challenges to come for the networks.

L31
OVERCOMING COMMON LIMITATIONS FACING PESTICIDE RESIDUE LABORATORIES BY APPLYING NEW ANALYTICAL METHODOLOGIES. EURL ACTIVITIES

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The high demand in increasing the scope of matrices and pesticide residues for routine control is driving to the control laboratories to develop improvements or even new analytical methodologies to satisfy such requirements. A first consequence is the introduction of small modifications in the previously validated methods such as: new clean up procedures, increasing the use of isotopically labeled standards, more efficient chromatographs etc. However, in many cases the best way to overcome these limitations is the application of new analytical methodologies. Obviously a critical balance about cost/benefit of these new methodologies is necessary before their broad application.
The EURLs for pesticide residues have, among other duties, to explore the possibilities, advantages and drawbacks that can appear with the use of new analytical methods. We present here a critical evaluation by showing a few examples of new methodologies developed an/or consolidated in the last years for overcoming common limitations such as: shortening the analysis run time, decreasing matrix effects, increasing the analytical scope and analysis and evaluation of high polar compounds.

Keywords: *pesticide residues, matrix effects, new analytical methods*

Acknowledgement: *The authors acknowledge funding support from the European Commission, DG SANTE (Grant decision SI2.726352).*

LECTURES

L32
EU REFERENCE LABORATORY FOR PESTICIDE RESIDUES IN CEREALS AND FEEDING STUFFS - ACHIEVEMENTS DURING THE PAST 10 YEARS AND CHALLENGES FOR THE FUTURE

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First of July 2006, the Technical University of Denmark, National Food Institute was designated as EU Reference laboratory for pesticide residues in cereals and feeding stuff with the overall task to improve the analytical performance of the EU National Reference Laboratories (NRLs). The aim has been pursued via Proficiency Tests (PTs), workshops, surveys, analytical and technical guidance, not only for the NRLs but also for the Official Laboratories (OfL) with an analytical scope of cereals and/or feed. All the achievements have been done in close cooperation with the three other EU Reference Laboratories on pesticide residues.
The EURL-CF has organised 11 PTs on cereals/feeds with both incurred and spiked pesticide residues. The PTs are offered to EU and EFTA NRLs and OfL (mandatory) as well as OfL from Third Countries. This results in a high number of participants (up to 178) mainly from EU. The PTs have elucidated specific analytical problems which then have resulted in recommendation in the SANTE 11945/2015: Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed. The PT results show that the robust standard deviation of the results has decreased from 31% to 17 % and the combined z scores, AZ^2, have also decreased. Unfortunately, it has not been possible to enhance the scope of the laboratories. Still, around 50% of the laboratories are not able to analyse for 90 % of the pesticides on the Target List. But this problem is more related to manpower and equipment at the laboratories which is out of influence of the EURL.
The EURL-CF has also conducted research and surveys in cooperation with the NRLs. One example is a study on particle size influence on the extraction efficiency, showing extraction efficiency can be improved up to 60% if cereal grains are milled to a particle size below 1 mm. The surveys also showed the milling equipment at NRL were very diverse and many also quite old. The result will hopefully result in upgrade of the milling equipment not only at the NRLs but also at the OfL, so the analytical results of pesticide residues in EU will be more correct and comparable.
During summer 2017 the four pesticide residues EURLs has conducted a comprehensive survey that will allow us to draw-up an interim résumé on our work and get new ideas on how to effectively continue our activities in the coming years. The outcome from this survey will be included in the presentation.

Keywords: *EU reference laboratory, pesticide residues*

L33
CHALLENGES ON THE DEVELOPMENT AND IMPLEMENTATION OF MULTICOMPONENT METHODS FOR THE ANALYSIS OF MARINE BIOTOXINS UNDER THE PERSPECTIVE OF THE EU REFERENCE LABORATORY (EURLMB)

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The transition from animal tests to chemical analysis is being a challenging task for the EURL for marine biotoxins. This challenge is mainly due to the complexity of analyte and matrix. The EURLMB activities with method harmonization also imply a critical role on the development, implementation and validation of analytical methods for these algal toxins. The main difficulties found when dealing with these activities are related with the complexity of the analysis of multiple congeners with different toxicities at trace levels, and also subjected to possible biotransformation. This is a very critical task for the analyst, in particular, when the activity is aimed to official control under specific regulations. Updated issues on multicomponent analysis are widely discussed in specific scientific and regulatory forums in the areas of food, environment, etc., and the need for improved method performance, improving in particular selectivity and sensitivity, is being also widely discussed and agreed. The present advances on instrumental development, especially regarding improved sensitivity on mass spectrometry analyzers, are being remarkable, nevertheless there is still a need for improved separation approaches to ensure the efficient extraction of the analyte from these complex matrices and also to minimize the critical matrix effect that so frequently compromise the reliability of the analytical measurement. This presentation will focus on the main challenges of the EURLMB in the method development for marine biotoxins, in particular for emerging toxins not yet included in the EU Legislation, and for which no standards or reference materials are commercially available.

Keywords: *EU reference laboratory, marine biotoxins*

L34*
DETERMINATION OF CHLORINATED PARAFFINS IN FOOD AND FEED USING GC-ORBITRAP-MS

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Short-chained chlorinated paraffins (SCCP) have been widely used as plasticisers or flame retardants for decades, leading to global pollution due to unsafe handling of products or waste [1]. Caused by efforts to ban SCCPs, medium-chained CP (MCCP) production increased in the last decade, often to replace SCCPs [2]. With the worldwide ban of SCCP by the Stockholm Convention in April/May 2017 [3], a robust but selective analysis is needed for enforcement. To this day there is no consensus on an analytical procedure for SCCPs and MCCPs in food samples, resulting in many different methods with barely comparable results. Amongst the multitude of methods currently in use, high resolution mass spectrometry is particularly valuable for in-depth studies of homologue patterns. Using full-scan acquisition and negative chemical ionisation (NCI) at 60,000 and 120,000 resolution (FWHM, *m/z* 200), experiments focused on assessing linear dynamic range, selectivity and sensitivity were performed.
Measurements of SCCP and MCCP standards resulted in an excellent linearity in a concentration range of 25-10,000 ppb. Spiking experiments with high levels of native mono- and di-*ortho*-PCBs and mixtures of MCCP and SCCP standards showed no significant effect on peak profiles of extracted homologues. While most times the $[M\text{-}Cl]^-$ ion was the most abundant ion species, contrary to expectation an increase of $[M\text{-}HCl]^-$ ions with decreasing chlorination degree of the homologues could be observed. Previously reported $[M+Cl]^-$ ions were only found with less than 0.5% relative abundance [4]. Additionally, an ion species preliminarily designated as $[M\text{-}HCl\text{-}H]^-$ was found with a relative abundance of 5-8%.
The analysis of salmon (*Salmo salar*) samples showed similar homologue patterns for salmons farmed in Norway while the pattern of a sample from Scottish aquaculture and a wild salmon clearly deviate. The Scottish sample especially was characterized by high amounts of SCCPs along with a higher relative abundance of hexachloropentadecane isomers compared to the other samples.
Our results demonstrate that GC in combination with high resolution, accurate mass Orbitrap-MS enables insights into the pattern and content of CPs without having to fear mass interferences from both other CP homologues and PCBs, indicating the same for other halogenated compounds.

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Keywords: *chlorinated paraffins, GC-Q-Orbitrap, persistent organic pollutants, simultaneous determination, mass spectrometry*

Acknowledgement: *We would like to thank the EU Commission for the financial support of the work of the EU-RL for Dioxins and PCBs in Feed and Food and Thermo Fisher Scientific, Runcorn/UK division, for their support with the GC-Orbitrap-MS measurements.*

L35
HARMONISATION AND IMPROVEMENT OF RESIDUE CONTROL – EXAMPLES OF THE WORK OF THE EURL

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REGULATION (EU) 2017/625 defines in article 94 the tasks of the European Reference Laboratories (EURL). These are among others the provision of suitable residue control methods, the assistance to NRLs in the implementation of these methods and the control of the successful implementation by inter-laboratory comparative testing. This includes the provision of pure standard substances for the implementation of methods and the production of incurred reference materials for performing proficiency tests and later on internal QA control.
Examples of the production of incurred reference materials and its use in inter-laboratory comparative tests are given. The use of really incurred material is here of high importance, since this material reflects real-life samples as far as possible (e.g. with respect to metabolites, conjugated residues and extraction efficiency).
The question of the implementation of new methods, efficient validation and the use of multi methods is also of high importance for the control laboratories. The EURL has long experience on the use experimental design based method validations according to Commission Decison 2002/657. This approach was now transferred to an experimental design based interlaboratory method validation study. The method was developed by the EURL and transferred to the participating NRLs in a preceeding training programme. The requirements of the validation plans were explained and the particiants were supported in its implementation. Experiences from this pilot validation study and first results and conclusions are presented.

Keywords: *EURL, validation, inter-laboratory comparisons, reference material*

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

L36
TECHNICAL CHALLENGES AND ACHIEVEMENTS OF THE EU REFERENCE LABORATORY FOR FOOD CONTACT MATERIALS

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Food contact materials (FCM) are an essential part of our lives as they include, among others, packaging, food production machinery and kitchenware. There is a great interest in the improvement of these materials and many chemical substances are being used for advancing their technological characteristics to the benefit of the consumers, while they must also be safe.
The FCM sector has a significant and continuously increasing importance in the food chain. For this reason, the Joint Research Centre of the European Commission had been appointed as EU Reference Laboratory for FCM in 2004.
The chemical safety of an FCM, either in packaging, food processing or kitchenware, relies on ensuring that no release of chemical substances occurs in amounts above the legal limits of EU Regulation 10/2011.
The main tasks of the EURL-FCM are, among others, the organisation of proficiency testing targeting to increase the comparability of analytical results in official controls, the coordination of the National Reference Laboratories´ (NRLs) network by disseminating information, trainings and technology transfer; the development of migration and analytical methods in priority areas for intended legislative developments in agreement with DG SANTE, ad-hoc contributions to the European Food Safety Authority (EFSA), and providing technical and scientific support to the EU policy framework regarding FCM. Additionally, the EURL–FCM has created databases for about 550 substances and 300 methods for regulated FCM substances for compliance testing and enforceability. Recent examples of completed and on-going projects with high impact will be outlined, such as testing of kitchen and table ware, monitoring of mineral oil in food and FCM proficiency testing results.

Keywords: *food contact, food safety, packaging, contaminants, official control*

L37
CHALLENGES AND ACHIEVEMENTS OF THE EU REFERENCE LABORATORY FOR DIOXINS AND PCBS

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Self-control of industry is an important pillar for safety of feed and food. In addition to the requirements of an accreditation of a laboratory according to the EN 17025, the specific requirements of the Commission Regulations have to be met prescribing analytical criteria for determination of dioxins, dioxin-like PCBs and non-dioxin-like-PCBs in feed and food. This clarification is part of the latest amendments in 2017 [1, 2].
The "Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry" [3] and the "Guide on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (jointly prepared with the other three EU-RLs for contaminants: PAHs, Heavy Metals and Mycotoxins) [4] have been finished. According to the above latest amendments in EU regulations for analytical criteria, the principles shall be followed when applicable.
When contamination incidents with PCDD/PCDFs and/or PCBs occur in the food chain, an important immediate step is the interpretation of the occurrence pattern for identification of the source. A database collecting patterns was built up and tools for interpretation developed [5].
In addition to the work on PCDD/F and PCBs, the EU Commission asked the EURL to cover also brominated flame retardants (BFRs) and chlorinated paraffins (CPs). A first proficiency test on determination of BFRs in cold liver and fish liver oil focussed on PBDEs, HBCDDs, TBBPA, brominated phenols and emerging and novel BFRs [6]. A second PT is being performed on PBDEs and HBCDDs in liver of cattle.
A demanding new task is the request to initiate new work on the analysis of short and medium chain chlorinated paraffins in feed and food. Part of these activities is the organization of an international interlaboratory study as a first step towards a harmonized analytical approach to chlorinated paraffins [7].
The inclusion of these and other new tasks are reflected by change of the name of this EURL to "EU Reference Laboratory for Halogenated POPs" starting from 1 Jan 2018.

[1] COMMISSION REGULATION (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014, Official Journal of the European Union L 92/9.
[2] COMMISSION REGULATION (EU) 2017/771 of 3 May 2017 amending Regulation (EC) No 152/2009 as regards the methods for the determination of the levels of dioxins and polychlorinated biphenyls, Official Journal of the European Union, L 115/22.
[3] Guidance Document on Measurement Uncertainty for Laboratories performing PCDD/F and PCB Analysis using Isotope Dilution Mass Spectrometry" (http://ec.europa.eu/food/safety/animal-feed_en).
[4] Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (http://ec.europa.eu/food/safety/animal-feed_en).
[5] Malisch, R. et al, Interactive data base of PCDD/F and PCB congener patterns to aid identification of contamination sources in feed and food, presented at "Dioxin 2017" in Vancouver, Canada, submitted for inclusion in Organohalogen Copounds 2017.
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[7] Kerstin Krätschmer, An international Interlaboratory Study as a first step towards a harmonized analytical approach to Chlorinated Paraffins, presentations at RAFA 2017.

Keywords: *halogenated POPs, food and feed, EU regulations for analytical criteria, proficiency test*

Acknowledgement: *We would like to thank the EU Commission for all support. Furthermore, the scientific contributions and the excellent cooperation with the network with the National Reference Laboratories (NRLs) for Dioxins and PCBs and experts is gratefully acknowledged, including several core-working groups formed on specific tasks.*

L38
IMPROVING ACCESSIBILITY TO FOOD AUTHENTICATION AND TRACEABILITY METHODS IN DEVELOPING COUNTRIES: THE ACTIVITIES OF THE JOINT FAO/IAEA DIVISION'S FOOD AND ENVIRONMENTAL PROTECTION LABORATORY

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Developing countries can face barriers to international trade whilst meeting increasing demands for authentic, quality food products from developed countries. The fast evolving world of food fraud presents a unique set of challenges to developing countries due to the high costs of state-of-the-art instrumentation and the lack of human capacity to implement analytical surveillance often imposed by markets such as the European Union. For developing countries to actively participate in food authenticity testing of produce from their domestic markets, and for export, there is a need to develop appropriate, affordable and rapid methods to screen foods for adulterants using accessible methods and instrumentation. Consequently, there is an increasing demand for strategic technical support that will enhance national food control systems and monitoring programs. Examples will be given from the work of the FAO/IAEA Joint Division of Nuclear Applications in Food and Agriculture's Food and Environmental Protection Laboratory to target food authentication and origin as a vital component of food quality and traceability.

Keywords: *food adulteration, authenticity, developing countries*

L39
IDENTIFICATION BY LC-Q-EXACTIVE MS OF PREVIOUSLY UNREPORTED PFAS'S IN WATER CLOSE TO A FLUOROCHEMICAL PRODUCTION PLANT

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Per- and polyfluoroalkyl substances (PFASs) are industrial chemicals that are produced for numerous industrial and consumer products. PFASs are global environmental contaminants and are found in the environment, food and drinking water. The production of perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) (and their precursors) has been phased out by main producers in North America and Europe. In the Netherlands, a fluorochemical production plant near the city of Dordrecht historically used PFOA until 2012, but is currently using the replacement chemical 'GenX' to produce fluoropolymers. This raises the question what current GenX levels in river water downstream from the plant are. In addition, studies reported on several other new polyfluoroalkyl homologues downstream fluorochemical plants, and it's unclear if such homologues are present downstream the Dordrecht plant as well. The presence in river water, which is used for drinking water production, could therefore be a potential source for exposure of the local population.
Legacy PFASs (PFSAs and PFCAs) were analysed in drinking and river water using our routine approach (SPE, UPLC-QTRAP-MS/MS), and this method was further optimised for inclusion of GenX. Emerging PFASs identification was performed on a UPLC-QExactive high resolution mass spectrometer (HRMS). The QExactive was operated in negative electrospray ionisation (ESI-) mode in full scan (100-1250 m/z) at a resolution of 140,000. MS/MS experiments were performed in order to obtain fragment information for structural confirmation, at a resolution of 35,000. Samples were screened for a database of compounds reported in the literature and potential other homologues differing CF2 (49.9968), CF_2CH_2 (64.0124), or CF_2O (65,9917) in mass.
GenX was detected in drinking water collected from 3 out of 4 municipalities in the vicinity of the production plant, with highest level at 11 ng/L. GenX was also detected up to 812 ng/L in downstream river water, being a possible source for drinking water contamination. Using HRMS, eleven polyfluoroalkyl acids belonging to the $C_2nH_2nF_2nO_2$, $C_2nH_2n+2F_2nSO_4$ or $C_2n+1H_2nF_2n+4SO_4$ homologue series were detected in downstream river water samples. Of these eleven compounds two chemicals belonging to the $C_2nH_2nF_2nO_2$ homologue group were detected in drinking water. Polyfluoroalkyl sulfonates ($C_2nH_2F_4nSO_3$) were detected in all collected river water samples, and therefore appear to be ubiquitous contaminants in Dutch rivers, and were also found in drinking water. Lack of analytical standards prohibits quantitation, but MS responses of these emerging PFAS were a multitude of the legacy PFASs, which may suggest high levels. The local population exposure to GenX via drinking water is estimated at 220 pg/kg/day, which was comparable to the daily intake via drinking water of the chemical it replaced, i.e. PFOA. These findings show that HRMS techniques are invaluable tools for identification of emerging PFASs.

Keywords: *GenX, PFASs, LC-Q-Exactice, fluorochemical plant*

L40
HUMAN BIOMONITORING OF MYCOTOXINS IN PHYSIOLOGICAL SAMPLES: ANALYTICAL CHALLENGES FOR INDIVIDUAL EXPOSURE ASSESSMENT

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The risk assessment of mycotoxins is mainly based on toxicity data from animal experiments in combination with reliable data on mycotoxin occurrence and food consumption. Based on this approach limits and regulations for mycotoxins in food samples are established in many countries. One major drawback of such risk assessments is a potential underrepresentation of subgroups with a regional, cultural or medically determined deviation in their nutritional habits, hence resulting in an altered exposure than estimated based on global consumption databases.
To overcome this problem the individual exposure assessment based on the analysis of physiological samples is the method of choice and could significantly contribute to an improved risk assessment. Limitations in the analysis of physiological samples, especially blood samples, are the laborious and time-consuming sample collection and clean-up.
Within the last years, the use of dried blood spots (DBS) or dried serum spots (DSS) gained more and more attention due to the development of new applications, besides the well-known use in clinical laboratories. Here, we present a multi-mycotoxin method using DBS in combination with HPLC-MS/MS for the quantification of 27 mycotoxins and metabolites in human blood samples [1-3]. The results show that 20 µl of human blood taken from finger-pricks are sufficient to quantify for example Ochratoxin A at levels as low as 0.026 ng/mL (LOQ) with just one extraction step for cleanup. The analysis of OTA in blood samples from a German cohort (n=50) using DBS revealed that all samples were positive for OTA with a mean value of 0.21 ng/mL [1].
Using a simple *„dilute-and-shoot"* HPLC-MS/MS approach, 31 mycotoxin biomarkers are currently detectable in urine samples based on our previous study [4]. The analysis of urine samples from a German cohort (n=101) identified deoxynivalenol (DON) and DON-glucuronide in 82% of the samples with mean values of 3.4 and 12.2 µg/L respectively.
As some mycotoxins or metabolites are mainly detectable in urine (e.g., DON-3-GlcA) and others such as OTA mainly in blood, the analysis of both matrices is recommended to evaluate the individual human exposure to mycotoxins. Besides *dilute-and-shoot* approaches used for urine samples, the use of DBS (or DSS) provides an optimal extension of human biomonitoring due to the minimal-invasive on-site sample collection and easy sample preparation.

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[4] Gerding J, Cramer B, Humpf H-U (2014) *Mol. Nutr. Food Res.* 58, 2358-2368.

Keywords: *human biomonitoring, mycotoxin, dried blood spot, urine, HPLC-MS/MS*

L41
MICROPOLLUTANTS AND RESIDUES IN FRENCH ORGANIC AND CONVENTIONAL MEAT

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Food safety is reported as the prime motivation driving the consumers of organic food, although very few scientific data are available to support this presumption of a health benefit. The present paper aims at providing a first reference study on the presumed health benefit of French organic meat products in regard to their possible chemical contaminant contents. In this context, the chemical contamination levels of both conventional and organic meats were assessed. Environmental contaminants (17 PCDD/F, 18 PCBs, 3 HBCD isomers, 6 mycotoxins, 6 inorganic compounds) together with chemical residues arising from production inputs (75 antimicrobials, 10 coccidiostats and 121 pesticides) have been selected as target compounds to be measured by the five corresponding French National Reference Laboratories. Selected analytical strategies specifically targeted trace levels of contamination (<< EU regulatory limits) to provide occurrence data in a context of chronic exposure. A dedicated sampling strategy, representative of the French production, allowing quantification of a large sample set (n=266) including both conventional and organic raw meat from three animal species (bovine, porcine, poultry) has been set-up. While contamination levels below regulatory limits were measured in all the samples, significant differences were observed between both species and types of farming. Several environmental contaminants (Dioxins, PCBs, HBCD, Zn, Cu, Cd, Pb, As) were measured at significantly higher levels in organic samples.

Acknowledgement: *The research work was funded by the French Agence Nationale pour la Recherche (ANR), contract n° ANR-12-ALID-004 « Sécurité sanitaire des viandes issues de l'agriculture biologique » SOMEAT (Safety of Organic Meat). http://www.so-meat.fr.*

L42*
PESTICIDE RESIDUES AND MYCOTOXINS DETERMINATION ON FEED SAMPLES: A FIRST STEP TO ASSURE MILK QUALITY

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Evaluation of undesirable compounds derived from agriculture is a very important issue concerning milk safety. The main source of such compounds is dairy cattle diet, so analyzing feed materials for pesticide residues and mycotoxin contamination is a major task to trace milk quality. Our previous studies showed that raw milk is susceptible to contamination with mycotoxins (aflatoxin M1 was found in 40% of 160 raw milk samples ranging 0.03-0.293 µg/kg) and pesticide residues (diazinon, chlorpyrifos, λ-cyhalothrin and others) [1,2]. So there is a need to evaluate the origin of such contamination, and also to enable collecting information on other many regulated and not regulated compounds, such as various groups of mycotoxins. Argentina established national MRLs for more than 400 pesticides a.i., and in the world there are more than 300 mycotoxins from many fungal species. Not only free mycotoxins but also metabolites and conjugates, along with natural toxicants of recent raised interest such as ergot alkaloids, beauvericin and enniatins, represent an emergent issue in food safety, challenged to be analyzed with the aid of modern, high throughput and sensitive sample preparation and instrumental determination methodologies. The aim of this work was to study contamination of typical feed used in dairy cattle diets in one of the most important milk production area in Argentina. For that purpose, multi-residue and multi-class QuEChERS sample preparation was performed followed by instrumental determination using UHPLC-MS/MS approaches (TQ-S and Q-LIT; ESI±) and GC-MS/MS (QqQ; EI). Over 450 compounds among pesticides, mycotoxins and metabolites were included in the methods. Analytical complexity was added by the different kind of feed samples that constitute the concentrated diets used in the study area (alfalfa and barley pastures, corn, wheat, soybean, various kind of silages and expellers, cotton seed, gluten, burlanda, commercial balanced feed and others), totalizing 54 samples. Pesticides were detected in 72% of the analyzed samples (5-500 µg/kg) and mycotoxins in 100% of samples, having at least one of the 39 compounds detected (2.5-2500 µg/kg). This kind of wide-scope study was not previously reported in concentrated feed from Argentina. Findings were relevant since multi-contamination in individual samples was frequent and in some cases with considerable levels, showing that more research in this area is needed and improvements on monitoring systems and management practices at farm level are highly recommended to protect animal and human health and to assure that this important food safety issue will not affect milk quality.

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Acknowledgement: *MINCyT, UNL, INTA (Argentina); MEYS, UCT Prague (Czech Republic)*

L43
ELUCIDATION OF NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) MIGRATING FROM POLYESTER-POLYURETHANE LACQUERS INTENDED FOR FOOD CONTACT MATERIALS

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In order to preserve food quality, internal surfaces of metallic food contact materials (FCMs) are coated with polymeric coatings. In terms of technical and economical performances, epoxy-resins based on bisphenol A (BPA) diglycidyl ether have been extensively used worldwide. Increasing regulatory pressure against BPA for FCM applications led can coating manufacturers to develop polyester-based alternatives. From a chemical food safety perspective, it is important to control any potential migration of substances from the FCM to the food. Besides intentionally added substances, which monitoring is not considered an analytical challenge, 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 can be impurities, degradation products, contaminants or oligomers. Generally, only NIAS with a molecular weight below 1,000 Daltons represent a chemical potential hazard since, above this threshold, substances are not supposed to be absorbed by the body. When dealing with polyester coatings, NIAS can be predictable oligomers (cyclic, linear or branched) originated from known monomers or unpredictable compounds obtained after reaction with solvent, reticulation or blocking agents used in lacquer formulation. Consequently, identification of NIAS is needed as a first step in the frame of proper risk assessment.
In theory, NIAS migrating from lacquer can represent a multitude of compounds. An innovative approach aiming at identifying such NIAS was applied to two polyester-polyurethane lacquers, which suppliers previously provided lists of monomers. Therefore, a non-targeted strategy was developed using liquid chromatography coupled to high resolution mass spectrometry. Hence, specific data processing was needed to address the amount of obtained data from lacquer extract fingerprints. Data were automatically processed using open-source R-environment to list detected features and deconvolute them in paired groups of same compounds. The most intense groups were investigated and compared to a homemade database using the exact monoisotopic mass. This database, populated with possible polyester oligomer combinations from a relevant selection of polyols and polyacids, enabled highlighting the presence of 14 and 17 cyclic predictable polyester oligomers in the two lacquers, including 3 mutual combinations explained by common known monomers. Among unpredictable NIAS, three additional monomers were hypothesised to explain several polyester oligomer series. Moreover, the presence of caprolactam cyclic oligomers in both lacquers was concluded as highly probable. Hypotheses were strengthened by chromatographic considerations and by the investigation of fragmentation patterns. Finally, it was possible to tentatively characterize about 80% and 90% of the cumulated feature intensities in the 2 lacquers.

Keywords: *polyester-polyurethane coatings, untargeted approach, oligomer, HRMS screening, chemical risk assessment*

L44
ENZYMATIC HYDROLYSIS OF FATTY SAMPLES – A NEW SMOOTH CLEAN-UP APPROACH FOR THE ANALYSIS OF LABILE COMPOUNDS

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The analysis of persistent organic pollutants (POP) in samples with high fat content is still of increasing interest. For a broad spectrum of analytes, we developed a new clean-up method which allows the replacement of typically used techniques like gel permeation chromatography (GPC) or fat oxidation with sulfuric acid. The new module is based on an enzymatic hydrolysis under physiological conditions and can be implemented in present manual or automated clean-up procedures.
We focused our method development on the matrix fish oil. In the last decade the consumption of dietary supplements containing fish oil became very popular. The reason is the high amount of omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are attributed to various health benefits. Because of their lipophilic behavior many POPs tend to accumulate through the aquatic food chain. Thus, it can be expected that fish oils also contain relatively high levels of non-polar compounds such as organochlorine pesticides or other POPs. But, fish oil is not just like fish oil. To get a defined fatty acid profile the triacylglycerides (TAG) of the raw fish material are usually esterified during the production process to obtain fatty acid ethylesters (EE). Consequently, the routine quality control business requires the analysis of TAG-fish oils as well as those containing only the EE which is a huge analytical challenge.
In the case of EE-fish oil the classical fat-clean-up approaches (GPC, sulfuric acid) are non-applicable. Treatment with sulfuric acid is impossible because of the instability of many POPs under these conditions. Otherwise, the low molecular weight of the EE prevents a separation via GPC.
This matrix problem was largely unsolved until now. We developed a completely new clean-up procedure for the reduction of fatty matrix compounds including also the EE. An amount of 0.5 g of the fish oil sample is hydrolyzed with a porcine pancreas lipase for several hours. During that time the EE and TAG are divided to the free fatty acids (FFA) under very smooth and specific conditions. After the hydrolysis we extract all compounds using a liquid-liquid-extraction with n-hexane and separate the FFA from the analytes by an optimized NH_2-SPE clean-up. Depending on the matrix further SPE-clean-up steps can be applied.
For the best of our knowledge we developed for the first time a method which uses an enzymatic hydrolysis under physiological conditions for the separation of fatty compounds in the trace analysis of POPs. The focus of the current method is on the analysis of organochlorine pesticides and PCB with a limit of detection of 0.01 mg/kg for most of the analytes of interest. For the future the implementation of the module for the analysis of other contaminants is planned. The simple procedure (only shaking) allows a robust and parallel processing of even high quantities of samples at low equipment and consumable costs.

Keywords: *fatty samples, persistent organic polutants, enzymatic hydrolysis, clean-up, trace analysis*

Acknowledgement: *We kindly thank Heidolph and Macherey-Nagel for their support and collaboration.*

L45*
DETERMINATION OF THE THERMAL DEGRADATION PRODUCTS OF TYLOSIN A IN HONEY USING AN MS BASED FOODOMICS APPROACH

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Veterinary drugs are regularly used in animal production to maintain health and productivity. As a result, veterinary drug residues are frequently detected in food of animal origin. During the storage or the thermal treatment (e.g. cooking) of food, the residues of these chemicals can undergo degradation, resulting in a decrease of concentration of the compound, and the general "conclusion" is that the human health risks would decrease proportionally. To date though, little is known about the identity of degradation by-products of most veterinary drug residues (e.g. antimicrobials) during food production/cooking, and the possible consequences of this degradation in terms of residual toxicity and antimicrobial activity.
Tylosin A is an antibiotic used in the control of American foulbrood, which is considered to be the most virulent brood disease known in honey bees. Although it has been proven to degrade in different thermal conditions, a comprehensive study of the behaviour of this compound in honey at high temperatures, including the characterization of its main degradation products, has never been reported. In this work, the thermal degradation of tylosin A in honey was studied using high resolution mass spectrometry. The degradation of this compound under thermal conditions was first studied in water solution at various pH and temperatures, and then different thermal treatments were applied to honey samples containing tylosin A. The analysis was performed by direct injection in HPLC-QTOF-MS/MS, with Dual AJS ESI ion source operated in positive ionization mode (ESI+) and using the All-ions MS/MS mode. Since both MS and MS/MS data were recorded for all ions, degradation products (e.g. Tylosin B) could be simultaneously isolated and identified under the various conditions.

Keywords: *veterinary drugs, foodomics, food safety, non-targeted analysis, mass spectrometry*

L46
INTERACTION BETWEEN PLANTS AND XENOBIOTICS: UPTAKE AND METABOLIZATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Pharmaceuticals are widely used in both human and in veterinary medicine and prescriptions are increasing continuously. A negative effect of this development is the fact that pharmaceutically active ingredients (AI´s) can be detected in the aquatic system. This is due to either incomplete uptake by the human body (so still active substances are excreted), the washing off of drug containing gels and lotions during personal hygiene, and unfortunately still due to improper disposal of pharmaceuticals. Although contaminated municipal wastewater is treated in wastewater treatment plants (WTP), many of these substances are unaffected by the treatment process currently used. Mainly in arid regions such reclaimed waters are more and more used for irrigation in agriculture. Thereby plants can come into direct contact with the AI´s, resulting in uptake or even metabolization of the drug by the plant. Particularly for edible plants the studying of such effects must be studied this mandatory.
In the present work we studied the uptake of pharmaceuticals in particular non-steroidal anti-inflammatory drugs (NSAIDs) by several edible plants namely cress (*Lepidium sativum*), onion (*Allium cepa*), lettuce (*Lactuca sativa*), pea *(Pisum sativum*), radish (*Raphanus sativus*), and maize *(Zea mays)*. A special focus was set on the transformation of the AI´s by the plant and the detection of the formed metabolites. After germination plants were cultivated in Petri dishes in the presence of drug containing water. After harvesting plant parts were separated and extracted. Subsequently the extracts were analyzed by a RP-HPLC and high resolution mass spectrometry (QTOF and Orbitrap) was used for detection in order to identify potential metabolites. For all NSAID´s in addition to the parent drug a series of metabolites could be detected including hydroxylation products as well as conjugates with glucose, glutamic acid, glutamine and malonic acid. Based on the information from QTOF MS^2 experiments, specific fragment ions were selected for each analyte for establishing a multiple reaction monitoring (MRM) method on a QqQ MS instrument. This allowed the analysis of plants treated with the NSAID´s at concentration as low as 1 µg L^{-1}. Additionally, employing a semi-quantitative approach involving the use of a deuterated internal standard, the distribution of the parent drugs and the metabolites in different plant parts was investigated.

Keywords: *xenobiotics, metabolization, environmental analysis, edible plants, nons-steroidal anti-inflammatory drugs*

Acnowledgement: *FWF Project I-3046 Pharmaceuticals in the Environment and their Interaction with Plants*

L47
THREE-, FOUR-, AND FIVE-DIMENSIONAL LIQUID CHROMATOGRAPHY-GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROCESSES IN FOOD ANALYSIS

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The on-line use of a liquid chromatography (LC) step, prior to a gas chromatography (GC) one, can be of great utility in the field of food analysis. The LC dimension can be exploited to perform group-type separations, and thus to deliver simplified sample fractions to the GC system, or to carry out automated rapid sample preparation. For example, normal-phase (NP) LC can very efficiently separate polar compounds from low-polarity ones (*e.g.*, oxygenated compounds from hydrocarbons in essential oils); additionally, NP LC can be used as a sample preparation step: *e.g.*, the bulk triglyceride fraction in vegetable oils can be retained, leaving mineral-oil contaminants (saturated and aromatic hydrocarbons) to reach the GC dimension.
The present contribution is based on the combination and employment of a various number of LC and GC dimensions. The use of mass spectrometry, in different forms, adds one, or more dimensions of separation. The potential of such multidimensional platforms (from 3 to 5 dimensions) will be highlighted in a variety of food applications.

Keywords: *multidimensional LC-GC, group-type separations, LC-GCxGC-MS*

L48
MULTIVARIATE CHEMOMETRIC APPROACH FOR ORIGIN BASED CLASSIFICATION OF IRANIAN PISTACHIO FRUIT SAMPLES VIA ICP-OES

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It is necessary to authenticate agricultural products and food specimens according to their composition and geographic origin basis as a part of ensuring safe food provision and guarantees offered by their protected designation of origin. Mineral composition analysis of foods provides a data base to evaluate their safety and origin. Chemical composition of nuts depends on both genetic factors and growth conditions. In the other words same cultivars which are grown in different geographical locations could show significant variations in the amount of their ingredients such as amino acids, carbohydrates, flavonoids, polyphenols etc. Several reasons related to safety, economics and health impact make it important to trace pistachio from producer to consumer. This capability may also be useful in documentation of the entire production chain for detection and traceability of batches of high-risk products and quality assurance. Among several analytical techniques, spectrometric methods are widely employed in data collection by which the origin evaluation of fruit products could be conducted. Inductively coupled plasma optical emission spectrometry (ICP-OES) is a technique which has found useful applications in the multivariate analysis as a valuable field of analytical applications for ICP-OES and ICP-MS techniques have been widely combined whit numerous chemometric tools for establishing a mineral profiles of food products in order to categorize the origin of different samples from several geographical sources. Origin discrimination of Iranian pistachio fruit was assessed by mineral element analysis. A total number of 16 Pistachio types (48 samples) from two different geographical areas of Iran (Qazvin and Kerman provinces) was investigated. Several major and minor elements were quantitatively determined in pistachio samples by inductively coupled plasma optical emission spectroscopy (ICP-OES) in terms of their concentration in mg/g. Principal component analysis (PCA) and least squares-support vector Machine (LS-SVM) methods were employed for sample classification. Models built were used to classify samples. LS-SVM was able to distinguish the geographical origin of pistachio samples. Error rate in training and test sets were 3.7% and 5.0%, respectively. The ICP-OES in combination whit chemometrics was confirmed to be reliable for discriminant analysis of pistachio sample.

Keywords: *pistachio, geographical origin, chemometrics, inductive coupled plasma, ICP-OES*

L49*
U-HPLC METHOD EMPLOYING SIMPLE SINGLE QUADRUPOLE MASS ANALYZER FOR A RAPID SCREENING OF MULTIPLE FOOD ADDITIVES

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Food additives involve a wide range of chemicals, both natural and synthetic, largely differing in their physico-chemical properties. These compounds are intentionally added to foodstuff to perform specific technological functions such as to extend shelf life, to color, to sweeten, to modify flavor and texture etc. Despite their technological benefits, food additives are also a source of concern for some population groups, such as those suffering from specific health disorders (e.g. allergy, phenylketonuria…), or consumers favoring ´natural´ or organic food. In the EU, the use of certain food additive, identified by an E number or name, has to be declared on the food label, however, in some cases, fraudulent practices might be experienced and the use of additive is undeclared, or in case of those, that are regulated, maximum concentration limit is exceeded. No need to emphasize that the availability of a fast and broad scope screening method is needed for efficient food control, as well as fraud detection.
So far, a number of analytical methods based on various principles have been developed for the determination of different groups of food additives; HPLC technique employing conventional detectors such as UV/VIS is currently the most common in routine laboratories. With regards to limited selectivity, mass spectrometry (MS) appears to be a better option, nevertheless, the instrument equipped either with triple quadrupole or high resolution mass analyzer (TOF, Orbitrap) are still fairly expensive, which increases analysis cost. As a compromise, the use of simple quadrupole MS analyzer seems to be a conceivable option. In our study, we demonstrate the application of a relatively cheap QDa detector (Waters) for a rapid simultaneous screening of 21 most common food additives including 12 synthetic dyes, 7 sweeteners and 2 preservatives together with 2 flavourings and 2 purine alkaloids. Further analytes, including those used for adulteration, are continuously added on the analytes list. The performance characteristics of the validated U-HPLC-MS procedure when employed on various food matrices will be discussed and compared with other HPLC-based methods.

Keywords: *mass spectrometry, U-HPLC, food additives*

Acknowledgement: *This study was supported by the "Specific University Research" project (MSMT No 20-SVV/2017.)*

L50
METABOLOMIC FINGERPRINTING OF GREEN AND ROASTED COFFEE BEANS BY LC-ESI-QTOF

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Coffee is the world's second most consumed drink after water. With the rise of coffee consumption, coffee quality has received extensive attention. There is a wide variety of factors involved in coffee quality such as growth condition, processing, and storage. Among these factors, the roasting process must be highlighted since it severely affects the development of the broad array of complex flavors which make coffee enjoyable to drink. During the roasting process, the green coffee beans undergo many changes in their chemical and physical properties including formation of compounds responsible for the coffee quality. Therefore, the search for chemical markers which are characteristic of the roasting degree appears as a very good strategy to guarantee the coffee quality. The implementation of metabolomics approaches provides the tools needed to face this challenge. Despite metabolomics being a powerful tool to comprehensively characterize a process, to the best of our knowledge, only one contribution devoted to the study of the roasting process of coffee has been reported, which was based on NMR [1].
In this work, LC-ESI-QTOF-based metabolomics using a C18 column was used to monitor the substantial changes on the composition of coffee samples (*Arabica coffee*) during three different roasting processes (light, medium, and dark) in an untargeted analysis. To obtain the maximum number of molecular features, different extraction protocols were evaluated for these three roasted coffee samples and for green coffee. Under the optimized conditions 280 and 522 molecular features were initially observed for ESI+ and ESI-, respectively. To reduce the complexity of the information obtained from metabolomic analysis, data were analysed using both non-supervised (PCA) and supervised multivariate statistical methods (PLS-DA). The metabolite selection criteria chosen was the variable importance on projection (VIP) values obtained from the pairwise PLS-DA models. Results display two clear trends. On the one hand, some molecular features displaying high VIP values in each pairwise PLS-DA model increased or decreased their levels as the roasting degree increased. On the other hand, there were other molecular features which had high VIP values in only a PLS-DA comparison, i.e. they are characteristic from only a specific roasting degree. The influencing features were identified through their retention times and MS/MS spectra and taking into account metabolites databases. The results showed that some of the statistically significant metabolites belong to the groups of hydroxycinnamic acids and flavonoids.
[1] F. Wei, *et al., J. Agric. Food Chem.* 60 (2012) 1005-1012

Keywords: *metabolomic fingerprinting, coffee, roasting degree markers, liquid chromatography time-of-flight mass spectrometry*

Acknowledgement: *Authors thank financial support from the Comunidad of Madrid (Spain) and European funding from FEDER program (project S2013/ABI-3028, AVANSECAL-CM). R.P.M. thanks the University of Alcalá for her pre-doctoral contract. M.C.P. and M.P. thank the Spanish Ministry of Economy and Competitiveness (MINECO) for their "Ramón y Cajal" (RYC-2013-12688) and "Juan de la Cierva" (IJCI-2014-22143) research contracts, respectively. Authors gratefully acknowledge "Café Fortaleza" for providing coffee samples.*

L51*
ADVANCES IN HONEYDEW HONEY ANALYSIS FOR THE DETERMINATION OF ITS BOTANICAL ORIGIN

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Honeydew which is excreted by aphids feeding on conifers in Germany and Central Europe is an important food source for bees in late summer. The honeys produced of it are of a dark color and have an especially spicy, malty taste. Many consumers like these properties and accept the high prices.
So far, honeydew honeys are distinguished mostly by their sensory properties. The classical microscopy pollen analysis failed due to the missing pollen. In order to protect the quality and the authenticity of these rare expensive honeys, a project was launched and funded in Germany by The Federal Ministry of Food and Agriculture (BMEL) called "BoogIH" – Botanical, zoological and geographical Identification of Honeydew honey. Aside of the TU Dresden, the Apicultural State Institute (Dr. Schroeder, University of Hohenheim), the University of Wuppertal (Prof. Dr. Lohaus, Molecular Plant Research/Plantbiochemistry), HYDROISOTOP GmbH (Dr. Voerkelius, Schweitenkirchen), and Intertek Food Services GmbH (Dr. Rimkus, Bremen) are involved. The aim is to provide an accurate definition of honeydew honeys using objective chemical-analytical methods in order to promote their marketing.
For this purpose, Professor Speer's working group has developed a multi-method for the extraction of various honey constituents. To the best of our knowledge, this is the first report on the simultaneous extraction of honey components from one honey solution. The final method allows for the determination of polar, nonpolar as well as charged compounds followed by chromatographic analysis using
(a) HPLC-ELSD for sugar analysis,
(b) UHPLC-PDA-MS/MS for phenolics and other hydrophobic components, and
(c) HPLC-PDA-MS/MS for amino acids and other nitrogen-containing substances of honey.
The chromatographic profiles of authentic honey samples, especially of firs, spruces, and pines were compared and characteristic substances were identified by using chemometrics. A differentiation will be achieved by means of a discriminant analysis. The first results of our study will be presented.

Keywords: *honeydew honey, authentication, chemometrics, UHPLC-PDA-MS/MS, ELSD*

Acknowledgement: *The BoogIH project (Project No. 2816500314) is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.*

L52
RAMAN HYPERSPECTRAL IMAGING AND SPECTRAL SIMILARITY ANALYSIS FOR QUANTITATIVE DETECTION OF MULTIPLE ADULTERANTS IN WHEAT FLOUR

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Recent food safety incidents and public health concern related to detrimental food additive issues have driven the need to develop fast, sensitive, and reliable methods to detect food hazards and adulteration. Raman microscopic imaging has been used adequately for quality and authenticity analysis of food products; however, the application of line-scan Raman imaging is at early stage. Here, we assess the applicability of line-scan Raman hyperspectral imaging (RHI) technique for simultaneous detection of three potential chemical adulterants (brighteners) in wheat flour in the concentration range of 0.05 – 1.5% for each adulterant. Adulterated wheat flour samples packed into an aluminum sample holder (80 mm × 40 mm × 2 mm) and illuminated using a 785 nm line laser to generated Raman scattering. Raman hyperspectral images were collected at 0.2 mm step size and 1s exposure time. Reference Raman spectra were obtained for all three adulterants including: benzoyl peroxide, alloxan monohydrate, and L-Cysteine. The collected Raman data were firstly preprocessed by adaptive iteratively reweighted penalized least square (airPLS) and median filter for fluorescence baseline removal and to mitigate high frequency noise, respectively. Spectral angle mapper (SAM) as a tool for matching the separated endmember with pure spectra was applied to preprocessed Raman data to classify adulterants' pixels from the flour background using the pure endmember (adulterant spectrum) as an input. SAM images for each adulterant were converted to the binary images by applying threshold value to effectively visualize and quantitatively detect the adulterants pixels in wheat flour. The pixel based calculated proportion of adulterants in wheat flour were in agreement with added concentration. The applicability of developed technique was further validated for the repeatability purpose and the result demonstrate that RHI in combination with SAM provides a novel, elegant way for rapid and noninvasive analysis of powdered food for quality and authenticity evaluation.

Keywords: *food safety, raman hyperspectral imaging, wheat flour adulterant, spectral angle mapper, quantitative analysis*

L53
FOOD SAFETY MONITORING AND RISK ASSESSMENT: PAST, PRESENT AND FUTURE IN CHINA

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The implementation of the Food Safety Law of the People's Republic of China since 2009 greatly promoted the application of the risk analysis framework in China.This paper is intended to review the progresses in national food safety risk monitoring/surveillance and risk assessment works in which China National Centre for Food Safety Risk Assessment (CFSA, established in 2011) has played the role of technical support and guidance. The contribution of monitoring/surveillance and risk assessment to the development of riskmanagement in China, including food safety standard system development, is described. However, in comparison with risk management needs and practices in developed countries, China should further strengthen capacity building in food safety monitoring/surveillance and risk assessment. Moreover, the Lecture thoroughly introduces the capacity building of risk analysis in China. Progress is particularly evident in carrying out food safety risk monitoring/surveillance and risk assessment work. Risk management work has somewhat improved, especially a step-wise approach was followed in reviewing, simplifying and integrating food safety standard based on risk assessment, leading to the integrated National Food Safety Standard (NFSS) framework, which anchored China NFSS in scientific evidence and created the sky for their future evolution. However, the implementation of risk communication is still weak.

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Keywords: *food safety, monitoring, surveillance, risk analysis and risk assessment, China*

L54
FOOD ALLERGY RESEARCH PROGRAM IN CHINA

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Food allergy is becoming one of the serious problems of China´ food safety and public health emergency, which needs to be included in the national major research program. Currently, limited work indicated that the prevalence of food allergy in China is about 6% in children, supporting the global prevalence of an increasing trend. Therefore, it is necessary to set up a food allergy work group in China as soon as possible, to carry out food allergy epidemiological studies for allergen labelling in foods. It aims to identify allergic foods need to be labelled in Chinese food labels, and in a view of scientific frontier, such as the molecular characterization of food allergens, to establish food labeling system for food allergen that is more in line with China´ national conditions. During the 13th Five-Year Plan period, in the food safety major project, the following researches are planned to be carried out, including: multi-center epidemiological investigation on food allergy in Chinese population; research and development on food allergy diagnostic criteria and diagnostic reagents; the relationship between epitope structure and allergenic property of food allergens; multi-level technology system for allergenicity assessment; the validation technology of the testing of food allergens and related product development; and research on food allergy risk management measures.

L55
DEVELOPMENT OF FOOD SAMPLE PRETREATMENT METHODS AND APPLICATION TO CHEMICAL POLLUTANT DETERMINATION

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Although chromatographic instruments have enjoyed great technological advances, the food sample pretreatment step remains a critical component to improve detection sensitivity, selectivity, reproducibility and accuracy. Recently, plant growth regulator (PGR) has received more and more attentions in the field of food safety. But the simple and sensitive method for simultaneously analyzing multiple classes of PGR remains poorly investigated. PGRs can be labelled selectively by fluorescence labelling reagent and detected by FLD at specific excitation and emission wavelengths. Thus, the labelling procure could significantly decrease the interference of primary and secondary metabolites in real samples and improve the detection selectivity. A new pre-column fluorescence labelling method using 2-(11H-benzo[a]carbazol-11-yl)-ethyl-4-methyl-benzenesulfonate (BCETS) as the labelling reagent has been developed for simultaneous determination of seven PGRs (i.e., indole-3-acetic acid, 3-indolybutyric acid, 3-indolepropionic acid, jasmonic acid, gibberellin A_3, 1-naphthylacetic acid and 2-naphthaleneacetic acid) by HPLC with fluorescent detection (FLD). The proposed method offered the LOD of 0.34–0.73 ng/mL for seven PGRs, which were significantly lower than the reported methods. The crude extract without complex pre-treatments and purification was directly labelled by BCETS and analysed by HPLC-FLD, which facilitates the high-throughput sample screening. This method was proven to be inexpensive, simple, selective, sensitive, accurate and reliable for trace PGR determination. Porous materials have received burgeoning attentions over the past decade in the field of sample preparation because of their outstanding performance. We reported a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for determination of trace polycyclic aromatic hydrocarbons (PAHs) in food samples by employing a new core-shell nanostructure magnetic covalent organic framework hybrid microspheres (Fe_3O_4@COF(TpBD)) as the sorbent followed by HPLC-DAD. Fe_3O_4@COF(TpBD) was prepared with the retention of colloidal nanosize, larger specific surface area, higher porosity, uniform morphology and supermagnetism. By taking advantage of its merits, the as-prepared materials earned an excellent adsorption ability to PAHs, and the enrichment efficiency of Fe_3O_4@COF(TpBD) could reach 99.95%. The obtained materials had fast adsorption kinetics and realized adsorption equilibrium within 45 min. The eluent was further analyzed by HPLC-DAD. Good linearity was observed in the range of 1-100 ng/mL with the linear correlation being above 0.9990. The limits of detection (S/N=3) and limits of quantification (S/N=10) for 15 PAHs were in the range of 0.83-11.7 ng/L and 2.76-39.0 ng/L, respectively. The obtained materials were first employed for the enrichment of trace PAHs in food samples, and exhibited superior enrichment capacity and excellent applicability.

Keywords: *sample pretreatment, chemical derivatization, magnetic solid phase extraction, plant growth regulator, polycyclic aromatic hydrocarbons*

Acknowledgement: *This work was supported by The National Natural Science Foundation of China (No. 21677085, 21537001 and 81472986), and the Project funded by China Postdoctoral Science Foundation (No 2016M590071).*

L56
SIMPLE, RAPID, AND ENVIRONMENTALLY FRIENDLY METHOD FOR THE SEPARATION OF ISOFLAVONES USING ULTRA-HIGH PERFORMANCE SUPERCRITICAL FLUID CHROMATOGRAPHY

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Isoflavones are natural substances that exhibit hormone-like pharmacological activities. The separation of isoflavones remains an analytical challenge because of their similar structures. We show that ultra-high performance supercritical fluid chromatography can be an appropriate tool to achieve the fast separation of 12 common dietary isoflavones. Among the five tested columns the Torus DEA column was found to be the most effective column for the separation of these isoflavones. The impact of individual parameters on the retention time and separation factor was evaluated. These parameters were optimized to develop a simple, rapid and green method for the separation of the 12 target analytes. It only took 12.91 min using gradient elution with methanol as an organic modifier and formic acid as an additive. These isoflavones were determined with limit of quantitation ranging from 0.10 to 0.50 μg/mL, which was sufficient for reliable determination of various matrixes.

Acknowledgement: *This study was supported by the National Science and Technology Major Project of "Study on the Quality Detection Technology for Import and Export Medicine and Food Dual Purposes Products"(Project number:2017YFF0211000).*

L57
HUMAN BIOMONITORING OF DEOXYNIVALENOL AND ZEARALENONE IN THE CHINESE POPULATION

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Mycotoxins are secondary metabolites or biotransformation products of fungi. They commonly occur in various cereal crops and processed grains, and can also be found in animal-derived food as a consequence of a carry-over from contaminated feeds. Humans are easily exposed to mycotoxins through the diet. Assessment of human exposure to mycotoxins is conventionally performed by analysis of food contamination levels and calculation of intake based on consumption data. In recent years, biomarker-based strategies have gained increased acceptance.
A pilot study on human urinary biomonitoring of deoxynivalenol (DON) and zearalenone (ZEN) was recently conducted for the residents in Henan province located in the central part of China, where wheat as the staple food are highly consumed. High-throughput and sensitive UPLC-MS/MS methods following 96-well μElution solid-phase extraction were developed and validated for the determination of DON biomarkers (DON, DOM-1) and ZEN biomarkers (ZEN, α-zearalenol, β-zearalenol, α-zearalanol, β-zearalanol and zearalanone), using ^{13}C-DON and ^{13}C-ZEN as internal standards for accurate quantification. Urinary samples collected from 301 healthy volunteers aged 0-84 years were processed with and without enzyme hydrolysis to determine total and free biomarkers, respectively. DON, and DOM-1 to a le lesser extent, can be frequently detected in these samples both with and without enzyme hydrolysis. Free DOM-1 was detected at low level in human urine for the first time. Total DON was detected in all samples with a mean concentration at 52.8 ng mL^{-1}. The mean and median probable daily intakes (PDI) for the whole participants estimated to be 1.83 μg/kg bw and 1.14 μg/kg bw both exceeded the PMTDI (1 μg/kg bw/day), indicating a high potential risk for the residents in this area, especially for children and adolescents. For ZEN biomarkers, ZEN, α-zearalenol, β-zearalenol and zearalanone were detected in 71.4% urine samples in the range from 0.02-3.7 ng mL^{-1} after enzyme hydrolysis. The mean PDI was estimated to be 0.025 μg/kg bw, largely below the PMTDI set by JECFA (0.5 μg/kg bw/day) and the TDI set by EFSA (0.25 μg/kg bw/day).

Keywords: *deoxynivalenol, zearalenone, biomarkers, biomonitoring, risk assessment*

Acknowledgement: *This work was supported by National Natural Science Foundation of China (31471671 and 31501400) and CFSA "523" High Level Talents Development Project.*

L58
DIETARY INTAKE AS IMPORTANT PATHWAY FOR HUMAN EXPOSURE TO ISOMERIC PERFLUOROALKYL SUBSTANCES (PFASS)

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The presence of perfluoroalkyl substances (PFASs) have been well studied in human daily intakes from various exposure matrices. However, little is known about the isomeric compositions of PFASs in daily intakes and their influence on the isomeric profiles in humans. In this study, the occurrence of PFASs with isomeric analysis in multiple human exposure matrices including foodstuffs, tap water and indoor dust was investigated. Perfluorooctanesulfonate (PFOS) and/or perfluorooctanoate (PFOA) were predominant in these matrices collected in Tianjin, China. Dietary intake contributed >99% of the estimated daily intake (EDI) for the general population. In fish and meat, linear (*n*-) PFOA was enriched with a percentage of 92.2% and 99.6%, respectively. Although *n*-PFOS was higher in fish (84.8%) than in technical PFOS (ca. 70%), it was much lower in meat (63.1%) and vegetables (58.5%). The isomeric profiles of PFOA and PFOS in human serum were predicted based on a one-compartment, first-order pharmacokinetic model. The isomeric percentage of *n*-PFOA in the EDI (98.6%) was similar to that in human serum (predicted: 98.2%, previously measured: 99.7%) for Tianjin residents. The results suggest that direct PFOA intake plays an important role on its isomeric compositions in humans. For PFOS, the predicted *n*-PFOS (69.3%) was much higher than the previously measured values (59.2%) in human serum. This implies that other factors, such as indirect exposure to PFOS precursors and multiple excretion pathways, may contribute to the lower percentage of *n*-PFOS in humans than of technical PFOS.

Acknowledgement: *We acknowledge financial support from the Natural Science Foundation of China (NSFC 21325730, 21277077).*

LECTURES

L59
INTERACTIVE SEMINAR: STEP BY STEP STRATEGIES FRO FAST DEVELOPMENT OF SMART ANALYTICAL METHODS

Moderators:

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This educative seminar is intended for young scientists but all other RAFA attendees are also welcome! It will provide interactive demonstration of general approaches to fast development and troubleshooting of analytical methods for food quality and safety control. The moderators will introduce several 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. Each time, the attendees will identify the most suitable solution using an anonymous electronic voting system, followed by an interactive discussion about each presented option. In the end of the seminar, groups of participants will compete against each other by proposing optimal solutions for particular analytical problems. The best ideas will be awarded by special prizes!

We encourage you to attend this informal and interactive seminar, which was very well received at last RAFAs. Come to join the discussion, outline your vision, learn something new and have some fun!

L60
TOWARDS A MULTI-OMICS APPROACH AS THE NEW FRONTIER FOR MYCOTOXIN RESEARCH

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The chemical diversity of mycotoxins and their (co)occurrence in food and feed, poses a great challenge for mycotoxin research.
According to recent reports, almost 100% of crops are contaminated with one or more mycotoxins [1]. In addition, extreme weather events related to climate change are often responsible for unexpected changes in the pattern of mycotoxins occurring in food commodities worldwide. Therefore, besides human and animal health-related issues, mycotoxin contamination is responsible for severe market loss, as well.
Thanks to the tremendous progress made in mycotoxin analysis, a wide range of solutions for multi-toxin analysis have become available over the last decade, allowing the collection of more representative occurrence data as a base for risk assessment [2].
However, the toxicological evaluation of mycotoxin mixtures as well as a more personalized approach to multiple-toxicant exposure, remain as a major challenge.
Metabolomics has emerged as the one of the most promising "omics" disciplines, offering a tool to deciphering the ultimate response of biological systems to genetic or environmental changes.
Such approach is often used in the mycotoxin field, from one side to provide a more comprehensive picture of the plant-fungus cross-talk occurring in the field, and from the other side to explore animal metabolism leading to the formation of biomarkers of exposure.
In addition, latest "omics" techniques, such as lipidomics, may provide insights into the cellular mechanisms related to toxicity and, therefore, may support the identification of biomarkers of effect.
Little to nothing is known, finally, on the role played by epigenetics factors and microbiome-driven phenomena in the physiological impairing related to mycotoxin exposure.
The frontier of mycotoxin research is therefore moving towards the integration of multi-omics data for a deeper understanding of the inter-relation between mycotoxin occurrence, molecular events at cellular level, and the response of the living organism [3].
This contribution will cover the current use of "omics" methodologies in mycotoxin research, and will define future challenges and existing gaps of knowledge.

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Keywords: *metabolomics, high resolution MS, toxic effects, chemical mixtures*

L61
BEYOND AFLATOXINS: USING UNTARGETED HRMS TO ENABLE DISCOVERY AND IDENTIFICATION OF EMERGING MYCOTOXINS IN ASPERGILLUS FLAVUS

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Cyclopiazonic acid (α-cyclopiazonic acid, α-CPA) is an indole-hydrindane-tetramic acid neurotoxin produced by various fungal species, including the notorious food and feed contaminant *Aspergillus flavus*. Despite its discovery in *A. flavus* cultures approximately 40 years ago, its contribution to the *A. flavus* mycotoxin burden is consistently minimized by our focus on the more potent carcinogenic aflatoxins also produced by this fungus. Here, we report the screening and identification of several CPA-type alkaloids not previously found in *A. flavus* cultures.
Our identifications of these CPA-type alkaloids are based on a dereplication strategy involving accurate mass high resolution mass spectrometry data and a careful study of the α-CPA fragmentation pattern. In total, 22 CPA-type alkaloids were identified in extracts from the *A. flavus* strains examined. Of these metabolites, 13 have been previously reported in other fungi, though this is the first report of their existence in *A. flavus*. Two of our metabolite discoveries, 11,12-dehydro α-CPA and 3-hydroxy-2-oxo CPA, have never been reported for any organism. The conspicuous presence of CPA and its numerous derivatives in *A. flavus* cultures raises concerns about the long-term and cumulative toxicological effects of these fungal secondary metabolites and their contributions to the entire *A. flavus* mycotoxin problem.

Keywords: *cyclopiazonic acid, ergot-like alkaloid, dereplication, HRMS, Aspergillus flavus*

Acknowledgement: *Valdet Uka was financially supported by Project Basileus V (Erasmus Mundus Action 2) funding from the European Commission.*

L62*
EMERGING FOOD CONTAMINANTS: INVESTIGATING ALTERNARIA MYCOTOXINS BY LC-MS/MS

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The fungal genus *Alternaria* causes considerable agricultural losses due to both, pre- and post-harvest infestations and contamination of crops with secondary metabolites, known as *Alternaria* toxins. Several proved acute toxicity, genotoxicity, mutagenicity and estrogenic properties. However, due to the lack of comprehensive data on occurrence patterns and their toxicological potential, no regulatory guidelines have been established in the European Union to date. To evaluate whether maximum permitted levels for foodstuffs are required, the urgency for further research was highlighted by the EFSA in 2016.
In this study, a high performance liquid chromatography tandem mass spectrometric method for the simultaneous quantification of 18 *Alternaria* toxins in three food matrices of high relevance (wheat flour, tomato sauce and sunflower seed oil) is presented. Target analytes include the most relevant parent toxins (derivatives of tetramic acids, dibenzopyrenes, perylene quinones and others) and selected phase II metabolites (sulfates and glucosides) of alternariol (AOH) and its monomethyl ether (AME). Due to the lack of certified reference materials, the method validation was based on the fortification of blank matrices.
A survey on food products purchased from Austrian supermarkets gives first insights about *Alternaria* toxin contamination patterns in the different commodities and agricultural production types (conventional vs. organic production). Highest *Alternaria* toxin contaminations were found in tomato sauce products (tenuazonic acid up to 250 ng/g, AOH up to 30 ng/g, AME up to 3.5 ng/g). Interestingly, also modified forms of AOH and AME could be detected.

Keywords: *alternaria mycotoxins, LC-MS/MS, food matrices*

L63*
LC-ESI-MS/MS MULTI-CLASS METHOD FOR THE ANALYSIS OF COMPLEX ANIMAL FEED: EVALUATION AND REDUCTION OF ABSOLUTE AND RELATIVE MATRIX EFFECTS

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Due to several pre- and post-harvest conditions like climate impacts, processing or storage, a variety of contaminants as well as residues can enter the food chain. Liquid chromatography tandem mass spectrometric methods represent effective tools for the analysis of mycotoxins, pesticides or veterinary drugs. However, it is well known that this analytical state of the art approach is prone to signal suppressions or enhancements (SSE) as a result of matrix effects. Based on a divergent composition of different ingredients, animal feed represents a very complex matrix model, resulting in a high number of co-eluting matrix components.
In this work, a liquid chromatography-electrospray ionization tandem mass spectrometric method was developed first, to allow a simultaneous quantification of about 700 fungal metabolites, 500 pesticides, 100 veterinary drugs as well as several bacterial and plant toxins. Further, a survey on animal feed products was conducted to prove the methods applicability and give first insights about analytical identification data of this matrix. It might be expected that significant variations of SSE and extraction recovery between individual samples of a given feed type influence the method performance. Therefore different strategies, such as dilute and shoot, matrix matched calibration or the use of isotopically labelled internal standards were evaluated. Based on data deriving from spiking experiments of both, the compound feed formula as well as the individual ingredients, this work aims to discuss effective reduction strategies of absolute and relative matrix effects in animal feed based on a LC-ESI-MS/MS approach.

Keywords: *LC-MS/MS, matrix effects, animal feed, residues, contaminants*

Acknowledgement:
The competence center FFoQSI is supported by BMVIT, BMWFW and the federal states of Lower Austria, Upper Austria and Vienna within the framework of COMET Competence Centers for Excellent Technologies. The COMET program is managed by FFG.

L64*
UNTARGETED LIPIDOMICS TO DECIPHER FUMONISINS ACCUMULATION IN MAIZE

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Climate change is significantly affecting the mycotoxin contamination of crops worldwide and as a consequence, fungal infection and related pathogenic disease causing significant yield losses, quality reduction and mycotoxins accumulation is rising concerns. On this account, fumonisin-contaminated maize products are a major food concern due to their toxic effects in humans and animals; indeed, FB1 has been declared as a class 2B carcinogen by the International Agency for Research on Cancer.
In order to reduce the economic and health impact, several cultural practices have been proposed so far. Breeding and growing less susceptible plant genotypes is one possible strategy to reduce these effects and counteract micotoxin accumulation. In particular, recent studies related the amount of total fatty acids to fumonisin accumulation in maize. According to this study, maize hybrids with higher linoleic acid content showed a high fumonisin contamination. In addition, oxylipins profile and phytoceramides were found significantly changed within the hybrids.
Although the correlation between fumonisin accumulation and plant lipid profile is certain, the data collected so far cannot define a cause-effect relationship but open up new perspectives. In this view, the study of the lipidome seems to be the best approach to decipher the multifactorial nature of the resistance and plant-pathogen interaction mechanisms. Through the present field study, a global view of differential lipid metabolites accumulated during resistance and mycotoxin accumulation was obtained, giving additional information about the mechanisms and pathways conferring resistance in maize. At the same time, untargeted UHPLC-QTOF merged with chemometrics analysis demonstrated to be a powerful tool in order to distinguish low (< 1 mg/kg) and high (> 10,000 mg/kg) fumonisins-contaminated samples. The results were confirmed in a 2-year data analysis and several lipid markers (i.e. oxylipins, phytoceramides, diacylglycerols) were identified.
These evidences highlighted, indeed, the crucial role played by lipid signaling as an integrated part of the complex regulatory network in plant.

Keywords: *lipidomics, fumonisins, maize, oxylipins*

L65
TROPANE ALKALOIDS IN FOOD. RESULTS OF AN EUROPEAN WIDE SURVEY FOR EFSA

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Tropane alkaloids (TAs) are a broad group of secondary metabolites found in several plant families, but most notably in the Solanaceae (nightshades). Atropine and scopolamine are the most well-known TAs, as they can be present as contaminants in food and feed, and for this reason have been the subject of two EFSA opinions (2008 and 2013) and regularly the subject of RASFF notifications. Due to the lack of available occurrence data (only data for atropine and scopolamine in children's cereals were available), in 2014 a large survey was initiated and funded by EFSA, to evaluate the occurrence of a broad range of TAs in a wide variety of food products.

Based on a literature study that was part of the project, the most relevant food products at risk of contamination with TAs were identified comprising single flours (buckwheat, sorghum, millet), cereal-based products (bread, pasta, breakfast cereals, cookies), vegetable-based products (beans, stir-fry mixes) and herbal teas. A specific group of TAs, the calystegines, was selected because they are inherent to many of the edible species of the Solanaceae family, most importantly potatoes and aubergines.

An LC-MS/MS method was developed and validated for the analysis of 24 TAs in cereal- and vegetable based food samples and herbal teas. A second LC-MS/MS method was developed to quantify 6 calystegines in potato and aubergine. LoDs ranged from 0.05-1.0 µg/kg for the TAs and from 0.25-1.0 mg/kg for the calystegines.

Between June 2015 and August 2016 1700 samples were collected from retail stores in 9 European countries, of which 1300 were analysed for TAs and 400 for calystegines. One or more TAs were detected in 22.5% of the samples, most often in herbal teas (70%), least often in breakfast cereals (6.8%) and pasta (0%). Overall 19 from the 24 TAs were detected, of which atropine (17%) and scopolamine (13%) the most frequently. The TA patterns differed between the food categories. In flours and cereal-based products, atropine and scopolamine were the major TAs. Mixed vegetable products often contained the low molecular weight TAs tropine and pseudotropine. The herbal teas contained TAs from all types, most notably atropine, scopolamine, convolvine, tropine and pseudotropine. The maximum TA concentration detected was 4358 µg/kg in a herbal tea sample, but also vegetable products could contain substantial amounts of TAs (up to 2216 µg/kg).

The average content of calystegines in potatoes was 161.6 mg/kg fresh weight (max: 507.3 mg/kg). The average content in aubergine was 21.1 mg/kg FW (max: 181.5 mg/kg). Calystegine A_3 was the major TA in potatoes, followed by calystegine B_2. In aubergine calystegine B_2 was predominant, followed by calystegine B_1 and A_3.

TAs appear to be compounds that are often present in foods. Nevertheless, toxicological data on TAs other than atropine and scopolamine, is very scarce. Inasmuch exposure to these TAs, including calystegines, poses a risk will be briefly discussed.

Keywords: *tropane alkaloids, occurrence, food, calystegines, LC-MS/MS*

Acknowledgement: *This project was funded by EFSA, GP/EFSA/CONTAM/2014/01: 'Occurrence of Tropane Alkaloids in food'. The sole responsibility of the information presented here lies with the authors and EFSA cannot be held responsible for any use that may be made of the information presented therein.*

L66
ANALYTICAL PERFORMANCE OF AN LC-MS/MS BASED MULTI-CLASS METHOD COVERING > 1000 ANALYTES

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Multi-analyte methods based on either LC-MS/MS or LC-HRMS have been successfully introduced in the field of both natural (mycotoxins, plant toxins, and marine biotoxins) as well as anthropogenic contaminants (e.g. pesticides and veterinary drugs). As most of these methods include only a very crude QuEChERS-based or even no clean-up of the acidified methanol/water or acetonitrile/water extract, additional analytes may be included without any need for modification as long as they are amenable to the chosen generic conditions.
The obvious step forward is to merge all these approaches in order to cover most of the relevant food and feed contaminants by using one single method. Such an analytical tool enables to generate data on the co-occurrence of contaminants deriving from different classes, which would provide a priority list for the determination of combinatorial effects of different contaminants.
This presentation aims to highlight the limitations of tandem mass spectrometry related to the number of analytes that can be monitored within a single chromatographic run. The effectiveness of different unspecific clean-up approaches will be discussed in terms of reduction of matrix effects. Finally, the feasibility of the method in view of accurate quantitative analysis will be evaluated.

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012.*

L67
HOW SAFE ARE YOUR INFUSIONS? ANALYSIS OF PYRROLIZIDINE ALKALOIDS IN PLANT BASED PRODUCTS USING UHPLC-MS/MS & HIGHLIGHTING OF THEIR TRANSFER RATE DURING BREWING

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Pyrrolizidine alkaloids (PAs) and their N-oxides (PANOs) are natural toxins, exclusively biosynthesized by a wide variety of plant species (>6000). They are secondary plant metabolites against herbivores and are believed to be one of the most widely spread natural toxins. Human poisoning cases have been documented; they are characterized by acute and chronic liver damage, pulmonary hypertension, cardiac or kidneys injuries, and can lead to death. Therefore, the development of efficient analytical methods is required to detect PAs/PANOs even at low levels.
The Belgian Scientific Institute of Public Health has directed a project aiming to report occurrence levels of PAs and PANOs in targeted food items to perform a dietary exposure assessment to these natural contaminants and evaluate if they can pose a health problem, filling a data gap at Belgium's level. Analytical methods based on UPLC separation and MS/MS detection were developed for the analysis of 16 PAs and 14 PANOs in honey and honey based snacks, meat and meat products, milk and dairy commodities, plant based products and food supplements. A structured sampling plan has been established for more than 1,000 samples available on the Belgian market. The presentation will focus on the interesting case of PAs and PANOs in plant based products: dry (herbal) teas and "as consumed" teas (brewed), but also less targeted matrices such as salads, spices and food supplements.
On one hand, the sample preparation for dry plant based products (freeze dried salads, spices, (herbal) teas and food supplements) involved organic acidic extraction of the analytes followed by Envi-Carb SPE purification to avoid loss of sensitivity due to matrix effect. On the other hand, tea samples were brewed following ISO3103 standardized method, the infusion was then purified using C18 SPE cartridges. Several analytical steps were optimized in order to obtain the highest sensitivity. The separation of PAs/PANOs was performed on a C18 stationary phase LC column. The analytical methods were validated in-house and the method's performances were within the criteria allowed by Directive 2002/657/CE and SANTE/11945/2015. The LOQs achieved (0.1 $ng.g^{-1}$ for dry products, 0.01 $ng.mL^{-1}$ for brewed teas) were lower than those proposed by EFSA (2011).
Surprisingly, high levels of heliotrine-type PAs/PANOs (>500 $ng.g^{-1}$) were detected in 2 Mediterranean spice mixes and certain salad mixes were also contaminated with significant levels of senecionine-type compounds. More than 85% of plant based food supplements contained PAs/PANOs, with levels up to 557,000 $ng.g^{-1}$. An original approach enabled us to demonstrate that only a part of the PAs/PANOs contamination is transferred during the brewing process of (herbal) teas (15-25%). This transfer rate contrasts with the conventional strategy consisting of applying a dilution factor to the PAs concentration found in the dry plant product to estimate the contamination level of the infusion.

Keywords: *pyrrolizidine alkaloids, transfer rate, teas, salads, spices*

Acknowledgement: *The research that yielded these results was funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT 14/10 PASFOOD.*

L68*
MULTIPLE HEART-CUTTING TWO DIMENSIONAL LIQUID CHROMATOGRAPHY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY OF PYRROLIZIDINE ALKALOIDS

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Pyrrolizidine alkaloids (PAs) and their corresponding *N*-oxides (PAs-ox) are genotoxic plant metabolites which can be present as unwanted contaminants in food products of herbal origin like tea and food supplements. PAs and PAs-ox come in a wide variety of molecular structures including many structural isomers. PAs and PAs-ox have distinct toxicities and, particularly relevant for the present work, differences in toxicity between isomers are observed. For toxicity assessment it is important to determine the composition of a sample and to resolve all isomeric PAs and PAs-ox, which is currently not possible in one liquid or gas chromatographic (LC or GC) run. In this study an online two dimensional liquid chromatography quadrupole time-of-flight mass spectrometry (2D-LC QToF-MS) method was developed to resolve isomeric PAs and PAs-ox. After comprehensive column and mobile phase selection a polar endcapped C_{18} column was used at pH 3 in the first dimension, and a cross-linked C_{18} column at pH 10 in the second dimension. Injection solvents, column IDs, flow rates and temperatures were carefully optimized. The method with column selection valve switching described in this study was able to resolve and visualize 20 individual PAs/PAs-ox (6 sets of isomers) in one 2D-LC QToF-MS run. Moreover, it was shown that all isomeric PAs/PAs-ox could be unambiguously annotated. The method was shown to be applicable for the determination and quantification of isomeric PAs/PAs-ox in plant extracts and could be easily extended to include other PAs and PAs-ox.

Keywords: *two dimensional liquid chromatography, isomeric pyrrolizidine alkaloids, multiple heart-cutting 2D-LC, mass spectrometry*

L69*
ERGOT AND ERGOT ALKALOID SCREENING METHODS BY PSPE-FLD AND HPTLC-FLD/MS

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Ergots (*Secale cornutum*) are the permanent form of the parasitic fungus *Claviceps purpurea* and are mainly growing on rye. The infestation with *Secale cornutum* is a serious problem because the ergots are responsible for toxic effects caused by ergot alkaloids. Despite the toxicity and the infestation of rye grain with ergots, at present there are no maximum limits established for ergot alkaloids in grain and grain-based food in the European Union.
However, limits for the total ergot alkaloid content should be defined soon. In this respect, the determination of ergot alkaloids as the sum is a meaningful new approach. Therefore, the planar solid phase extraction (pSPE) concept was applied for a rapid ergot alkaloid screening in rye, based on high-performance thin-layer chromatography (HPTLC). After extraction with acetonitrile/ammonium acetate buffer and liquid-liquid partition (LLE) in toluene, determination was performed in a single zone after chromatographic concentration. For selective and sensitive detection, the native fluorescence was scanned at UV 254/>400 nm. In addition, HPTLC–MS offers the identification and determination of the quantitative ratio of the alkaloids in a single mass spectrum [1].
Furthermore, a rapid screening method for the common lysergic acid moiety of all alkaloids was developed for rye flours by means of HPTLC. After sample extraction and LLE according to Oellig and Melde [1], ergot alkaloids were selectively transformed with a mixture of methanol and Superhydride solution. Released lysergic acid amide (LSA) from peptide ergot alkaloids and unaffected ergometrine were separated on HPTLC silica gel, and the fluorescence was scanned for selective determination of the total ergot alkaloids. For calibration, the LSA standard was simply obtained from ergocristine transformed to LSA under identical conditions. With LOD and LOQ far below the currently applied quality criterion limit for alkaloids in rye and near-100% recoveries, a reliable screening for the total ergot alkaloids was guaranteed [2].
Apart from the toxic ergot alkaloids (~0.08% of the ergot mass), ergot lipids are useful chemical markers for *Secale cornutum* impurities in cereal [3]; *Secale cornutum* comprises 10% of ricinoleic acid as the key component [3]. Therefore, a sensitive screening for the determination of ricinoleic acid in rye by HPTLC–FLD was developed as a rapid alternative to time-consuming GC–FID analyses. After lipid extraction, transesterification and SPE clean-up, the ricinoleic acid methyl ester was selectively derivatized with 2-naphthoyl chloride and separated by HPTLC. The enhanced fluorescence allowed quantitation far below the maximum level of 0.05% *Secale cornutum* [4].

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Keywords: *ergot alkaloids, ergots, screening, pSPE-FLD/MS, HPTLC-FLD*

L70
SCREENING FOR MARINE ICHTHYOTOXINS WITH DYNAMIC CLUSTER ANALYSIS: A UNIQUE APPROACH TO HPLC-TOF-MS DATA FILTERING FOR SULPHUR, CHLORINE, AND BROMINE CONTAINING COMPOUNDS

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Aquaculture is an essential portion of the food supply chain, particularly in light of depleting wild fish stocks in the world's oceans. Open water aquaculture facilities are an efficient means of maturing marine species for human consumption however, these open water settings are exposed to environmental conditions which can have adverse effects. This is particularly the case for farmed fish, which can be exposed to harmful (ichthyotoxic) algae blooms (HAB). To investigate the ichthyotoxic metabolites produced by a notorious HAB forming species of microalgae, *Prymnesium parvum*, an algorithm was developed to aid in the screening of crude extracts. This algorithm uses a unique approach to filtering data produced by high performance liquid chromatography (HPLC) coupled time-of-flight mass spectrometry (TOF-MS), which allows for the filtering of molecular features which contain A+2 elements. This method is unique in its ability to specifically filter HPLC-TOF-MS data for sulphur containing chemical features, and also allows for the screening of chlorine and bromine containing features across an unprecedentedly wide *m/z* range. Applying this algorithm across crude extracts of 10 strains of *P. parvum*, it was possible to rapidly distinguish the 16 previously reported [1], large (> 1800 Da), ichthyotoxic, chlorine containing polyketides of this species, known as prymnesins. In addition to this, more than 30 other previously unreported prymnesin-like molecular features were discovered across the 10 strains [2]. This method of analysis utilises isotope pattern variations in a unique way which allows for previously unattainable specificity, and allows for an algorithm which can be applied in a generalised automated way without the need of user defined compound specific parameters.

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Keywords: *A+2, marine toxins, high resolution, mass spectrometry*

Acknowledgement: *This study was funded by the Danish Council for Strategic Research through the project "HABFISH" (Project No. 0603-00449B). Agilent Technologies is acknowledged for the Thought Leader Donation of the 6545 UHPLC-QTOF.*

L71*
A COMBINED IN VITRO/IN SILICO PERSPECTIVE ON THE TOXICODYNAMIC OF TETRODOTOXIN AND ANALOGUES - A TOOL FOR SUPPORTING THE HAZARD IDENTIFICATION

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Tetrodotoxin (TTX) is a potent neurotoxin found in terrestrial and marine animals. It targets the voltage-gated sodium channels and it has caused many fatalities over the years. Historically, TTX has raised food safety concerns mainly in the Asian countries due to the consumption of the pufferfish-based delicacy *fugu*. However, today TTX is spreading in terms of geographical areas and TTX-bearer species threatening a broader range of consumers than before. For instance, bivalve mollusks and Pacific oysters harvested in Europe have been found contaminated by TTX, and some TTX-bearer species have been found adapted to the Mediterranean areas. Nonetheless, the toxicity of the TTX-related toxins group is not fully understood as: i) few of the structural analogues found with TTX have been assessed for toxicity; ii) the effects of human metabolism on TTX toxicodynamic and toxicokinetic are still understudied. Therefore, in the light of harmonizing the risk assessment with other natural foodborne toxicants, TTX toxicity has to be investigated following the group-based approach, assessing structural analogues and metabolites. However, the high costs in sourcing TTX analogues make practically unfeasible the wide-scale assessment using conventional experimental trials. Conversely, the toxicological assessment using a combined *in vitro/in silico* approach may succeed in extending data on compounds poorly affordable, hierarchizing them to focus experiments and supporting the early step of hazard identification.
In this framework, this work investigated the toxicodynamic of TTX, some main analogues and human metabolites by using a molecular modeling approach. The model dealt with the toxin-sodium channel interaction and it was trained and validated relying on the few toxicity data available for the TTX analogues so far. Then, their structure-activity relationship was analyzed providing the rationale of the toxicity found experimentally under a toxicodynamic perspective. Afterward, the model was challenged with additional TTX analogues never tested before (n=10) and putative human metabolites (n=13, including phase-I and phase II metabolites) to identify further forms possibly mediating a relevant toxic stimulus *in vivo*. While the TTX analogues appeared to be less toxic than TTX, four metabolites were found preserving a relevant toxicity, suggesting that human metabolism may partially fail in preventing the recognition of the biological target. Therefore, their actual formation in *in vivo*-like conditions was checked *in vitro* through LC-MS/MS analysis. Overall, our results indicated that some metabolites likely circulating *in vivo* might be involved in mediating the toxic stimulus in living organisms. This remarks the need for further investigating both the dynamic and kinetic aspects of TTX group toxicity to provide a more informed perspective for assessing the TTX-related risk.

Keywords: *tetrodotoxin, marine drugs, toxicodynamic, tetrodotoxin metabolites, in silico toxicity*

Acknowledgement: *We would like to acknowledge Prof. Pietro Cozzini for the kind access to all the facilities of the Molecular Modeling Laboratory (Department of Food and Drug, University of Parma, Italy).*

L72
HUMAN BIOMONITORING IN FOOD SAFETY RISK ASSESSMENT

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EFSA is an integral part of the EU's food safety system. Its mission is to contribute to the safety of the EU food and feed chain and to a high level of protection of human life and health (Regul. 178/2002). EFSA's core responsibilities are the delivery and communication of advice on general scientific assessment priorities, and the evaluation of food and feed products that require a safety assessment before they can be used on the EU market.
EFSA carries out risk assessments using the four steps in the paradigm of risk assessment: hazard identification, hazard characterisation, exposure assessment and risk characterisation. When considering exposure assessment, external exposure is most frequently used as an easily quantifiable estimate, based on levels of a given substances, such as a contaminant, pesticide, or micronutrient in food. A more pertinent quantification of exposure closer to the ultimate health effect can be achieved by using data from human biomonitoring.
EFSA has used human biomonitoring to inform its risk assessments, such as for bisphenol A, cadmium, lead, the mycotoxin deoxynivalenol (DON), and pesticides. EFSA recently commissioned a 'Review of human biomonitoring (HBM) and its application to exposure assessment for food safety' and a project on 'HBM data collection from occupational exposure to pesticides'.
Next steps for greater uptake of human biomonitoring into risk assessment for EFSA are: *Depending on biomarkers used, HBM data have been used in EFSA to "validate" dietary exposure estimates but also to detect health effects; HBM data are particularly important in exposure assessment and could play an important role in post market monitoring; Further development of biomarkers of exposure to monitor substances of interest for EFSA is needed; HBM data need to be combined with other data and tools for interpretation in risk assessment, such as information on dietary intake (FFQ, 24h recalls); Access to individual HBM data is needed, which requires a data format compatible with EFSA's format for chemical concentration and intake data.*
An appropriate data model comprises 3 components: the structure in which the data can be reported, the catalogues to describe the terms in a standardized way, and the business rules to check the validity of the reported data. Currently the Standard Sample Description template is already implemented for chemical contaminants, pesticide residues, food additives and food contact materials, whereas for veterinary drug residues implementation is in progress.
To improve its work, EFSA is closely following developments in human biomonitoring such as the HBM4EU project, a joint effort of 26 countries, the European Environment Agency and the EC (https://www.hbm4eu.eu) and the EU JPI on biomarkers (http://www.healthydietforhealthylife.eu).
Whereas it can improve risk assessment, any biomarker requires (scientific as well as analytical) validation, standardisation and harmonisation before it can be used.

Keywords: *EFSA, biomonitoring, biomarkers, food safety, risk assessment*

Acknowledgement: *Contributors to EFSA's outputs on biomarkers and biomonitoring, as published on the EFSA website, are greatly acknowledged.*

L73
CHARACTERIZATION OF EXPOSURE TO PESTICIDES THROUGH UNTARGETED INVESTIGATION OF THE URINARY EXPOSOME

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The presence of chemical contaminations in the environment can lead to human exposure to various molecules, which could be responsible for adverse effects. Generally, this exposure is estimated by indirect methods, and although biological monitoring allowed improving the exposure assessment, it usually concerns a limited number of compounds analyzed in a targeted way, whereas untargeted approaches should provide a wider and more relevant exposure assessment.
In this context, we developed an exposomics workflow, which was applied to the assessment of exposure to chemicals in a non-targeted way from Human cohort samples. Urine samples were analyzed using reversed phase LC coupled to ESI-HRMS (LTQ Orbitrap XL). Following the HRMS detection, data were processed in two ways: (i) validation of suspected metabolites by MS^n experiments using a list of known or putative metabolites generated from almost 100 representative pesticides, and statistical analyses based on identified metabolites, or (ii) statistical analyses of all features, and MS^n identification of discriminant metabolites (including metabolites of other xenobiotics).
A first example will illustrate the characterization of pesticide exposure of population groups differentiated by cereal crop surrounding their place of residence. A total of 490 compounds were screened, corresponding to pesticides usually used on cereals, and their known and putative metabolites. The negative ionization mode was the most informative due to the large proportion of conjugated metabolites present in urine. From HRMS data, more than 70 features matched the suspected compounds, among which about 30 could be confirmed by MS^n experiments. Since most compounds were not available as standards, metabolism studies were conducted on laboratory animals exposed to suspected pesticides, allowing the bio-synthesis of the suspected metabolites. Results show that the exposure to pesticides of the various population groups could be characterized according to their residence place.
The second example will focus on dietary exposure to pesticides through analysis of urine samples from organic or non-organic food consumers. From the 131 ions matching the theoretical *m/z* ratio of suspected pesticide metabolites, several compounds were successfully identified by MS^n experiments. At the same time, a non-targeted analysis has also been performed to identify unexpected metabolites that could differentiate the two population groups. The OSC-PLS-DA statistical analysis pointed out 242 features, corresponding to almost 100 xenobiotic metabolites, responsible for the separation of the two groups. Interestingly, by using this untargeted analysis, discriminating

metabolites did not only correspond to pesticides, but also to other chemicals that were not considered initially. These complementary results illustrate that the combination of untargeted and suspect screening approaches, constitutes an efficient tool for exposomics studies.

Keywords: *pesticides, exposome, mass spectrometry, urine*

Acknowledgement: *The authors acknowledge financial support from the EHESP School of Public Health, France in the context of N.B. PhD thesis and the French National Research Agency in the context of the BioNutriNet project (ANR-13-ALID-0001).*

L74
HUMAN BIOMONITORING TO ASSESS THE BODY BURDEN OF MINERAL OIL (MO)

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Mineral oils (MO) have important uses in a variety of food applications and in lubricating food processing and packaging machinery. The past few years significant public and regulatory concerns have been raised that exposure to MO through food, but also through the use of cosmetics, may pose a health risk. The 'MO' detected in food has been described by the acronyms MOSH (MO Saturated Hydrocarbons) and MOAH (MO Aromatic Hydrocarbons). These terms refer to chromatographic fractions used as indication of the presence of mineral hydrocarbons in food. MOSH has been linked to accumulation and inflammatory liver granulomas, while MOAH is suspected of being potentially carcinogenic. MOSH and MOAH fractions however do not distinguish MO or waxes approved for use in food contact applications from other hydrocarbons that may be true food contaminants.
An in-depth review of key studies on MO and waxes generated over the last 40 years including the 2017 recently published EFSA External Scientific Report allow to challenge the MOSH and MOAH hype.
Compositional and manufacturing data show that there are two types of MOAH. Carcinogenic MOAH are the 3-7 ring polycyclic aromatics which are effectively removed by refinement operations and controlled by current regulatory standards. The second MOAH type are mainly highly alkylated 1-2 ring structures that are biologically inactive but due to their molecular weight give the impression of high aromatic content in high viscosity products. As MOAH is unspecific to its composition, contamination of food should thus focus in controlling the 3-7 ring PCA.
Regarding MOSH, a review of past and recent data unequivocally demonstrates that liver epithelioid granuloma formation is a specific adverse response of the F344 rat strain to n-alkane exposure, which would include the n-alkanes ubiquitously present in fruit, vegetables and vegetable oils. The SD rat strain does not show this response. However, the F344 data is currently used for the risk assessment and setting of ADI's for MO (composed primarily of iso- and cycloalkanes) and waxes (n-alkanes). Moreover, F344 wax data interpretations are extrapolated to MO despite their compositional difference. These findings show that differences between the rat strains and the structural sub-classes of MOSH should be taken into account and that the SD rat should be the model of choice when establishing ADI's for this type of substances, especially because the formation of epithelioid granuloma after alkane exposure is not seen in humans. In addition, comparative toxicokinetics studies in F344 and SD rats and human volunteers show that although the elimination kinetics of MO are comparable, the uptake/retention is much less in SD rats and humans than in F344 rats. Further human biomonitoring data will facilitate the risk assessment of alkane fractions found in tissues (e.g. preferential retention) and the interpretation from data from toxicity tests on complex substances such as MO.

Keywords: *MOSH, MOAH, F344 rats, mineral hydrocarbons, ADI*

L75*
HUMAN PLASMA LIPIDOMICS AS A TOOL FOR BIOMONITORING OF ENVIRONMENTAL BURDEN ON MOTHERS AND NEWBORNS

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Lipidomics, a subgroup of metabolomics, focuses on a comprehensive characterization of a large set of low-mass lipid species present in an organism. Currently, it is a well-established tool in clinical analysis, enabling uncovering of potential biomarkers of diseases, number of studies have also employed lipidomics to assess the effect of dietary intervention. Rather surprisingly, until now, extensive lipidomics-based studies concerned with the impact of human exposure to environmental pollutants have not been performed.
Plasma represents one of the most suitable biological matrices for such types of studies, therefore, a lot attention has been paid by our group to finding out the optimal analytical approach for plasma analysis, with a special focus on a lipidome. Various strategies were tested, the most common, reverse phase ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-HRMS), was our first choice, since this technique is a gold standard when it comes to untargeted analysis of lipids. The other separation option, hydrophilic interaction liquid chromatography (HILIC) which allows for different mode of separation and is more beneficial when performing targeted lipidomics was tested, too. We also employed supercritical fluid chromatography (SFC) as it is capable to separate compounds on both non-polar and polar stationary phases. The latter feature actually outperforms HILIC in the analysis of lipids, as SFC is more compatible with highly non-polar compounds. Comparing SFC-HRMS and UHPLC-HRMS analysis, we could conclude, that these techniques provide almost equivalent results, the statistical analysis of the data gave the same patterns and same markers, even when using slightly different plasma extraction methods.
In the current study, we have used untargeted lipidomics as a part of a broader project focused on changes induced by genotoxic polycyclic aromatic hydrocarbons (PAHs) on the genome of newborns and their mothers from differently polluted localities. We also investigated whether lipidome was altered. For this purpose we decided to use UHPLC-HRMS method. Plasma samples obtained from almost 350 pairs of mothers and newborns were investigated. The generated data were subjected to multivariate statistics to search for specific patterns of compounds for sample groups, which were defined by the sampling locality and season. This enabled us to identify metabolites impacted by polluted environment during particular season. For example, one of the key compounds contributing to the separation of the two groups of newborns from polluted and unpolluted localities in winter, was hydroxy-eicosatetraenoic acid. The concentration of this metabolite was below the detection limit in plasma of newborns from South Bohemia, while its level was fairly higher in the plasma of newborns from Silesia. Higher oxidation stress in Silesia during the winter season might be the conceivable cause.

Keywords: *lipidomics, mass spectrometry, supercritical fluid chromatography, ultra-high performance liquid chromatography, environment pollution*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015).*

L76*
EVALUATION OF THE HAIR ANALYSIS IN HUMAN BIOMONITORING STUDIES

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Human biomonitoring is a useful tool for the assessment of a population´s exposure to environmental contaminants by directly measuring substances or their metabolites. Blood is commonly considered as an ideal matrix because being in contact with different organs and tissues. Nevertheless, its collection is invasive connected with several limitations (for example pain, more difficult sampling for children, the elderly, or chronically ill people). From this reason, the interest in non-invasive samples represented by hair is increasing. Several advantages are associated with this matrix, such as its easiness of sampling and can be stored for a longer time without loss of properties. Moreover, hair can provide information about short-term and long-term exposure (months or even years), which is not always possible for biological fluids (blood, plasma, urine).
One of the most recurrent criticisms concerning hair analysis lies in the possibility of external deposition of chemicals on the hair surface. Although the biological mechanisms of substances incorporation in hair are still debated, it is admitted that they are mainly incorporated from blood into living cells in the hair bulb while external contamination is likely to remain on the surface of hair scales (i.e. cuticle). Nevertheless, no standardized "universal" procedure for the decontamination of hair before analysis has been set up to date and in most studies the individual approaches are selected with the respect to target analytes.
The main aim of the present study was to implement and validate the multi-analyte methods enabling quantitative analysis of multiple contaminants (pesticides residues, per- and polyfluorinated compounds, metabolites of phthalates and monohydroxylated metabolites of polycyclic aromatic hydrocarbons) occurring in hairs. For analysis, ultra-high performance liquid chromatography coupled to tandem mass spectrometry (MS/MS) with triple quadrupole mass analyser was used, for non-target fingerprinting, high resolution mass spectrometry (HRMS) was employed. Extensive experiments focused on a hair sample preparation prior to target / non-target analysis were realized, special attention was paid to a selection of suitable solvent for a washing step aimed the removal of possible external contamination. Fingerprints of small molecules (less than 1,200 Da) isolated from hair strands by washing solvents were compared with those obtained from the analysis of extracted disintegrated washed hairs with the aim to assess extractability of various substances, when not only the major components representing by lipids, but also some minor compounds (contaminants, steroids) were screened. The results obtained in this study not only documented how complicated matrix hairs are when considered for biomonitoring study, but also suggestions for further challenging research in the field of sample preparation were addressed.

Keywords: *hair, human biomonitoring, high resolution mass spectrometry, liquid chromatography*
Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). „Financial support from specific university research (MSMT No 20-SVV/2017)" is also acknowledged.*

L77
SMARTPHONE ANALYZERS FOR ON-SITE TESTING OF FOOD QUALITY AND SAFETY

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Imagine how many random and suspect samples are being taken for food quality and safety testing within the European Union (EU): millions each year again and again. Typically, all these samples are taken on-site at farms, slaughterhouses, border inspection points, retail shops, etc., documented, transported to a control laboratory, screened for target substances such as food contaminants and drug residues, and finally the few suspects from the screening methods have to be confirmed by validated instrumental methods in order to declare the sample non-compliant or compliant. Despite all these efforts, we are still facing frequent food incidents and fraud issues. A paradigm shift in food quality and safety testing is required in order to free resources for an intensified combat against fraud in the food chain. As an enabling technology solution to the problem, the H2020 project FoodSmartphone proposes the development of smartphone-based (bio)analytical sensing and diagnostic tools, for simplified on-site pre-screening of quality and safety parameters and wireless data transfer to servers of relevant stakeholders. Bioanalytical chemists, biologists, physicists, micro/nanotech engineers, mathematicians, organic- and food chemists will work together on the joint supra-disciplinary goal. FoodSmartphone is a so-called Marie-Curie training network in which 11 PhD students work on individual research projects in order to achieve the following overall objectives:
(1) To study the supra-disciplinary challenge of smartphone-based analysis systems having advanced biorecognition, signal transduction, microfluidic sample handling and image data handling solutions.
(2) To develop user-friendly, rapid integrated sample preparation and smartphone-compatible Apps, to ultimately ensure adequate field implementation for both professionals and future Citizen Science.
(3) To develop a unique range of smartphone-based on-site screening demonstrators for food quality and safety issues of concern, viz. for pesticides, allergens, mycotoxins, food spoilage organisms and marine toxins.
(4) To deliver, through high level training, a group of multidisciplinary scientists who can integrate (bio)analytical chemistry, physics, micro-engineering and ICT knowledge into a common supra-disciplinary goal, to combat major socio-economic challenges, such as maintaining a healthy, safe and fair food supply.
(5) To substantially improve the career prospects of early-stage researchers across academia, public research institutes and private industry sectors, including SMEs.
The scientific training in novel smartphone-based technologies plus the complementary skills training provided, is expected to have a major impact on future EU monitoring practices and, moreover, pave the road for Citizen Science. For more information and/or signing-up in our stakeholder database:
Website: www.foodsmartphone.eu
Blogs: www.foodsmartphone.blog
Twitter: @foodsmartphone

Keywords: *food testing, smartphone, diagnostics, on-site monitoring, biorecognition*
Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325.*

L78
PHASMAFOOD: A FOOD SENSING DEVICE AND AN OPEN SOFTWARE ARCHITECTURE DELIVERING FOOD-TECH INNOVATION

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PhasmaFOOD is an EU collaborative R&D project funded by the Horizon 2020 Programme. It aims at delivering a miniaturized multi-sensor optical sensing device for the detection of food safety threats such as food spoilage, adulteration and aflatoxins. The system is based on heterogeneous visible and near infrared spectroscopy technologies and is supported by a software architecture that delivers fast characterisation of foods, encompassing an extendable framework for the deployment of smart chemometric algorithms, data fusion strategies and reference laboratory measurements. The built-in algorithms address data mining and data analysis methods from non-destructive, non-invasive instruments and are independent of the food type and food-tech application. We argue that the framework proposed by PhasmaFOOD can lead to new forms of food-tech applications and growth, especially in the rural regions of Europe where the technological and broadband penetration is still low, while the economic growth coming from food value-chains is rapidly growing.
One example application is aflatoxin detection and is already been developed by the PhasmaFOOD consortium. This application has significant impact in the supply chain of agricultural products such as corn and grains. Governments and private businesses are spending considerable amounts of money to meet food safety criteria enforced by the law. Other food safety applications with substantial economic development opportunities include: fruits and vegetables; livestock and fish productions; milk production; and, edible oils.
PhasmaFOOD is addressing a market where similar devices are already positioned and customers and the general public are increasingly becoming familiar with food sensing technologies. Strong R&D and product-driven development are important strengths of PhasmaFOOD and the consortium is capitalising upon them seeking synergies with similar development efforts. In addition, rapid exploitation opportunities are pursued via pilot applications addressing niche food markets.

Keywords: *food sensing, food safety, food-tech innovation*

L79
NANOARRAYS FOR FOOD DIAGNOSTICS

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Contaminant monitoring from microbiological, chemical and fraudulent sources in agri-food production is an important yet complex issue. A huge investment in time and effort is placed on these activities by regulatory and industrial laboratories. Although sophisticated techniques such as chromatography and spectrometry provide accurate and conclusive results, screening tests allow a much higher low cost throughput of samples with less operator training. Biosensors combine a biological recognition element with a transducer to produce a measurable signal proportional to the extent of interaction between the recognition element and contaminant. Different uses of biosensing instrumentation available are extremely varied, with agri-food analysis an emerging and growing application. The advantages offered by biosensors over traditional immunoassay screening methods with respect to food analysis, include automation, improved reproducibility, speed and real time analysis. The miniaturisation of immunoassays and biosensors towards nanosensing offers not only enhanced sensitivity but portability and multiplexing capabilities. As fresh demands from consumers and regulators grow to improve the integrity of food the need for improved smart nano-technologies has never been greater. Progress has been made to the development and validation of nanoarrays that can detect both single and multiple contaminants in food samples to offer a holistic approach to agri-food safety. ELISA spot and planar waveguide technologies have been developed for the rapid and multiplex analysis of marine biotoxins, antibiotic resudes and mycotoxins to be compatible with food control procedures.

Keywords: *nanoarray, chemical contaminants, mycotoxin, marine toxins, antibiotic residues*

L80
NIR SPECTROSCOPY IN FOOD SENSING – A COMBINED SENSING APPROACH

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The increasing issues of food fraud and food hazards in a globalized economy and a general trend towards the control of our environment by individuals have brought about a growing demand for devices which are able to measure the food quality that is relevant to the end user. With the first hardware solutions already in the market, there is yet room to improve the stability and informative value of such measurements. Existing devices have reached a small community of explorative-minded users but not the broad public, whose acceptance requires a higher degree of reliability of the measurements. Currently, information about the food is gained via near-infrared (NIR) spectroscopy in various limited wavelength ranges. PhasmaFOOD is a new EU-funded collaboration (H2020 no. 732541) which sets out to widen the data basis on which decisions about food safety are made and to open up more dimensions of data collection. In its current initial phase, the PhasmaFOOD project combines several sensing methods into a single, portable device, to be used for on-the-spot food quality sensing.
Fraunhofer IPMS, with a longstanding experience in miniaturization and integration of optical detection technology, is part of the PhasmaFOOD consortium. We present early stage insights into the development of the PhasmaFOOD prototype as well as first measurements and analysis. The PhasmaFOOD device includes NIR spectroscopy over a broad spectral range of 1000 to 1900 nm. With this NIR spectrometer, we integrate UV-visible (UV-vis) spectroscopy as well as a vis camera, in order to supplement NIR data but also as sensors with their own tasks and roles. Miniaturized components have recently become available for all sensors used here, driven by integration needs which come from outside the food sensing market. We give an overview of the state of the art in miniaturized optical spectrometers, in particular NIR, and discuss the optical requirements for their combined use. In combination, these sensors will enable a multidimensional view at food quality and safety. Here, our system development is guided by function. Therefore, the first measurements span three distinct use cases which are used to test the equipment. Particular focus lies on the detection of food hazards in nuts, spoilage of vegetables and fish and food fraud in oils. The validation of the basic spectroscopic functionality in these cases sets the fundament for all later work such as developing algorithms for a combined bio-chemical data analysis and transforming the results into clear and understandable information for end users. Finally, we will look out to the future of the PhasmaFOOD system and illustrate our visions for a commercial food sensing device.

Acknowledgement: *This work has received funding from the EU-H2020 project PhasmaFOOD under grant agreement No. 732541.*

L81*
GOLD NANOPARTICLE NANO-SENSORS FOR THE DETERMINATION OF PATHOGENIC DNA AND ENZYME BIOMARKERS

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Nano-sensor development has become an integral part of food safety research due to the need for highly sensitive, specific, simple, fast, on-site and miniaturised systems. These features overcome the limitations of conventional techniques such as polymerase chain reaction, enzyme-linked immunosorbent assay and plate culturing. Gold nanoparticles (AuNPs) have emerged as an excellent candidate in nano-sensor design owing to their unique optical and catalytic properties. Herein, we present two nano-sensors utilising these unique properties for the detection of pathogenic DNA derived from *Campylobacter jejuni*,[1] an important foodborne pathogen, and enzyme biomarkers, *i.e.* protease and lipase [2] which are important indicators of human and animal health as well as food safety.

The first approach exploits the localised surface plasmon resonance of AuNPs, resulting in the ultrasensitive detection of *C. jejuni* DNA. The method utilizes RNA-functionalized AuNPs which form DNA-RNA heteroduplex structures through specific hybridization with target DNA. Aggregation is controlled by an endonuclease enzyme which specifically cleaves the RNA within the heteroduplex structure, resulting in aggregation of AuNPs upon exposure to a high electrolytic medium. Target DNA is detected visually (pM level) and spectroscopically in 3 hr (40.7 fM and 2.45 fM as measured by UV-vis and dynamic light scattering (DLS), respectively). The application to a food matrix also successfully detected target DNA at a concentration of 1.2 pM (by UV-vis) or 18.0 fM (by DLS).

The catalytic activity of AuNPs was also investigated for the development of nano-sensors to detect protease and lipase biomarkers. AuNPs can catalyse for the oxidation of 3,3',5'5-tetramethylbenzidine (TMB) in the presence of H_2O_2, however we found for the first time when the AuNP surface is coated with casein or Tween 20, this intrinsic peroxidase-mimicking activity is supressed by up to 87.4%. In the presence of protease or lipase, the enzymes bind and catalyse the degradation of their respective coating layers, resulting in the recovery of catalytic activity. This is shown visually (blue coloured product) or spectroscopically as an increase in absorbance at 370 nm and 650 nm. This mechanism allows the detection of protease and lipase at 51.6 ng/mL and 22.1 ng/mL, respectively, using their corresponding substrate-coated AuNPs in 90 min. The limit of detection for protease spiked in ultra-heat treated milk and synthetic urine was 1.4 µg/mL and 0.15 µg/mL, showing great potential in food safety and clinical diagnostics.

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Keywords: *gold nanoparticles, DNA detection, aggregation, peroxidase-mimicking, enzyme biomarkers*
Acknowledgement: *The author C.M. and N.L. thank the PhD studentship support from the Department of Employment and Learning for Northern Ireland (DEL); C.C. thanks the strong support from the Central Research Support Funds of Queen's University Belfast via a start-up grant.*

L82*
DETECTION OF FOOD SPOILAGE USING SPECTROSCOPY- AND MULTISPECTRAL IMAGING-BASED SENSORS

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The development and application of "smart" sensors, allowing for food spoilage detection based on rapid analytical technologies, are expected to be of great value throughout the food supply chain. In this framework, the aim of the present work was the evaluation of spectroscopy and image analysis methods as means of detecting spoilage of fish and fresh produce. Aquacultured whole ungutted sea bream (*Sparus aurata* L.) samples were stored aerobically under controlled isothermal conditions (0, 4 and 8°C) for a maximum time period of 11 days. Moreover, ready-to-eat fresh rocket (*Eruca sativa* L.) salad samples were stored in their original packaging under both isothermal (4, 8 and 12°C) and dynamic temperature (periodic temperature changes from 4 to 12°C) conditions for a maximum time period of 12 days. At regular time intervals during storage, duplicate fish/rocket samples were analysed using (i) conventional microbiological analyses, and (ii) rapid analytical methods based on spectroscopy (Fourier transform infrared spectroscopy as well as spectroscopy in the near-infrared and/or visible spectral regions) and on multispectral imaging. The selected data were used for the training and validation of appropriate models using spectral/imaging data as input variables and total mesophilic microbial populations as output variables. As demonstrated by the results of this study, both spectroscopy- and multispectral imaging-based techniques appear to be promising for the rapid and non-invasive detection of the microbial spoilage of both animal and plant origin perishable foods.

Keywords: *food spoilage, spectroscopy, image analysis, sensors*

Acknowledgement: *This work has been supported by the project "PhasmaFOOD", funded from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 732541.*

L83
SIZE CHARACTERIZATION OF PARTICULATE FOOD ADDITIVES/NUTRIENT SOURCES AND THEIR NANOFRACTIONS BY SINGLE PARTICLE ICP-MS

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Thorough characterisation of nanomaterials is essential for establishing their physicochemical identity and for assessing their functional properties and potential toxicological hazards, which are both dependent on physicochemical characteristics.
Inorganic nanomaterials (metals, their compounds, and oxides) – e.g. Ag, SiO_2, TiO_2, ZnO, TiN, Fe oxides/hydroxides, nanoclays – are a prominent class of nano-sized materials with a number of current or projected applications in the agri-food sector. They include use as food additives, nutrient sources, antibacterial agents, additives for food packaging. It is thus essential to have analytical methods available to characterize them as such ('pristine materials'), and in food.
State-of-the-art mass spectrometric techniques for the analytical determination of inorganic nanoparticles in dispersion and (after proper sample preparation) in complex matrices have recently become available. Being based on atomic mass spectrometric, they are element-specific (i.e. provide information on the chemical identity) and have the potential to measure size, size distribution, number and mass concentration of particles, and concentration of the dissolved fraction (if present). Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) is based on time resolved analysis of diluted nanoparticle dispersions using short dwell times (≤10 ms). Each particle gives rise to a signal clearly distinguishable from random background noise and, by means of appropriate algorithms, signal frequency distributions are converted into size frequency distributions. In principle, the signal arising from ionic (i.e. soluble) forms of the element constituting the particles, if any, can be distinguished from that due to the presence of the particles themselves. Therefore, sp-ICP-MS is a particle-specific technique with sizing capability.
We describe the size characterization of several currently used food additives and nutrient sources, which are composed by small particles (sub-micron range), including a variable fraction of nano-sized particles (<100 nm), by sp-ICP-MS. The materials investigated include anatase and rutile TiO_2 (food colours, E171), yellow iron oxide (FeO(OH)) and red iron oxide (Fe_2O_3) (food colours, E172), and zinc oxide (a source of zinc in food supplements).
The pristine materials were studied after dispersion with a standardized protocol and were all found to contain an appreciable fraction of nanoparticles. Particle size and morphology were independently characterized by electron microscopy. The same materials were then determined by sp-ICP-MS in a number of food products and supplements, with a range of different compositions. The selection of sample preparation approaches for analysing real-world food samples and of operating parameters for obtaining accurate number-based size distribution with low size LoDs are discussed.

Keywords: *nanoparticles, food additives, nutrient sources, physicochemical characterisation, single particle inductively coupled plasma mass spectrometry*

L84
DEVELOPMENT OF A METROLOGICALLY VALIDATED SEM BASED METHOD TO CHARACTERIZE NANOPARTICLES SIZE: APPLICATION TO DIFFERENTS ADDITIVES UNDER THE NANOFORM CONTAINED IN FOOD AND COSMETICS PRODUCTS

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Nanomaterials containing commercial products are already part of our everyday life, as for example in the food or cosmetics sectors. Even if clarifications are still awaited today regarding what kind of substance has to be qualified as a nanomaterial from a regulatory perspective, reliable characterization of main nanomaterials parameters as described in ISO/TR 13014/2012 appears as crucial to support decision-making in very different aeras. Indeed optimization of products performances and production process, quality control procedures, risk assessment studies and enforcement of the regulatory framework (labelling) depend mainly on objective and comparable measurement data. Among the different nanomaterial's parameters to be measured (size, size distribution, shape, aggregation/ agglomeration, charge, surface, chemical composition…), dimensional measurement appear as the most critical one, while determining nanomaterials size with an appropriate accuracy remains today a real challenge. Indeed a large set of analytical techniques are available on the market (DLS, PTA, A4F, sp ICP-MS, AFM, SEM, TEM…), each of them having its own advantages and limitations. Electron microscopy appears however as the gold standard technique because it allows to access primary particles information without any ambiguity in most cases and is therefore consider as a confirmatory technique in the tiered approach supported by the NanoDefine consortium.
LNE developed over the last years a hybrid approach based on the combination of SEM (Scanning Electron Microscopy) and AFM (Atomic Force Microscopy) instruments to obtain reliable and traceable results on (nano)particles size characterization. Each step of the measurement process has been optimized and validated on reference samples (FD304, NIST RM8013…) to control analytical bias:
- Extraction of particles from consumer products (food or cosmetics),
- Sample preparation based on the use of a spin-coater apparatus and specific conditions (pH…) in order to reduce agglomeration of (nano)particles,
- Calibration/instrument qualification to obtain the metrological traceability and assess measurement uncertainties,
- Data acquisition (SEM voltage…),
- Raw data processing with the development of dedicated software (PLATYPUS / Pollen Metrology) based on artificial intelligence and pattern recognition to increase statistical counting.

A large set of food (candy, cookie, ice-cream, aroma…) and cosmetics products (toothpaste, sunscreen, mascara…) have been analysed by applying the methodology developed for SEM. In some cases, pure additive before its introduction in the consumer product was available. Its characterization gave us useful data to validate the robustness of the sample preparation step and shows that food matrix doesn't have any significant impact on the results when measurements uncertainties are taken into account. Main findings on TiO_2, SiO_2 and other additives (Fe_2O_3, Ag...) will be presented.

Keywords: *SEM, nanometrology measurement, process reliability, primary particles*

L85
ENGINEERED NANOMATERIALS IN DRINKING WATER SOURCES

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Nano-enabled consumer products are a growing market over the last decade, leading to advances in science and engineering, and to releases of engineered nanomaterials (ENM) to the environment including potential drinking water sources. A number of studies have already shown the release of nanoparticles from commercial products. Predicted environmental concentrations have been calculated but there is a need for real measurement data to validate these calculations. However, the detection of engineered nanoparticles in environmental matrices is challenging because of the low predicted environmental concentrations, which are in the ng/L range, compared to the much higher natural background of minerals. In this study nanosized Ag, CeO_2 and TiO_2 have been measured in multiple surface water samples collected along the rivers Meuse and IJssel in the Netherlands using single-particle ICP-MS as measurement technique. Validation of the analytical method showed its capability to quantitatively determine nanoparticles at low concentrations. The results of the study confirm the presence of nano-sized Ag and CeO_2 particles in surface waters in the low ng/L range. Micro-sized TiO_2 particles were also identified in surface waters in concentrations in the µg/L range. Conventional drinking water treatment processes remove sub-micron particles, including natural nanomaterials (e.g. viruses), however, the novel shape, configurations and properties of ENMs may enable their persistence. The presentation reviews the knowledge of ENM production and release and reports on their detection and concentrations in aquatic environments and in drinking water.

Keywords: *nanoparticles, environment, surface water, drinking water*

L86
DETECTION OF LEAD NANOPARTICLES IN GAME MEAT BY SINGLE PARTICLE ICP-MS FOLLOWING USE OF LEAD-CONTAINING BULLETS

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Lead (Pb) is a toxic metal that accumulates in the body and seriously affects the developing central nervous system in young children, but may also induce *e.g.* negative cardiovascular effects and nephrotoxicity in adults [1]. Today, the consensus is that there is no safe level of Pb, especially for infants, children and the fetuses of pregnant women [2]. While game meat constitutes a very small part of the diet for average citizens, game meat can be a major food commodity and therefore source of Pb exposure for high consumers. This is because game meat may contain very high levels of Pb in the form of fragments from the Pb core bullet used to cull the game. As a result, consumption of game meat has been associated with increased Pb levels in blood [3], even though the reported bio-accessibility of the ingested fragments is low [4]. During the penetration of the game body, expanding Pb core bullets typically release hundreds or thousands of fragments of varying size that can be visualized using X-rays [5]. In the process, a significant part of the kinetic energy of the bullet is converted to plastic deformation and heating, causing some of the exposed Pb to melt on or even vaporize from the surface of the expanding bullet and its fragments [5]. Potentially, upon cooling, this may cause Pb particles to be formed through re-solidification, some of which are too small to be detected by X-ray techniques.
Here, we investigate the prevalence and size distribution of Pb nanoparticles (PbNPs) from ammunition in game meat, using inductively coupled plasma-mass spectrometry in single particle mode (spICP-MS) and a modified version of an earlier described enzymatic digestion method [6]. Close to the wound channel, PbNPs in the size range of 40 to 750 nm were detected with a median diameter of around 60 nm. The particle mass concentration ranged from 290 to 340 ng/g meat and the particle number concentrations from 27 to 50 million particles/g meat. The size limit of detection (LOD) strongly depended on the level of dissolved Pb and was in the range of 40 to 80 nm. In game meat sampled more than 10 cm away from the wound channel, no PbNPs above the size LOD were detected. To the best of our knowledge, this is the first time that the occurrence of nanometer-sized Pb particles in bullet-shot game is reported. An improvement of the method towards lower size LODs will be required to assure that no smaller PbNPs are present in game meat intended for consumption. This is of particular importance since it is well known that some hunters also consume the meat close the wound channel.
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Keywords: *lead nanoparticles, single particle ICP-MS, lead ammunition, game, enzymatic digestion*

Acknowledgement: *We thank Erik H. Larsen and Marianne Hansen for their assistance in planning and performing the work.*

L87*
DETECTION OF NANOPLASTICS IN FOOD BY ASYMMETRIC FLOW FIELD-FLOW FRACTIONATION COUPLED TO MULTI-ANGLE LIGHT SCATTERING: POSSIBILITIES, CHALLENGES AND ANALYTICAL LIMITATIONS

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Micro- and potentially also nanoplastic particles are increasingly found in marine products but also other food items like honey as a result of the widespread pollution with plastics. Health effects of these particles are largely unknown, but may be attributed to both/either the particles and/or chemicals adsorbed onto the particle surface. A considerable number of methods are available for identification and quantification of microplastics in food and occurrence data is available. In contrast to microplastics, no methods have been developed yet for monitoring the occurrence of nanoplastics in food and hence we cannot estimate the human exposure.
Asymmetric flow field flow fractionation (AF4) coupled to different online detectors is a powerful method for detection and characterisation of nanoparticles in complex matrices such as food. In order to test the suitability of AF4 coupled to multi-angle light scattering (MALS) for detection of nano-sized plastics in food, polystyrene nanoparticles (PSNPs) of known size (100 nm) and mass concentration were spiked to homogenized fish fillet. Two sample preparation strategies were tested in order to remove the organic food matrix: acid digestion with nitric acid/hydrogen peroxide and an enzymatic digestion using Proteinase K. Both procedures were found suitable for degradation of the organic matrix. However, the acid digestion procedure resulted in considerable aggregation/agglomeration of the PSNPs. The presence of large particulates (> 1 µm) was not observed after enzymatic digestion, and consequently it was chosen as a sample preparation method.
The results demonstrated that it is possible to separate PSNPs from the enzymatically digested fish matrix. No elevated light scattering signal was obtained for blank fish fillet samples. The eluting particles could be easily detected by following their light scattering signal (90° angle MALS signal) with a limit of detection (LOD) of 5–6 µg (PSNPs)/g (fish fillet) which corresponds to ~1^{10} particles/g of fish. The fractograms obtained for PSNPs spiked to fish were similar to the ones of pristine PSNPs. The root mean square (rms) diameter values based on MALS were of 80-90 nm for the main elution peak (in agreement with the ratio of rms and geometric diameters for a solid sphere of 0.77). This confirmed that the sample preparation did not affect the particles. We expect that the LOD can be decreased to a minimum of 250-350 µg/g by increasing the injection volume. This LOD is specific for 100 nm PSNPs, as the intensity of scattered light decreases with decreasing diameter of the particles to the power of six.
The next steps will be to test the developed AF4-MALS method for the detection of a different type of nanoplastics (e.g. polyethylene) and for the screening of nanoplastics in real food items. The talk will also describe the challenges and limitations related to this methodology, namely quantification and identification of nanoplastics.

Keywords: *nanoplastics, asymmetric flow field-flow fractionation, nanoparticles, multi-angle light scattering, enzymatic digestion*

L88
SIMPLE HIGH-THROUGHPUT SCREENING OF TRACE ORGANIC CONTAMINANTS IN FOOD MATRICES BY HS-SPME AMBIENT MASS SPECTROMETRY

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Food safety, which includes the monitoring of a diverse range of food contaminants, often present at trace levels but significant with respect to human consumption, is a topic of worldwide concern. Mass spectrometry is a very powerful technique to analyze a broad range of compounds with very high sensitivity and selectivity. However, especially for complex matrices such as food, sample preparation steps often represent the bottleneck, as they can be quite tedious and time-consuming. Ambient mass spectrometric techniques were developed to overcome this drawback, allowing a fast and direct analysis, with minimal or no sample preparation. Due to these obvious benefits with respect to more established methods, ambient mass spectrometry has established itself as a competitive, or even superior technique for a variety of applications in numerous areas. This approach has also gained significant interest in the field of food analysis, which can enormously benefit from a faster analysis, requiring minimal sample preparation.
We here present a simple and high-throughput screening method for organic residues in food matrices by directly coupling a very efficient dielectric barrier discharge (DBD) ambient ionization source (developed and optimized in our research group [1]) to solid-phase microextraction (SPME). The versatility and sensitivity of our source and method were previously demonstrated, for example for the sub-pg/mL detection of pesticides in water samples [2]. The enrichment of compounds of interest, as well as the omission of chromatographic separation allows us to achieve a very simple, sensitive, and high-throughput analysis. This approach was here applied to a fully automated headspace (HS)-SPME analysis of organic trace contaminants in food matrices. Primary HS-SPME conditions (e.g., extraction time and temperature, fiber coating, water content, etc.) were optimized, and HS extraction results were compared to solid-liquid extraction. Results show that our method is successful at quantifying trace levels of organic residues in food matrices. For example, organophosphate pesticides (e.g., sulfotep, phorate, and thionazin) can be detected in fruits (e.g., apples, apricots, prunes) in the low µg/L range with virtually no sample preparation and a total analysis time of less than 20 minutes per sample. The simplicity, sensitivity, and speed of this method underline its great potential towards the application to food residue screening.

[1] Maryia M. Nudnova, Liang Zhu, Renato Zenobi, Rapid Communications in Mass Spectrometry, 2012, 26, 1447-1452.
[2] Mario F. Mirabelli, Jan-Christoph Wolf, and Renato Zenobi, Analytical Chemistry, 2016, 88, 7252-7258.

Keywords: *SPME, ambient mass spectrometry, high-throughput*

Acknowledgement: *The authors would like to acknowledge CTC Analytics and Plasmion GmbH for the support provided.*

L89
COMPARATIVE UNTARGETED HR-MS APPROACH FOR SALMON SALAR AUTHENTICATION

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In the present communication, the potential of untargeted HR-MS analysis for fish authenticity and traceability will be presented. *Salmon salar* discrimination based on geographic origin and farming condition was accomplished by untargeted HR-MS analysis of both the polar and non-polar fraction of salmon fillet extracts on Orbitrap-based mass spectrometers. In particular, two different approaches will be presented the first based on Direct Analysis in Real Time (DART) MS and the second based on conventional LC-MS acquisitions, full scan HR-MS acquisitions were performed in both positive and negative modes. As for DART-HR-MS analysis, the recorded spectral intensities were pre-treated and the resulting peak lists intensities of detected ions were used for statistical evaluation by principal component analysis (PCA) of the discriminating potential of the untargeted approach in terms of Salmon geographical origin and farming condition.
As for LC-HRMS analysis, the gathered spectra were processed via a commercial software Compound Discoverer 2.0 in order to identify the main discriminant peaks with an accuracy ≤ 2ppm. The study was carried out by setting two grouping factors either geographic origin or farming condition and data were pretreated by means of the retention time alignment of extracted chromatograms and background subtraction. Volcano plots combining the statistical significance (p-value from ANOVA model) of the identified compounds in discriminating the different groups and magnitude of change in the extracted peak areas were investigated in order to highlight a sub-set of compounds as potential markers for salmon authenticity/traceability. Edited compounds list constrained by means of p-value thresholds were subjected to further statistical evaluation by PCA in order to reducing the number of variable explaining most of the variance among groups.
A critical comparison of the two HR-MS approaches will be provided highlighting the potential and the limitation of each approach.

Acknowledgement: *The research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688. The equipment used in this work was funded by the project BioNet – PTP*

L90
AMBIENT IONIZATION FOR DIRECT FOOD ANALYSIS BY (TRANS)PORTABLE MASS SPECTROMETRY

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In the near future detection of residues and contaminants in food will, next to the laboratory, also be performed on-site at the farm, in industry and retail or even by consumers at home. This type of analysis requires different equipment than the high-tech facilities operated by skilled technicians in our laboratories. For example, for mass spectrometry (MS) we will need (trans)portable MS, elimination of sample clean-up, simplified sample introduction and data evaluation. Sample introduction can in theory be replaced by one of the (currently seventy) described Ambient Ionization MS (AIMS) techniques. In this research, three different portable AIMS techniques have been critically investigated for their applicability towards the analysis of pesticides, β-agonists, steroids, toxins, analgesics and antibiotics in various food matrices. The three AIMS techniques explored are Matrix Assisted Ionization (MAI), Desorption Atmospheric Pressure Chemical Ionization (DAPCI) and ID-CUBE Direct Analysis in Real Time (DART).
First, MAI is one of the most simple ionization options since no high voltage, lasers or gas supplies are needed. With MAI the sample or extract is mixed with a matrix. This mixture is tapped on the opening of the heated inlet of the MS, the mixture is transferred to the inlet where vacuum ionization occurs. Secondly, a handheld battery powered DAPCI device using ambient air was used to ionize analytes directly from a sample surface. DAPCI uses a high direct current voltage on a needle to generate a corona discharge and thereby are gas-phase reagent ions formed which are pneumatically transferred to the surface of the sample where the analytes are desorbed and ionized. Third, a relative simple DART setup was also tested, a modified version of the discontinued ID-CUBE. With this technique the sample is transferred onto a metal mesh sample card with the size of a credit card. The card is placed into the ion source where the metal mesh is heated resulting in desorbtion of the analytes. Ionization of the analytes occur through a metastable helium gas flow generate by the small and simple DART device, so obviously for on-site applications a small helium cylinder would be required. All three devices mentioned are coupled to various conventional mass spectrometers and also to a compact transportable single quadrupole MS to demonstrate its applicability in on-site testing.
All tested devices were able to successfully ionize some of the tested analytes in various food commodities. A general drawbacks of these ambient ionization techniques is the poor reproducibility, but isotope dilution of the sample may compensate that. Detection limits will be much higher when compared to the conventional MS facilities so applications will be limited to substances having relatively high maximum limits values. However, for some applications the ambient ionization with (trans)portable mass spectrometry can work for already direct analysis of food products.

Keywords: *AIMS-MS, ambient ionization, on-site detection, direct food analysis*

L91
RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY FOR HIGH-THROUGHPUT SCREENING OF RACTOPAMINE IN MEAT PRODUCING ANIMALS

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Ractopamine is a β-agonist substance that may be used in some parts of the world as growth promoter in livestock, although forbidden in a number of countries. Rapid evaporative ionization mass spectrometry (REIMS) was explored for the rapid detection of meat samples from treated livestock. In this context, the combination of REIMS with untargeted metabolomics workflow was investigated to identify carcasses from pig treated animals on the basis of a modification of indirect metabolites profile. REIMS strategy generated specific lipidic fingerprint patterns which enabled differentiating meat samples collected from pigs fed with ractopamine. Furthermore, the strategy was found successful when tested on different muscle types (loin, shoulder and ham), which further expand its applicability. Classification performances were 0% false negative and 10% false positive, which fully answers requirements of a screening strategy. This research indicates that REIMS implemented in an untargeted-metabolomics workflow can be considered as a high-throughput and powerful strategy for real-time meat classification in relation to ractopamine treatment in pig. This approach may subsequently be implemented as rapid screening test, at the slaughterhouse or at boarder inspection points, to detect such practice.

Keywords: *ractopamine, β-agonist, livestock, pig muscle, REIMS iKnife, metabolomics biomarkers, fraud, chemical food safety, public health*

L92*
TARGETED AND UNTARGETED SCREENING OF FOOD SAMPLES BY SPME USING A MATRIX COMPATIBLE COATED MESH COUPLED TO DART-MS

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Analysis of food commodities and environmental samples often requires multiple and laborious sample preparation/separation steps that significantly decrease the analytical method throughput. Compared to conventional sample preparation strategies, solidphase microextraction (SPME) constitute a convenient and green alternative, able to provide faster analysis throughput and the introduction of cleaner extracts into the analytical instrumentation. Ambient mass spectrometry techniques have also shown to be suitable for the fast determination of contaminants in food commodities. However, quantitation capabilities at the trace level (low ppb to ppt) are usually limited by the lack of sample preparation/analyte-enrichment. In recent years, SPME has proven to be an ideal technique for direct coupling to ambient ionization methods since it enables not only rapid and simultaneous extraction/enrichment of target analytes but also minimizes matrix interferences. In this study, we report the successful implementation of SPME- transmission mode (TM) and direct analysis in real time (DART) for the multiresidue analysis of food samples. The SPME-TM device consists of a mesh uniformly coated by hydrophilic-lipophilic balance (HLB) particles embedded in polyacrylonitrile (PAN) that gives the device antifouling properties. Milk, grape juice and orange juice samples spiked with a mixture of 20 pesticides and 3 deuterated internal standards were analyzed without any sample treatment prior to extraction. Analytes selected for the evaluation belong to different classes the pesticides and encompassed a broad range of polarities. SPME-TM-DART-MS/MS attained remarkable performance for the multicomponent systems containing trace levels of pesticides with only 1 minute of extraction/enrichment. Satisfactory linearity (>0.99) over 3 orders of magnitude in concentration (from 0.1 to 100 ng/g) was obtained for most of the analytes tested. Limits of detection (LOD), determined according to FDA standards, ranged between 0.1 and 5 ng/g. Analytical accuracy, determined at three different concentrations, ranged between 70 and 130%, confirming the suitability of the method for fast quantitative routine analysis in a concentration range that includes the MRL values imposed by EU regulations for pesticide residues in food commodities (10 ng/g). SPME-TM-DART was also coupled to a high-resolution MS system (Orbitrap) to demonstrate the potential of this approach for retrospective non-targeted analysis. Additionally, by coupling SPME-TM to portable single quadrupole MS system via DART, rewarding results were obtained for the rapid screening of pesticides in grape juice with LODs achieved in the low parts-per-billion level. Furthermore, we explored the suitability of this technology for on-site rapid molecular profiling of complex matrices. As a proof-of-concept, we demonstrate the rapid identification of milk samples from assorted animal and vegetal sources.

Keywords: *SPME-MS, DART-MS, pesticides analysis, molecular profiling, sample preparation*

Acknowledgement: *The authors thank the Natural Sciences and Engineering Research Council (NSERC) of Canada, Millipore Sigma (Merk), IonSense Inc., and VBM Science Ltd. for the financial support provided for this study. We acknowledge Waters*

Corporation for kindly providing the HLB particles used in this study. We would like to extend special thanks to Dr. Brian Musselman (IonSense) and Dr. Dragan Vuckovic (VBM Science) for providing us with access to the Waters-QDA instrument and to Robert Gougen (IonSense) for his help in setting-up the DART-qDA system and performing the preliminary experiments. Our gratitude is also extended to Dr. Nathaly Reyes-Garcés and Dr. Erica Souza-Silva for valuable scientific discussions.

L93
VACUUM ASSISTED SORBENT EXTRACTION FOR THE ANALYSIS OF FLAVOR COMPOUNDS IN FOOD

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Volatile and flavor compounds are extracted from foods using number of methods. Headspace analysis is the most popular approach, as it allows isolation of volatiles from various matrices, both liquid and solid without interfering non-volatile compounds that are usually co-extracted from matrix when liquid/liquid extraction is used. Most headspace techniques, provide also a significant analyte preconcentration, which enables analysis of flavor compounds in trace concentrations. Of the headspace analysis methods solid phase microextraction (SPME) and other sorbent based approaches (i.e. SBSE, P&T) are predominant in the analysis of food flavor compounds.
The positive effect of low pressure on SPME extraction was demonstrated in several applications, as lowering the sampling pressure results in improvement of extraction kinetics, especially for less volatile compounds with lower K_H values. Based partially on these findings vacuum assisted sorbent extraction (VASE) was developed recently as a novel method for the isolation of volatiles. It relies on extraction of volatiles and semivolatiles into dedicated sorbent traps under low pressure with their subsequent desorption in modified injection port of GC/MS. The lecture will be focused on the application of this technique for the analysis of flavor compounds in different matrices.
Use of VASE will be discussed for the analysis of phenolic compounds in smoked beers and peated whiskies representing liquid matrices. Method development, matrix effects and quantitative analysis will be demonstrated. The other type of matrix discussed will be plant tissues – broccoli, which represents Brassica vegetables. Method development for solid matrix will be presented for the analysis of compounds formed in the enzymatic reactions during tissue disintegration in raw vegetables as well as for the analysis of volatiles in cooked ones. The usefulness of the method for analysis of sulfur compounds, characteristic for Brassica vegetables will be discussed. Results obtained by VASE will be compared to that produced using SPME extraction.

Keywords: *vacuum assisted sorbent extraction, flavors, volatiles*

Acknowledgement: *Financing by the National Science Centre (Poland) under Grant 548 2015/18/M/NZ9/00372 is acknowledged.*

L94
HYPERSPECTRAL AND CHEMICAL IMAGING INSTRUMENTATION FOR FOOD SAFETY EVALUATION OF AGRICULTURAL PRODUCTS

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Researchers at the Beltsville Agricultural Research Center have developed spectral-imaging-based systems and techniques that target the reduction of food safety risks in post-harvest production and processing. Research objectives include comprehensive safety and quality inspection for online processing of fresh fruits and leafy greens, authentication of food ingredients and detection of food contaminants, and improvement of cleaning and sanitation efficacies in food processing facilities. For improved online screening of fresh produce, imaging methods and prototype instrumentation have been developed for inspecting whole surfaces of round fruits and relatively flat leafy greens. The prototype systems for produce inspection incorporate multi-view and multitask imaging methods for simultaneous reflectance and fluorescence imaging, thus allowing for screening of nearly 100% of the surface areas for fecal contaminants and defects during processing. To detect adulterants, a high throughput line-scan Raman imaging system was developed to rapidly scan large surface areas of food ingredients for authentication and for adulterant detection. In addition, a fluorescence-based handheld imaging technology for contamination detection has been developed for applications such as contamination and sanitation inspection in commercial meat and produce processing facilities. Collectively, these spectral-imaging-based systems and techniques will facilitate the rapid, non-destructive detection of contaminants or adulterants in food. The current states of the above spectral imaging technologies are presented.

Keywords: *spectral imaging, adulterant detection, food safety inspection, Raman chemical imaging, nondestructive sensing*

Acknowledgement: *Jianwei (Tony) Qin, Byoung-Kwan Cho, Diane Chan, Kuanglin (Kevin) Chao, Alan M. Lefcourt, Walter F. Schmidt, Julie Nguyen, Insuck Baek, Leigh Broadhurst, Hyunjeong Cho, Sagar Dhakal, Hoonsoo Lee, and Mirae Oh.*

L95
HEALTH RISK OF PATULIN AND PYRETHROIDS IN FRUIT JUICES CONSUMED IN THAILAND

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The mycotoxin patulin (PAT) is well-known as a natural contaminant of apple- and other fruit-based products. Pesticides are a group of chemicals abundantly used in agriculture to maximize productivity by protecting crops from pests and weeds. Due to their harmful health effects, PAT and pesticides are strictly monitored. The current study was undertaken to investigate the significance of PAT and pyrethroid insecticide contaminations in a variety of fruit juices in Bangkok. In order to do this, a total of two hundred fruit juice samples consisting of 40 samples each of apple, apricot, peach, pineapple and grape juice were collected from supermarkets in Bangkok, Thailand. PAT contamination in a variety of fruit juices was detected using a validated liquid chromatography-tandem mass spectrometry, whereas pyrethroid insecticides including cypermethrin, cyfluthrin, and flumethrin were analyzed using a gas chromatography equipped with micro-electron capture detector. The survey found that PAT concentrations were lower than the maximum residue limit established by European Union. The results of the present study suggest that the risk of exposure to harmful levels of PAT, cypermethrin, cyfluthrin, and flumethrin in fruit juices appears very low in urban areas of Thailand.

Keywords: *patulin, cyfluthrin, cypermethrin, flumethrin, fruit juices*

Acknowledgement: *This work was financially supported by the Center for Advanced Studies for Agriculture and Food, Institute of Advanced Studies, Kasetsart University under the Higher Education Research Promotion and National Research University Project of Thailand.*

L96*
DETECTION AND QUANTIFICATION OF ALLERGENS IN COMPLEX AND PROCESSED FOOD PRODUCTS

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Worldwide, food allergy is a major health problem and, to avoid a reaction, allergic populations have to exclude the incriminated ingredients from their diet. However, to be able to exclude the allergen implies a mandatory labelling of food allergens. However, the undeclared putative presence of allergens in food products is still widespread.
To protect allergic customers, reliable analytical methods for food allergen detection is essential. In agreement with VITAL recommendations, the limit of quantification (LOQ) for these methods should be lower than 2.5 mg milk and tree nut proteins, 0.75 mg egg proteins, 5 mg peanut proteins or 25 mg soybean proteins per kilogram of food (portion size: 40 g of food).
A UHPLC-MS/MS method for the detection of 10 allergens will be presented, with the ultimate goal of improving the food labeling. Milk, egg, soybean, peanut and tree nuts (hazelnut, walnut, almond, cashew, pecan nuts and pistachio) were incurred and processed in chocolate, ice cream sauce and cookies. Food allergen peptides were then analyzed by UHPLC-MS/MS (Xevo TQS – Waters). This routine method, running within a day, used a single protocol for the detection of 10 allergens. To the best of our knowledge, to date this method is still the most sensitive one for the detection of allergens by mass spectrometry in processed food products. We obtained a limit of quantification (LOQ), defined by a signal to noise ratio higher than 10, of: 0.5 mg of milk proteins, 2.5 mg of peanut, cashew, hazelnut and pistachio, 3 mg of egg proteins, and 5 mg of soy, almond, walnut and pecan proteins per kg of incurred foodstuffs.
Moreover, several quantification strategies will be presented and compared with ELISA for the quantification commercially samples.

Acknowledgement: *We thank Waters Corporation, the Walloon Region (FirstDoCA project: Allermass convention 1217881) and the Technological Platform Mass Spectrometry Service (MaSUN, UNamur) for scientific support and for their financial contributions to this project.*

L97
APPLICATION OF SEAWEEDS IN FOOD AND FEED - ANALYSIS OF TOXIC ELEMENTS AND IMPLICATIONS FOR FOOD/FEED SAFETY

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Seaweed is the common term for marine macroalgae plants, which may be divided into green, red and brown algae types. There is an increased industrial interest to increase the exploitation of marine macroalgae for commercial purposes including the use in relation to food and feed production. Certain seaweeds have the potential to accumulate various trace elements and contain consequently relatively high levels of both essential and toxic elements. Seaweeds can even be used for bioremediation purposes in order to remove trace elements from the environment.
There is consequently a need to document the levels of toxic elements in seaweeds and to gain more knowledge about this bioressource. The toxic elements (incl species) of highest concern are iodine, cadmium and inorganic arsenic. Furthermore, a better understanding of how biological and environmental factors, like seaweed type, geography, season etc affects the levels of trace elements is called upon, in order to be able to select seaweeds with optimum characteristics for commercial use in the food and feed sector.
The present lecture will include:
1) examples of the use of seaweeds in various food and feed applications
2) examples of the analysis of toxic elements (incl species) in various seaweed samples by (HPLC-)ICP-MS
3) results from studies on iodine bioavailability from seaweed and the seasonal variation of iodine in kelp
4) discuss the results in relation to food and feed safety assessment.

Keywords: *food safety, arsenic speciation, iodine, seaweed*

L98
CRITICAL EVALUATION OF HIGH RESOLUTION LC-MS/MS2 WORKFLOWS IN TERMS OF CONTEMPORARY IDENTIFICATION CRITERIA

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Modern hybrid high resolution mass spectrometers (QToF, QOrbitrap) offer various workflows. The best way of pesticide residues analysis is simultaneous acquisition in MS and MS^2. Typically, MS data are used for detection and quantitation whereas MS^2 for identification. In MS the only reasonable workflow for a multiresidue analysis if full scan. However, in MS^2 mode there are several approaches. Differences between various MS^2 workflows include number of fragmentations per analyte, quadrupole isolation window, data dependent or independent character of the data acquisition etc. In consequence, the analyst has to search for a workflow that will provide good sensitivity, selectivity and obtained data will fulfil all the criteria for detection and identification.
Contemporary pesticide residues identification criteria include parameters as retention time, number of detected ions, mass error, ion ratio, coelution factors. All of the mentioned parameters (except retention time) in some way depend on the conditions of MS analysis. Selected accumulation time, resolution and especially quadrupole isolation window can be crucial for correct identification of a pesticide.
In this presentation, some important aspects of pesticides identification will be discussed. The reproducibility of MS^2 fragmentation without precursor ion isolation (all ions fragmentation) is still not clear. In other words, is not certain how the complexity of the matrix influences the ion ratio. In workflows as SWATH and vDIA an important question is what is more substantial for correct identification: resolution or number of mass segments. Another problem is how to identify a pesticide having only one MS^2 scan per compound.

L99
INNOVATIVE DNA-BASED METHODS - THE RESULTS OF THE EUROPEAN DECATHLON PROJECTS

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The European Decathlon (Development of Cost efficient Advanced DNA-based methods for specific Traceability issues and High Level On-site applicatioNsproject) project (december 2013 – december 2016) has focused on the development of DNA-based methods for the three areas of the project: 1) food pathogens, 2) GMOs (genetically modified organisms, with a focus on unauthorised GMOs), and 3) customs issues (with a focus on tobacco and endangered species identification).
The project has lead to important developments in all three areas, with a focus on both multimethods that can be applied in laboratories with advanced facilities, as well as on on-site methods that may be used outside of such facilities in the (near) future. In close cooperation with the stakeholders in the respective fields minimum performance paramaters were identified for the methods to be developed with a clear focus in all cases on cost efficiency. Also, methods have been developed that can be applied in different types of samples ranging from single plant or animal materials up to complex microbial or food/feed products. This presentation will provide an overview of the main achievements of the project, with emphasis on the most innovative DNA approaches combining DNA target enrichment strategies with Next Generation Sequencing.

Keywords: *DNA-based methods, genetically modified organisms, species identification, food pathogens, on-site methods*

Acknowledgement: *The DECATHLON project has been funded with support from the European Commission in the context of the Seventh Framework Programme (FP7).*

LECTURES

L100
MULTI-PARAMETER FOOD ANALYSIS AT THE POINT-OF-USE ENABLED BY THE VERSATILE CENTRIFUGAL "LAB-ON-A-DISC" PLATFORM

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Over the last two decades, centrifugal microfluidic systems have rapidly emerged as user-friendly and compact sample-to-answer solutions for decentralised bioanalytical testing. A particular strength roots in their capability to integrate and automate powerful sample preconditioning with common assay protocols and typically optical or electrochemical detection schemes. Centrifugal systems further excel with their rather rugged, modular setup which is essentially constituted by a compact instrument hosting a low-cost spindle motor and detection module, and a single-use, microstructured polymer disc for processing the liquid biosample and encapsulating the final waste for safe disposal. Numerous proof-of-concepts have been demonstrated and several products have entered the market, especially for point-of-care diagnostics, drug discovery, environmental monitoring and life science research.
The recently launched "FPC@DCU" - the Fraunhofer Project Centre for Embedded Bioanalytical Systems at Dublin City University offers time- and cost-efficient development of a variety of point-of-use applications towards high-technology-readiness levels (TRLs). Seamless scale-up of manufacture from early demonstrators is achieved in collaboration with the renowned Fraunhofer Institute for Production Technology (FhG-IPT). In these often highly interdisciplinary projects, FPC@DCU operates as a one-stop-shop for its partners and contractors.
FPC@DCU's proprietary centrifugal "Lab-on-a-Disc" (LoaD) technology uniquely enables very robust liquid handling that permits high-level integration and automation of multi-step test protocols, concentration series, combinatorial mixing and multi-parameter analyte panels. Due to FPC@DCU's versatile platform concept, the LoaD can readily be customised for a broad spectrum of targets such as small molecules, proteins, nucleic acids, cells, bacteria and other bioparticles. FPC@DCU have specifically demonstrated a system for the DNA-based detection of plant pathogens towards point-of-use monitoring in food safety.
As a pilot application we used a custom instrument controlled by pulse-actuated centrifugo-pneumatic valving [1], we have fully automated DNA purification, spatial multiplexing and on-disc LAMP amplification for detecting common plant pathogens [2]: Boty, Tomato leaf curl virus (TYLCV), bacteria Clavibacter michiganensis (CMM), Pepino mosaic virus (PepMv), a ssRNA virus, Potato spindle tuber viroid (PSTVd), and Phytophthora infestans (fungus) (Mod Phy).

[1] Kinahan *et al.*, *Proc.* μ*TAS 2014,* pp. 1437-1439.
[2] Chung *et al.*, *Proc.* μ*TAS 2016,* pp. 848-849.

Keywords: *plant pathogen, centrifugal microfluidics, Lab-on-a-Disc, sample-to-answer, FPC@DCU*

Acknowledgement:
This publication has emanated from research conducted with the financial support of Science Foundation Ireland (SFI) and Fraunhofer-Gesellschaft under the SFI Strategic Partnership Programme Grant Number 16/SPP/3321. Authors also acknowledge support from the European Union (FP7-KBBE-2013-7-613908-DECATHLON).

L101
USE OF NGS COMBINED TO ENRICHMENT TECHNOLOGIES FOR GMO DETECTION

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Next Generation sequencing (NGS) is a new way to detect and characterize genetically modified organisms (GMOs). However, the technology is not always able to cover the sequence of the whole genome due to the fact that the genome size can be very different from one plant species to another and because some regions of the genome can be deeply sequenced while other are not covered. This issue is increased if the sample to analyse contains a mix of several plant species. The strategy proposed here is an enrichment of the regions of interest prior to sequencing. These sequences of interest are structural elements that can be met in transgenic constructs. In this work, the sequences of 10 promoters, 6 terminators and 20 genes present in GM constructs were used to create a library of DNA sequences. Capture probes were designed to cover the sequences of the DNA library and to fish the fragments of interest. The fragments were then sequenced on Illumina Hiseq2500. Two subsequent approaches were followed: first, the detection of GMOs on the base of the assignation of the reads to the different sequences composing the DNA library and second, the evaluation of the possibilities to create contigs with the sequences obtained to characterize the GM construct.
Analysis of the NGS outputs also requires some new development in bioinformatics due to the huge amount of data. Bioinformatic scripts and pipeline analysis were created to align the reads on reference genomes and the DNA library, filter the reads in function of their alignment scores and proceed to a statistical analysis of the results to determine if they can be distinguished from the noise band and be assimilated to positive results. In a second step, a pipeline to create contigs was developed.
This work was realized on DNA extracted from several GM plants. After bioinformatics treatment, the targeted elements were positively detected if present. Only few of them presented problems due to the similarities with sequences that can be naturally present in some plants and were removed from the next version of the DNA library used for enrichment. Contigs can be used to characterize the GM constructs and this approach permits to determine the junction between the elements present in the transgenic construct. The quality of the results however decreases as a function of the GM percentage.

L102
IDENTIFICATION OF BERRY SPECIES AND CULTIVARS BY DNA BARCODING AND HIGH RESOLUTION MELTING (HRM) ANALYSIS

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Regular intake of berries has been associated with a reduced risk of chronic and degenerative human diseases. Thus, the consumption of berries and berry products has become very popular. Like other commercial foodstuffs, berry products have to be safe and authentic in order to comply with national and EU food regulations. However, studies report that berry products are frequently adulterated. Most commonly, berry products are adulterated by replacing berries of higher value by less expensive berries or even by other fruit species.
Analytical methods are required to verify if berry products actually contain the berries species declared. Studies have shown that DNA barcoding has a high potential for species differentiation in food. Papers dealing with the differentiation of berry and fruit species by DNA barcoding are, however, scarce.
In DNA barcoding, distinctive regions in the DNA, so-called "DNA barcodes", are analyzed with the aim to identify and differentiate organisms. DNA barcoding includes the following steps: selection of an appropriate barcode; the amplification of the selected barcode region by the polymerase chain reaction (PCR); and analysis of the amplicons, e.g. by sequencing or high resolution melting (HRM) analysis. In order to be applicable, a DNA barcode should contain a central variable part, allowing the discrimination of the species or cultivars of interest. The variable part should be flanked by conserved regions, making it possible to use a universal primer pair for amplifying the barcode sequence in different species/cultivars.
The aim of the present study was to develop HRM methods allowing to differentiate between bilberry (*Vaccinium myrtillus)* and blueberry (*Vaccinium corymbosum)*; American cranberry (*Vaccinium macrocarpon*), European cranberry (*Vaccinium oxycoccos*) and lingonberry (*Vaccinium vitis-idaea*); and pomegranate (*Punica granatum*) cultivars. The presentation will demonstrate the potential but also discuss the limitations of this technology.

Keywords: *berries, authenticity, DNA barcoding, high resolution melting*

Acknowledgement: *This work was financially supported by the Austrian Federal Ministry of Science, Research and Economy (Scientific & Technological Cooperation, Project Number MK10/2016) and CEEPUS (Network CIII-RO-0010-11-1617).*

L103
DEVELOPMENT AND VALIDATION OF A DNA METABARCODING METHOD FOR THE IDENTIFICATION OF TWENTY-ONE SPECIES IN FOOD USING A SINGLE MARKER SYSTEM

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According to national and international food regulations commercial foodstuffs have to be safe and authentic. Recent food scandals demonstrate that food adulteration is a problem all over the world. Food adulteration covers a variety of aspects, including substitution of food components, dilution, the use of non-authorized food processing steps and the declaration of an incorrect geographical origin. Meat products are particular prone to be adulterated by replacing high-value meat from more expensive animal species by cheaper ones (Ballin, Vogensen, & Karlsson, 2009). Therefore, food fraud is a considerable problem for consumers that attempt to avoid the consumption of certain meat species due to health, religious or ethical reasons (Ballin, 2010).
Several studies demonstrate that DNA barcoding has a high potential for species differentiation in food (Abdullah & Rehbein, 2017; Günther, Raupach, & Knebelsberger, 2016; Staats et al., 2016). In recent years, remarkable progress has been made towards developing DNA metabarcoding strategies, which involves next-generation sequencing of DNA barcodes for the simultaneous detection of multiple species in complex samples. Metabarcoding strategies can be applied to processed materials containing highly degraded DNA e.g. for the identification of species in processed food products (Arulandhu et. al., 2017).
A DNA metabarcoding method using a single marker region is presented, allowing the identification and differentiation of 21 animal species in foodstuffs. These 21 species (15 mammals and 6 poultry species) were selected because they are quite frequently contained in European foodstuffs. The method was developed on the MiSeq platform, targeting a mitochondrial 16S rDNA region recently found to be suitable to differentiate mammalian species. A novel primer pair for poultry species was designed and applied, in combination with the primer pair for mammals, in a duplex assay. The applicability and reliability of the method was demonstrated by analyzing DNA extracts from muscle meat, DNA extract mixtures and extracts from model sausages running a number of validation experiments. The species of interest can be differentiated and detected down to a proportion of 0.1%.
Since 96 samples can be sequenced in one Next Generation Sequencing run, the method has a high potential to be applied in routine analysis. The DNA metabarcoding method assessed in this study provides reliable and detailed data on

the composition of complex food products, including information on the quantity of species present. However, only when DNA metabarcoding has been demonstrated to be robust and transferable across laboratories can the method truly be implemented in routine testing.

Keywords: *next generation sequencing, meta barcoding, species differentiation, food authentication*

Acknowledgement: *We thank our PhD student (Stefanie Dobrovolny) for her phenomenal contribution and all colleagues who provided insight and expertise that greatly assisted the research. I am thankful for the aspiring guidance from professor Cichna-Markl from the University of Vienna, Department of Analytical Chemistry.*

L104
CONTRIBUTION OF DNA ANALYSIS FOR FURTHER FOOD AUTHENTICITY ASSESSMENT

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High quality and safety food on the shelves is the basic requirement of the consumers. Quality and safety relate to properly used raw materials and protection against contaminants of any origin. To ensure the food quality and safety, specific standards have been set for primary production, processing and final products placed on the market. Requirements invoke the need to carry out market controls. Controls have been carried out by the responsible authorities according to the valid legislative framework by appointed laboratories.
Control laboratories typically operate under the ISO 17025: 2005 quality system and use standardized methods. A number of procedures based on instrumental analysis that have been validated and widely applied to detect and quantify even negligible traces of residues, metals and other demanded and undemanded ingredients. Various instruments are used, as HPLC, Gas Chromatography, Mass spectrometry or NMR being the most known.
A special group of techniques target on identification and quantification of DNA molecules present in the food, that fully reflect origin of raw materials used. As DNA is a relatively stable molecule it can be analyzed even partially damaged. Several procedures have been tested that allow for effective applications. Methods based on the multiplication of specific DNA segments using polymerase chain reaction (PCR) are the most spread for control purposes. Conventional PCR can distinguish the DNA coming from individual species, RT-PCR allows for relative quantification, digital PCR makes absolute quantification possible. Other variants, those that use the length variability of short repeating sequences (SSR analysis) are used to distinguish varieties/breeds and individual genotypes. Further Sanger sequencing in combination with PCR fis applied for *bar coding* to distinguish bacterial, animal and plant species. The New Generation Sequencing (NGS) is new perspective method for unambiguous determination of food authenticity. All the methods have to be validated, in house verified and full fill performance criteria given by specific standards and EU guidelines.
We have demonstrated practical applications of the techniques on foods containing GMO (PCR, qPCR), unknown origin of ingredients (Sanger sequencing and bar coding), determining variety authenticity (SSR analysis), groups of genotypes (SNPaSHOT) and their use in market control.

Keywords: *DNA analysis, food authenticity*

Acknowledgement: *The work was supported by Ministry of Agriculture, project NAZV QJ1530272 and Institutional support by the Ministry of Agriculture RO017.*

L105
PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANE SULFONATE (PFOS) RISK TO BEEF CONSUMERS PART I: RAPID RISK EVALUATION FOR THE CONSUMPTION OF PFOA AND PFOS CONTAMINATED BEEF

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At a site in the Southeastern United States, biosolids containing perfluorinated compounds (PFCs) were applied to agricultural lands. As these lands included cattle grazing areas, cattle were likely exposed to PFCs which could lead to subsequent PFC exposure for beef consumers. A rapid risk evaluation was needed to determine if beef from PFC exposed cattle presented a risk to consumers. A model was developed to estimate the PFC concentrations in cattle that were grazed on the contaminated lands and the risk associated with consumption of beef from these cattle. Due to the absence of PFC concentration data in edible tissues from cattle in this location and the absence of bovine PFC absorption, distribution, metabolism, excretion (ADME) data, the model utilized PFC, specifically perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), environmental concentrations and rat ADME data to estimate PFC concentrations in beef for this scenario. The estimated concentrations ranged from 0.7 to 2.0 ng/g PFOA and 5.6 to 17 ng/g PFOS. These estimated PFC concentration estimates were significantly less than beef concentrations of concern for acute exposure. For sub-chronic exposure, approximately 3% of estimated PFOS (and 0% of PFOA) residues exceeded the concentration of concern. Recognizing that the availability of bovine PFOA and PFOS ADME data would likely improve the accuracy of future risk evaluations for similar PFC environmental contamination scenarios, the USDA FSIS requested that the USDA Agricultural Research Service (ARS) conduct such studies.

L106
PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) RISK TO BEEF CONSUMERS PART II: ADSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION STUDIES FOR IMPROVING RISK EVALUATIONS

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Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are industrially produced chemicals used as surfactants and coatings in many industrial, commercial and consumer products. These compounds are ubiquitous in humans and the environment. PFOA and PFOS have been observed in biosolids from wastewater, suggesting exposure of agricultural animals to these chemicals may occur through application of biosolids to cattle pastures and animal food crops. Possible accumulation of PFOA and PFOS in edible tissues of agricultural animals could be a risk to consumers. As such, the United States Department of Agriculture (USDA) determined the absorption, distribution, metabolism, and excretion (ADME) of PFOA and PFOS in beef cattle following an oral dose. These studies determined the magnitude and distribution of these chemicals in beef cattle and data were used to improve risk evaluations conducted by the USDA.
Initially, 4 Lowline Angus steers were provided single bolus doses of ^{14}C-PFOA at 1 mg/kg body weight (bw) and 3 of the 4 steers also received a simultaneous unlabeled PFOS dose at 10 mg/kg bw and were held for 28 d. The one steer not receiving PFOS was used for control purposes. The second study consisted of 2 Angus steers and 4 Angus heifers given single bolus doses of PFOS at 0.098 mg/kg bw and 9.1 mg/kg bw, respectively, and maintained during the course of the 343 d study. Plasma was collected at various time intervals during the studies from all animals. Urine and feces were quantitatively collected each day during the 28 d study. After 28 days, 105, and 343 days, animals were sacrificed and tissues collected. ^{14}C-PFOA analysis was completed by liquid scintillation counting (LSC) for liquid samples and sample oxidation with LSC for solid samples. PFOS was analyzed by an ion pairing extraction method with quantification using liquid chromatography mass spectrometry (LC-MS).
From the 28 d study, peak concentrations of ^{14}C-PFOA in plasma occurred between 24 and 36 h post-dose with the elimination half-life in plasma being 19.2 ± 3.3 h. However, plasma PFOS elimination half-life could not be determined from the 28 d study, but 30-40% of the dose was still circulating in the plasma on day 28. From the 343 d study, PFOS plasma half-lives were 120 d and 106 d for steers and heifers, respectively. Quantitative elimination of ^{14}C-PFOA in urine was observed for the 28 d study at 100.8 ± 3.3% of dose, while elimination via feces was <5%. PFOS excretion via urine was minimal, <1% over 28 d, but feces contained 11% of the PFOS dose. As for tissue distribution, ^{14}C-PFOA was not detected in any tissues at the conclusion of the 28 d study. PFOS concentrations in tissues (excluding plasma) were found to be highest in liver, kidney, and muscle for both studies and tissue half-lives were 116 d, 385 d, and 165 d, respectively. No metabolism was observed for either compound.

Keywords: *PFOA, PFOS, ADME, risk, beef*

L107
THE DISTRIBUTION OF ENVIRONMENTAL CONTAMINANTS AND PHARMACEUTICALS AMONG SKIM MILK, MILK FAT, CURD, WHEY, AND MILK PROTEIN FRACTIONS THROUGH MILK PROCESSING

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Twenty-seven environmental contaminants and pharmaceuticals encompassing a wide range of physicochemical properties were utilized to determine the effects of milk processing on xenobiotic distribution among milk fractions. Target compounds included radiolabeled antibiotics [ciprofloxacin (CIPR), clarithromycin (CLA), erythromycin (ERY), oxytetracycline (OTET), penicillin G (PENG), sulfadimethoxine (SDMX), and thiamphenicol (TAP)], anthelmintics [ivermectin (IVR), praziquantal (PZQ), and thiabendazole (THIA)], analgesics and antipyretics [acetaminophen (TYL), aspirin (ASP), flunixin (FNX), ketoprofen (KETO) and phenylbutazone (PBZ)], a hormone (estrone, E1), and environmental contaminants and/or metabolites [brominated diphenyl ether-99 (BDE-99), bisphenol A (BPA), glyphosate (GLY), β-hexabromocyclododecane (β-HBCD), imidacloprid (IMI), polychlorinated biphenyl-118 (PCB-118), tetrabromobisphenol A (TBBPA), methylsulfone PCB-101 ($MeSO_2$ PCB), triclocarban (TCC), 1278-tetrachloro dibenzo-*p*-dioxin (1278-TCDD), and 1378-hydroxy TCDD (OH-TCDD)]. Radioactive distribution into skim milk, milk fat, curd, whey, and the permeate or retentate from whey passed through 10 kDa filters was quantified for each chemical. Greater than 75% of radioactivity was distributed into milk fat for β-HBCD, BDE-99, IVR, $MeSO_2$ PCB, PCB118, TCC, and TCDD. About 50% of the phenolic chemicals such as BPA, OH-TCDD and TBBPA were distributed into milk fat. In contrast, polar compounds including ASP, CIPR, CLA, ERY, GLY, IMI, KETO, OTET, PENG, PZQ, SDMX, TAP, THIA, and TYL were poorly (<5%) distributed into milk fat. Log D values were better indictors than log P values for predicting the distribution of chemicals between fat and skim fractions, but was less effective at predicting distributions between insoluble (curd) and soluble (whey) proteins. When whey was separated into retentate and permeate (~1:2 v/v), > 85% of IVR, PCB-118 and TCC remained in retentate indicating these chemicals had high affinity for whey proteins. Compounds associated with < 10 % whey protein included ASP, GLY, IMI, OTET, PENG, TAP, and TYL. These data may be beneficial for predicting the distribution of xenobiotics into dairy products during milk contamination events.

Keywords: *milk, distribution, enviromental contaminants, curd, whey*

Acknowledgement: *The skillful technological assistance provided by Dee Ellig, Patrick Harland, Lindsey Fransen, Amy McGarvey, Jason Neumann, Colleen Pfaff, Jason Holthusen, and Michael Woodworth is greatly appreciated.*

L108*
OVERVIEW OF A NOVEL LC-MS/MS METHOD FOR THE DETERMINATION OF SULFITE IN FOOD AND BEVERAGES: DEVELOPMENT, VALIDATION, AND INVESTIGATION OF PROBLEMATIC MATRICES

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Sulfites are food additives used in a wide range of food and beverage products to prevent browning or oxidation. While their consumption has no side effects for the majority of the population, a small subset of the population has been shown to have an allergic-like response. For this reason, the US Food and Drug Administration (US FDA) requires that sulfites be labeled as an ingredient on all products that contain greater than 10 mg/kg (ppm) SO_2. The current regulatory method, AOAC #990.28 Optimized Monier-Williams (OMW), is successful in quantifying sulfites in most matrices but is very time consuming and has a method detection limit at the regulatory labeling threshold. It also produces false positive results with vegetables from the *Allium* (garlic) and *Brassica* (cabbage) genera due to extraction conditions that are thought to cause endogenous sulfur compounds to release SO_2. Recently, an LC-MS/MS method was developed that was applicable for a wide range of sulfite containing matrices ranging from dried fruits and vegetables to frozen seafood. This method converts free and reversibly bound sulfite to the formaldehyde adduct, hydroxymethylsulfonate, which can then be separated from matrix constituents using a HILIC analytical column and then detected with tandem mass spectrometry. A multi-laboratory validation was conducted with 11 laboratories in the United States and Canada. Four matrices were spiked at varying concentrations and three additional commercially sulfited matrices were included. An abbreviated comparison study between the LC-MS/MS and OMW was conducted for select samples. Average recoveries for all matrices ranged from 86-114% with % RSD_r and % RSD_R of 4.5-17.5 % and 8.6-22.5 %, respectively. Proficiency samples were also tested in one laboratory by both methods to further compare the two methods. Method detection limits were determined in several matrices and all were found to be less than 1 ppm SO_2 which is a marked improvement to the OMW. The LC-MS/MS method reduces the false positive observed when *Allium* and *Brassica* vegetables are analyzed by the OMW titration and gravimetric method. The SO_2 concentration was below the 10 mg/kg regulatory threshold in all *Brassica* species analyzed and was slightly reduced in most of the *Allium* species investigated though significant differences from the OMW only existed in the garlic samples. There also does not appear to be any problems recovering added sulfite from these matrices using the LC-MS/MS method, making this a viable replacement method for the tedious and time consuming OMW methods. This LC-MS/MS method is a faster, more sensitive, and more selective way of determining sulfites in food and beverages. It shows promise for continuing to improve the enforcement of sulfite labeling requirements protecting those individuals who have sulfite sensitivity.

Keywords: *sulfite, multilab validation*

L109
SURVEY OF ARSENIC SPECIES IN THE TEN MOST CONSUMED SEAFOODS IN THE UNITED STATES

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Seafood is regarded as an important part of a healthy diet, as it contains various nutrients associated with beneficial effects. On the other hand, it is also known to be a major source of human exposure to dietary arsenic. Most arsenic monitoring in food products are based on the determination of inorganic arsenic, which is a Class I carcinogen. Unlike many food products (such as rice and fruit juices) which contain inorganic arsenic along with a few other species, seafood arsenic comprises dozens of chemical forms. While inorganic arsenic has been associated with long term health effects, in most seafood it accounts for only a very small percentage of the total arsenic. Most of the arsenic in seafood is known to be non-toxic; however, there are some species which require more research to determine their potential toxicological effects. The survey study reported here focused on the ten most consumed seafoods in the United States, which make up over 90% of the total seafood consumption. Samples were collected from local supermarkets in three states and analyzed using methods recently developed and validated by the US Food and Drug Administration. The analysis involved stepwise extraction of water-soluble and non-polar arsenic using hot water and a dichloromethane–methanol mixture, respectively. The arsenic in the aqueous extracts was speciated by ion exchange chromatography, and the non-polar arsenicals were collectively determined after digestion in acid. The results of the survey represent the most comprehensive analysis of arsenic species from seafood in the American diet to date.

Keywords: *arsenic, seafood, survey speciation*

L110*
MULTIPLEXED IMAGING SURFACE PLASMON RESONANCE (ISPR) BIOSENSOR ASSAY FOR THE DETECTION OF FUSARIUM TOXINS IN WHEAT

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Certain *Fusarium species* (*F. graminearum and F. verticilloides in particular)* infest grains and can produce a wide range of fungal(myco)-toxins, causing huge economic losses worldwide. A reproducible and sensitive imaging surface plasmon resonance (iSPR) assay was developed and validated for three important *Fusarium* mycotoxins, deoxynivalenol (DON), zearalenone (ZEA) and T-2 toxin. The assay was based on a competitive inhibition immunoassay where secondary antibodies conjugated with gold nanoparticles (AuNPs) were used for signal amplification. By using AuNPs, the signal was amplified 12 to 90 fold relative to signal from toxin-specific (primary) antibodies alone. The technique is based on antigen coated sensor chips that were used for more than 40 cycles with minimal reduction of signal (<12%). To determine *Fusarium* toxins in wheat, matrix matched calibration curves were constructed. In spiking studies mean recoveries ranged from 87% to 103% with relative standard deviations of repeatability of less than 15%. The limits of detection were 15 µg/kg for DON, 24 µg/kg for ZEA and 12 µg/kg for T-2 toxin, providing enough sensitivity to monitor for contamination in wheat. The assay was more sensitive than previously reported immunoassay and multiplexed SPR assays. Cut off levels were successfully validated for all three *Fusarium* toxins, which allowed separation of blank samples from those spiked at levels regulated by the European Commission (100 µg/kg for ZEA and T-2 toxin, or 400 µg/kg for DON, which was one-third of the EC level). To validate the cut off level, the method was also successfully applied to naturally contaminated wheat. This is the first reported iSPR assay that uses signal amplification with AuNPs in order to detect three agriculturally important *Fusarium* toxins in wheat.

Keywords: *mycotoxin, fusarium, iSPR, biosensor, wheat*

Acknowledgement: *This work was supported by USDA-ARS project number 5010-42000-0049-00D and the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy and the USDA.*

LECTURES

L111
DEVELOPMENTS IN RAPID MONITORING OF PATHOGENS IN POULTRY PRODUCTS AND THE PROCESSING ENVIRONMENT

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Monitoring of foodborne pathogens is largely based upon robust but time consuming procedures involving recovery, enrichment, and plating of samples on selective media. These microbiological tests are often followed by biochemical, serological, or genetic confirmation of species and serotypes. The food industry requires fast and reliable tools for more efficient microbiological testing that can significantly reduce the safety inspection time. Research performed in the Quality and Safety Assessment Research Unit, USDA-ARS, Athens, Georgia is aimed toward rapid and reliable detection of pathogens, either planktonic or in biofilm, using primarily optically based systems. A hyperspectral microscopy imaging (HMI) methodology whereby individual cells are identified based upon spectral signatures in combination with shape features is under development. HMI has the potential for reducing the incubation time necessary for bacteria cultures. Five foodborne Salmonella serotypes were accurately classified at the typical incubation time of 18-24 hours with 99% accuracy, and reduced incubation times as short as 8 hours with 96-99% accuracy. Once fully developed, this methodology may allow the classification of serotypes by HMI to be implemented as a rapid and early tool for presumptive detection of bacterial pathogens. A surface plasmon resonance imaging (SPRi) technique has been developed to detect foodborne Salmonella cells on a sensor surface modified with anti-Salmonella antibody spots. Salmonella was detected from chicken carcass rinse with no sample pretreatment. This technique shows promise for multiplex screening of foodborne pathogens because the sensor chip can be modified with dozens of spots each consisting of antibodies targeted at different pathogenic species or serotypes. Currently, biofilms on poultry processing surfaces are detected by ATP swabbing, a method that is effective in discriminating the biofilm from other, non-living, contaminants. The technique, however, is limited in the amount of surface area that can be reasonably tested. To rapidly detect biofilms potentially harboring pathogenic bacteria, a spectroscopic signature that discriminates the biofilm from co-contaminants is required. Biofilms have no intrinsic spectral signature, making it necessary to produce one. A series of generally regarded as safe (GRAS) dyes, as well as non-GRAS dyes, were tested for their abilities to bind to specific components of the extracellular polymeric matrix (EPM) formed by biofilms. Results indicate that most of the dyes studied are not suitable as biofilm disclosing agents due to either non-specificity or low binding to any of the EPM components. One dye, calcofluor white, does bind preferentially and strongly to the carbohydrate component of the EPM, and displays intense fluorescence when illuminated at 365 nm. This methodology shows promise for developing a rapid and efficient technique for disclosing biofilms over large surface areas.

Keywords: *hyperspectral, SPRi, biofilm*

L112
CRITICAL REVIEW, EXPERIENCES AND OUTLOOK WITH RESPECT TO METABOLOMICS DATA HANDLING OPTIONS

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Key Parameters in ensuring quality of LC-HRMS metabolomics data and models

- Overview of LC-MS based metabolomics workflow (Identification of critical steps)
- Quality of the samples (Key parameters in designing the experiment)
- Analytical Quality (Expectations and requirements in both sample preparation and fingerprinting steps)
- Data Analysis Quality (Tools for validating the models / markers)
- Examples and General recommendations to ensure robustness of the whole workflow

L113
DATA PROCESSING AND IDENTIFICATION OF SMALL MOLECULES IN LC–MS-BASED NON-TARGETED ANALYSIS WORKFLOWS

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Presentation will focus on:

- Overview of non-targeted workflows in food analysis
- LC-MS platforms and approaches to non-targeted data acquisition
- Requirements and assurance of HR-MS data quality
- Data mining, pre-processing and analysis
- Identification of small molecules: approaches and tools (Elemental formula generation, mass spectral libraries and chemical databases, prediction and interpretation of mass spectra…)
- Examples
- General recommendations

Keywords: *non-targeted analysis, LC-MS, identification, software tools*

L114*
PARAFAC MODELLING COMBINED WITH FLUORESCENCE SPECTROSCOPY FOR BEER CHARACTERIZATION

Lea Lenhardt Acković*[1], **Ivana Zeković**[1], **Tatjana Dramićanin**[1], **Bojana Milićević**[1], **Jovana Periša**[1], **Miroslav D. Dramićanin**[1]

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Beer is one of the most popular alcoholic beverages consumed worldwide. Because of large increase in beer production it is getting more and more challenging to test and control the quality of beer. Standard chemical analysis are time-consuming and expensive. For that reason there is a big demand for new rapid methods for the beer analysis and on line monitoring. Fluorescence spectroscopy coupled with parallel factor analysis (PARAFAC) and partial least squares discriminant analysis (PLS-DA) has proven to be fast, sensitive and intact method suitable for the food characterization. In this study excitation-emission fluorescence spectra of 111 beer samples were measured. Beer samples included 6 different types of beer (Pale lager, Wheat beer, Stout, Dark lager, Pale Ale and India Pale Ale) produced all over the world. All measurements were recorded on Fluorolog-3 Model FL3-221 spectroflurometer system (Horiba Jobin Yvon) in emission spectral range from 275 to 600 nm with a 2.5 nm interval step, and excitation spectral region from 255 to 550 nm with a 2.5 nm step. Results of PARAFAC analysis showed presence of five dominat fluorophores present in beer, and based on their excitation and emission maxima they could corespond to amino acids, different forms of the vitamin B group, iso-α-acids and phenolic compounds naturaly present in beer. Based on their relative concentrations it was possible to build classification model which had mean cross-validated classification error of 16.1% for different types of beer. Obtained results showed the potential of PARAFAC coupled with fluorescence spectroscopy to be used for extraction of pure spectra of most dominant fluorophores present in beer and their relative concentrations. Based on the results this technique is well suited for characterization and classification of beer samples.

Keywords: *beer, fluorescence spectroscopy, PARAFAC*

L115
SAMPLING GUIDELINES FOR BUILDING AND CURATING FOOD AUTHENTICITY DATABASES

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The detection of fraudulent foods is based on knowledge of what should, or should not, be present in an authentic sample. In simple cases data interpretation is easy, for example active mānuka honey should contain methylglyoxal and milk should not contain melamine. In more complex cases, for example whether the isotopic signature of a beef sample is consistent with coming from the UK, a database of known authentic measurements is required for comparison.
The detection of fraudulent and adulterated foods is even more challenging since an authentic product can often have a significant natural variation in composition. These variations can arise from natural factors such as seasonality, geographical origin and from differences in production methods. The generation of a robust database, that adequately covers these variations is not a trivial task.
Therefore, as part of the EU FoodIntegrity project, a scientific opinion is being prepared on guidelines and considerations for building, curating and using databases for food fraud detection. This presentation will provide an overview of the authors' opinions on critical aspects of generating and importantly, using, such food authenticity databases. The presentation will consider aspects that need to be taken into account before, during and after database creation. Importantly this will include factors such as defining the database scope, collection of authentic reference materials, identifying minimum information requirements for metadata, data collection and longer-term applicability, robust database validation, data storage and maintenance.

Keywords: *food authenticity, database*

Acknowledgement: *The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.*

L116
THE POTENTIAL OF BIG DATA SYSTEMS AND NOVEL ANALYTICAL APPROACHES TO BETTER ANTICIPATE NEW RISKS TO THE CONSUMER

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Analytical methods have historically been used to identify and help quantify food risks. They perform a valuable service in identifying new contaminants and associated metabolites as well providing a means of surveying the safety of the food supply. Outside of the research environment such methods are usually deployed *post hoc* as part of the information gathering and risk communication that inevitably occurs at the time of a food "incident", e.g. fipronil.
All stakeholders are interested in how they can better anticipate future risks so that mitigation can take place *before* the risk becomes an "incident". The latest advances in science and technology have the ability to identify problems much further down the incident pipeline thereby reducing the need for expensive post incident crisis management. Big data has a significant role to play in any such prediction system as it is well suited for identifying unusual changes in the foods trading and production environments. Early Warning Systems (EWS) using big data approaches that can interrogate and exploit, through machine learning, macroeconomic signals of food fraud events will be discussed. In addition strategies for using advances in non-targeted analysis as a means for detecting food risks earlier in the incident pipeline will be presented. Case studies showing how an EWS could have predicted the horsemeat incident of 2013, together with an example of how non-targeted analysis was successfully applied to meat production problem, will be used to demonstrate how potential risks can be identified before they impact on the consumer.

Keywords: *big data, machine learning, early warning system, non-targeted analysis, food fraud*

Acknowledgement: *The authors would like to acknowledge that this work has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688 (FoodIntegrity).*

L117
ANALYTICAL DATA ON FOODS AND FEEDS – SHOULD WE LINK THEM BETTER?

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High quality analytical data are essential for correct decision making on food safety at all levels – at the company, at the national and at the European Union (EU) level. The Regulation (EC) No 178/2002 lays down the general principles and requirements of food law and procedures with regard to food safety. In this regulation it is specified that when the decisions are taken they are taken based on available scientific evidence. It is well known that at the EU level, the European Food Safety Authority (EFSA) compiles the scientific evidence to the forms of risk assessments (opinions of EFSA) or to the forms of scientific reports of EFSA, such as updated exposure assessments. However, it also needs to be kept in mind that, at the moments when the decisions are taken at the EU level, other relevant factors such as societal, economic, traditional, ethical and environmental factors and the feasibility of controls, should also be taken into account. Therefore these aspects have also been included in the aforementioned piece of EU-legislation of Regulation (EC) No 178/2002.
At the national level the same applies and there the local interests and food cultures are taken into account when decisions are taken. The food business operators are then the ones who ultimately are responsible for the safety of their food products (and feeds), and the compliance with the EU food (and feed) legislation. Therefore, the accurate analytical data are essential for their decision taking, and similarly all the other information needed to guarantee the safety of the product should be error-free.
Taking decisions require that the available data and other information around them from different sources are linked together. At the end the customer makes his own decision based on his preferences and/or habits, and further expects that the food (and feed) he has purchased for himself or for his farm animals or pets is safe and that the information given on the product is accurate and not miss-leading.
In this presentation some thoughts are given with regard to the data and the fact that during the information generation process thorough thinking should be made how the produced results and information could be linked further and used in several ways to serve many different goals. We have already now a lot of information available and we generate it every day more and more in various forms. The overall outcome should be useful for many purposes and serve the heavily changing world when the natural resources are getting short and the population of the world only increases requiring more food for its dinner table.

L118
ANALYTICAL VALIDATION OF UNTARGETED METHODS IN FOOD METABOLOMICS AND GENOMICS: AN OVERVIEW ON THE STATE OF THE ART AND PERSPECTIVES FROM THE FOOD INTEGRITY PROJECT

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Food integrity, food traceability and food authenticity must be considered three priorities in food area and health. Authenticity and food quality can be assessed exploiting targeted or untargeted analytical methods, using different approaches. In the targeted methods area a number of organizations develop standards and guidance, unlike in untargeted methods.
Metabolomics comprises different methods and techniques useful to measure and profile small molecules in foods, leading to fingerprints able to describe a unique food composition. Moreover, genomic analysis (e.g. bar coding or Next Generation Sequencing) can generate big data useful in "food integrity" area. As pre-requisite, the harmonization of the untargeted analytical approaches requires a robust analytical validation, firstly considering the "post analytical processing" of data sets (e.g. spectral processing, spectral deconvolution) and the correct identification-detection of the "features". Many approaches based on the "artificial-intelligence" are dedicated to the data mining ("*deep analytics*"). Univariate and multivariate (supervised or unsupervised) methods represents the base of this complex analysis. When applied to untargeted profiles, these approaches allow the analysis, the extraction and the organization of large amounts of data in an "easy to read" form, that is useful and beneficial for the analytics software application. However, the criteria for the validation of untargeted methods are often not clearly identified, requiring more effort in their selection. The performance assessment, as well as the criteria based on the acceptance of data can be considered further targets in this area. Moreover, different problems (e.g. geographical origin, integrity and authenticity...) require customized solutions during validation process, also depending on the design of the system (one-class, multi-class).
The main activity of the INTELLItrace WP18 belonging the Food Integrity Project is focused on the development a Consensus Paper, useful as guideline to validate untargeted analytical methods.
This oral communication will deal with the description of the state of the art in this field, based on the literature previously published and on the Guidelines already prepared and disseminated worldwide [1-3]. Moreover, some examples of application and troubleshooting's will be discussed, showing the ongoing work of the research belonging Food Integrity Project.

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[2] Riedl, J., Esslinger, S., & Fauhl-Hassek, C. (2015). Review of validation and reporting of non-targeted fingerprinting

approaches for food authentication. *Analytica chimica acta*, *885*, 17-32.
[3] FCC 10 3S USP. Non-Targeted Methods for Adulteration Detection – Appendix XVIII pp 2053-2066

Keywords: *food authenticity and traceability, food integrity, non-targeted methods, analytical validation, metabolomics and genomics*

Acknowledgement: *The Project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant agreement No. 613688.*

L119
TARGET, SUSPECT AND NON-TARGET HRMS SCREENING APPROACHES FOR FOOD AUTHENTICITY AND QUALITY: FROM RESEARCH TO INDUSTRIAL APPLICATIONS

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During the last decade, the scientific community has faced a great breakthrough in food analysis due to the development of high-throughput omics technologies. Through the holistic foodomics approach, researchers are now facing the possibility of connecting food components with food quality and food safety. In this context, the MS-based metabolomics approach, being the characterization of the entire small metabolite composition of a particular food, has found a high number of applications in food science. In particular, high resolution mass spectrometry (HRMS) has proved to have excellent analytical performance, allowing the analysis of a wide range of micro-constituents and assisting the identification for both non-targeted and targeted ("suspect") compounds. The exploration of food adulteration and the assurance of geographical and botanological origin of food products are only a few research fields that MS-based metabolomics can apply to, holding promise for the discovery of potential (bio)markers in relation to food authenticity and food safety. In our laboratory, integrated target, suspect and non-target screening workflows based on LC–QToF–MS have been developed and optimized for the detection and identification of target, suspect and unknown compounds in an extensive variety of food matrices. Extensive databases were developed including targeted endogenous food metabolites (619 compounds), as well as a wide "suspect" MS-ready database, including thousands of natural products. QSRR models were developed for the retention time prediction of "unknown" compounds under RPLC and HILIC elution modes and a Retention Time Index (RTI) system was developed for interlaboratoy harmonization of LC–HRMS screening. Data analysis and evaluation in non-target HRMS screening workflow was significantly assisted using advanced data processing tools combined with advanced chemometric techniques, like Affinity Propagation for clustering, Ant Colony Optimization (ACO) for feature selection and Random Forest for prediction (ACO-RF/RF). The developed workflows have been implemented in different food authenticity studies, like the varietal discrimination or the discrimination of organic and conventional Extra Virgin Olive Oils, the characterization of honey based on its botanological and geographical origin and the detection of juice-to-juice adulteration. Furthermore, different applications of MS-based metabolomics have been used to answer challenging issues of the food industry, like questions on the packaging material migration in chocolate products, composite food analysis and new (bio)markers for fish freshness (in refrigerated products) and many more other examples.

Keywords: *foodomics, authenticity, high resolution mass spectrometry, advanced chemometrics, food industry*

L120
PESTICIDE RESIDUE ANALYSIS IN HUMAN HAIR: AN ALTERNATIVE OPTION FOR (DIETARY) EPOSURE ASSESSMENT?

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Exposure assessment of the consumer to pesticides residues is typically done based on residue data from food monitoring (mostly available for raw commodities only) and food consumption databases. To a lesser extent, total diet studies (market basket surveys) are done to estimate exposure. Human biomonitoring is another alternative and can bring added value for chemical risk assessment because it can reduce the assumptions regarding consumption rates and it integrates exposures from other sources (e.g. house-hold use, exposure from nearby agricultural activities) [1]. For biomonitoring several matrices can be used of which urine is most common. Hair is an alternative non-invasive matrix. Advantages compared to urine are easy of collection and storage, getting data on cumulative long-term exposure, and the ability to target parent compounds rather than metabolites.
The aim of this work was to develop a generic method for the simultaneous detection of multiple pesticides in human hair. The focus here was on 25 prioritized pesticides that so far have not been included in existing human biomonitoring programs. The method used for hair analysis involved washing/decontamination, pulverization, extraction (cleanup) and LC–MS/MS analysis. Several extraction methods were tested using naturally contaminated hair, and were compared for extraction efficiency and detectability. Acetonitrile with overnight ultrasonication was finally selected as best compromise. The final method was validated at the 0.5, 1.5 and 5 pg/mg level.
To gain insight in levels of pesticides, hair samples from 25 subjects were analysed. Multiple pesticides were detected in many cases. The most frequently detected pesticides included azoxystrobin, boscalid, imazalil, imidacloprid, and thiabendazole. Detectable levels were typically in the low pg/mg range, but in some cases as high as 36 pg/mg. An initial comparison was made between detection rates in hair vs detection rates in fruit and vegetables.
In hair, compounds tend to be stable for a long time which opens up possibilities to follow exposure in time by segmental analysis of hair strands. This was done for 30 cm hair strands from two subjects. Different trends were observed.
The results show that multi-residue analysis in hair is feasible and has potential as tool for exposure assessment. Comparison of levels of exposure between different populations (e.g. rural – non-rural, organic – non-organic diet) is in principle possible. For linking levels in hair to actual exposure, however, much more research on factors affecting incorporation, and toxicokinetics is needed.

[1] Choi J, Aarøe Mørck T, Polcher A, Knudsen LE, Joas A, 2014. EFSA supporting publications 2015: EN-724. [321 pp.]

Keywords: *pesticides, human biomonitoring, hair analysis, exposure assessment*

L121
INVESTIGATION OF THE PHENOLS ACTIVITY IN EARLY STAGE OXIDATION OF EDIBLE OILS BY EPR AND 19F NMR SPECTROSCOPIES USING NOVEL RADICAL INITIATORS

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A new dynamic method is introduced based on novel lipid Vanadium metal radical initiators for the investigation of the mechanism of oils oxidation at early stage. This method involves the addition of oil-soluble vanadium complexes in oils, in catalytic quantities, in order to activate the dioxygen contained in oils and generate radicals. These radicals are trapped by a-tocopherol generating a-tocopheryl radicals monitored by EPR spectroscopy *vs* time. The time interval until the occurrence of maximum peak intensity be reached (t_m), the height of the maximum intensity and the rate of the quenching of the a-tocopheryl radical are correlated with the total phenolic content, and consequently with the oxidative stability of the oils. The direct measurement of the alpha-tocopheryl radical *vs* time in oils consists an advantage of this method and is more suitable for the exploration of the kinetics of the oil oxidation compare with the indirect steady state methods performed by the addition of radical traps.
This study shows that in edible oil, vanadium complexes activate dioxygen to superoxide radical, and this is attributed to the prooxidant activity of phenols. The metal complexes act synergistically with the oil phenols as indicated by the variable temperature EPR spectroscopy, thus, the thermodynamically unfavored one electron reduction of the oxygen is facilitated. The superoxide radical first reacts with the lipids forming alkoperoxyl and alkoxyl lipid radicals. These radicals react with the most reactive phenol in oil, the alpha-tocopherol, resulting in the formation of alpha-tocopheryl radical and lipid alcohols. As evidenced by the ^{19}F NMR spectra double quantity of the radical initiator was needed in order the polar phenols to be oxidized. After the alpha-tocopherol is consumed the rest of the phenols eventually continues the work of alpha-tocopherol, quenching the radicals.
The alpha-tocopherol contained in the oils traps the radicals forming alpha-tocopheryl radical, and the kinetics of the reaction were monitored by EPR spectroscopy. The intensity of the signal and the lifetime of the radical depend on the type of oil, the storage-period and the phenol content of the oil. The radical trap experiments show that the mechanism of the reaction goes through the oxidation of the aliphatic unsaturated fatty acids. ^{19}F NMR experiments show that at the presence of metal ions the polar phenols of the oil rather directly react with the fatty acid radicals than recycling the a-tocopherol.

Keywords: *EPR/NMR, alpha-tocopheryl radical, olive oil, vanadium complexes, prooxidant/antioxidant*

Acknowledgement: *Supported by Research Promotional Foundation of Cyprus and the European Structural Funds ANAMISI/PAGIO/0308/32.*

L122
MARINE RESOURCES – ORIGIN FOR HEALTH BENEFICIAL COMPONENTS

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A variety of different marine resources is potential as future bioactives for health.
This includes marine invertebrates, microalgae, plants and marine bacteria together with biological rest raw material from fisheries and are sources for exploring novel bioactive components. In this context, marine proteins and peptides are very interesting.
The wide repertoire of biological functions that such natural peptides have, make them prosperous for bioprospecting and drug discovery. Among them, antimicrobial peptides (AMPs) are interesting because of their potent activity and different mechanisms of action.
The worldwide spreading of resistant antibiotic genes in bacteria resulting in less effect of commercial antibiotics to pathogenic bacteria makes it important to learn from nature and novel mechanisms of actions of marine AMPs or small secondary metabolites.
These molecules are important components of the innate defense system in invertebrates in addition to also be an important source for potential new drug leads of antimicrobials and anti-biofilm components. In general, small peptides have advantages in their rather specific activities like small size, short half-lives, usually non-immunogenic properties and low toxicity.
A majority of approved drugs today are based on knowledge from natural products (over 60%) from terrestrial sources. However, only 1% of these has marine origin. Thus, their unique bioactive properties are the focus of attention in addition of being usable as food or feed.
We have characterized several new cases of such AMPs and explored their mode of action. The organisms have been collected from the Arctic or/and sub-Arctic region. Novel bioactive peptides are isolated, characterised and explored by traditional bioassay-guided purification of organic extracts in combination with genetic approaches. In addition, a more extensive screening, performing biosensoric tests have revealed mechanisms of action of peptides towards the membrane or integral targets of bacteria. Defining the pharmacophores of the molecules by performing SAR studies, make it possible to design improved marine natural products mimics that are prosperous as candidates of novel drug leads with specific properties.

Keywords: *marine bioactives, antimicrobial, antibacterial peptides, anti-biofilm, marine natural products mimics*

Acknowledgement: *The ork was financed by theR research Council of Norway, Tromsø Forskningsstiftelse and the UiT The Arctic University of Norway.*

L123
ANALYSIS OF CANNABINOIDS IN DRIED PLANTS, CONCENTRATES AND FOODS: DEVELOPMENT AND VALIDATION OF A VERSATILE UHPLC–DAD/(HR)MS METHOD

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In the last few years, the popularity of *Cannabis* plants and cannabis-based products has significantly increased, as many countries around the globe adopted more liberal view towards the use of this plant for medicinal purposes. Among more than 400 compounds identified in Cannabis plants, most attention has been paid to cannabinoids, especially Δ^9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) due to their biological activities.
Gas chromatography with flame ionization detection (GC–FID) and high performance liquid chromatography with UV spectrophotometric detection (HPLC–UV) or mass spectrometric detection (MS) represent techniques most widely employed for analysis of cannabinoids in biological matrices. In general, LC-based analysis is preferred, as in contrast to GC, no thermally-induced decarboxylation of cannabinoid acids occurs and whole cannabinoid profile can be assessed.
This presentation will discuss development and validation of a versatile method employing ultra-high performance liquid chromatography (UHPLC) for quantification of 12 major cannabinoids in *Cannabis* dried plants and concentrates. The chromatographic separation system used is compatible with both diode array and mass spectrometric detection. While UHPLC–DAD was employed for quantification, quadrupole-time-of-flight high resolution mass spectrometer (Q-TOFMS) operated in full MS and MS/MS mode was connected in series with UHPLC–DAD during method development to provide high confidence analyte identification. This configuration also allowed monitoring other less abundant cannabinoids without reference standards. Finally, the use of this method with UHPLC and new compact single quadrupole mass detector for cannabinoid analysis in challenging matrices, such as foods, will be demonstrated.

Keywords: *cannabinoids, UHPLC, diode array detection, high resolution mass spectrometry*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and Financial support from specific university research (MSMT No 20-SVV/2017).*

L124*
METABOLOMICS ON CANNABIS SATIVA L. EMPLOYING ION MOBILITY Q-TOF LC/MS INSTRUMENTAL PLATFORM

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Cannabis sativa L. is a multi-use crop known for a wide range of bioactivities (including psychotropic). The spectrum of *Cannabis* phytochemicals is extremely complex, up to now, almost 600 secondary metabolites have been identified, among them, more than 200 endogenous cannabinoids. Nevertheless, *Cannabis sativa* L. is a hypervariable plant, the bioactive chemicals pattern fairly depends not only on the respective variety, but also on a geographic origin, growing practices and harvest year. Moreover, due to their relative instability, further changes of cannabinoids can be induced by post-harvest processing practices. Although being one of a largely investigated plant species, bioprospection of *Cannabis* is still of a high interest, mainly because of their potential use for medical and health care purposes. To perform a detailed chemical characterization of various *Cannabis* plants and products thereof, UHPLC-Q-TOF /MS instrumental platform equipped with a drift time ion mobility (DTIM) was employed for metabolomics fingerprinting / profiling of examined samples. For the profiling purpose, an extensive database of all phytocannabinoids and many other bioactive compounds, occurrence of which was reported in literature (e.g. terpenoids, alkaloids, flavonoids, etc.; altogether 330 compounds), was established. Thanks to three dimensional separation employed for samples analysis, several isomeric and isobaric constituents were discovered and thanks to improved spectral purity, achieved by ion mobility separation, even minor components could be identified. To support identification of target compounds, also measurement of the collision cross-section (CCS) values was performed. Critical assessment of the benefits and limitations resulting from integration of ion mobility into analytical platform will be further provided.

Keywords: *Cannabis sativa L., cannabinoids, metabolomics, ion mobility Q-TOF LC/MS, collision cross-section*

Acknowledgement: *This work was supported by the Technology Agency of the Czech Republic (project No TA04010331) and "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by specific university research (MSMT No 20-SVV/2017).*

L125
DEVELOPMENT OF LABEL-FREE ELECTROCHEMICAL SENSORS BASED ON SCREEN PRINTED ELECTRODES FOR BIOACTIVE COMPOUNDS MONITORING IN PLANT EXTRACTS

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Various plants and fruits contain high concentrations of several redox-active antioxidants, which can inhibit the oxidation of biomolecules by peroxide ions. Sensors and biosensors offer a real-time response with higher sensitivity and selectivity for bioactive compounds, some of which have been shown to act as antioxidants.
In this work different hydrosoluble extracts with antioxidant capacity were obtained via two extraction methods. Nanoparticle modified screen-printed carbon electrodes have been successfully used to develop sensitive, label-free electrochemical sensors for rapid and sensitive antioxidant detection and total antioxidant capacity monitoring of the studied extracts. Tyrosinase biosensors were also used for detection of phenolic compounds. Voltammetry and fixed potential amperometry measurements were performed using carbonic and gold nanoparticles; studies of pH and working potentials together with nanoparticles type and extract concentrations influence were carried out for sensors optimization.
The results were validated and correlated using the classical methods of spectroscopy (UV-VIS, FTIR and Raman). Highest antioxidant activity and optimized sensors configuration have been outlined. Best extracting method which conducts to the highest antioxidant activity of plant extracts and optimized sensors configuration have been highlighted.

Keywords: *antioxidants, nanoparticles, biosensors, plant extracts*

Acknowledgement: *This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-2801. We hereby acknowledge the structural funds project PRO-DD (POS-CCE, O.2.2.1., ID 123, SMIS 2637, No 11/2009) for providing the infrastructure used in this work.*

L126*
ONLINE EXTRACTION AND DETERMINATION OF BIOACTIVE COMPOUNDS FROM FOOD SAMPLES BY MEANS OF SUPERCRITICAL FLUID EXTRACTION-SUPERCRITICAL FLUID CHROMATOGRAPHY-MASS SPECTROMETRY

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Extraction and detection of bioactive compounds from food samples is a very hard task. Conventional extraction procedures are usually time consuming and can require a very high amount of organic solvents. Moreover, bioactive compounds can be photolabile and/or thermolabile, in this case must be immediately injected to avoid any kind of degradation.
The aim of this research was focused on the development of an online method coupling supercritical fluid extraction and supercritical fluid chromatography (SFC) for the extraction and detection of bioactive compounds (carotenoids) from food samples. The online nature of the system, compared to offline approaches, improves run-to-run precision, enables the setting of batch-type applications, and reduces the risks of sample loss and contamination.
Carotenoids were extracted and detected from red habanero peppers, and tamarillo. Regarding the red habanero peppers, 21 targeted analytes were extracted and identified by the developed methodology in less than 17 minutes, including free carotenoids, carotenoids monoesters and carotenoids diesters, in a very fast, and efficient way. Whereas 17 targeted analytes were extracted from the tamarillo sample. Multiple extractions, until depletion, were performed on the same sample, in order to evaluate the extraction yield and to obtain quantitative data. The online supercritical fluid extraction supercritical fluid chromatography method developed was then compared with the traditional solid-liquid extraction HPLC-MS giving comparable results.

Keywords: *SFE-SFC-MS, carotenoids*

L127
THE SAFETY ASSESSMENT OF BOTANICALS AND BOTANICAL PREPARATIONS FOR USE IN FOOD OR FOOD SUPPLEMENTS

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Botanicals and derived preparations made from plants have become widely available on the EU market in the form of food supplements. Examples include ginkgo, garlic, St. John's Wort and ginseng. Such products are typically labelled as natural foods and a variety of claims are made regarding their possible health benefits. They can be bought over the counter in pharmacies, supermarkets, specialist shops and via the internet. While most of these products have a long history of use in Europe, some concerns exist about their safety and quality. Indeed, plants contain naturally occurring substances that can be of concern for human health. In order to be able to assess the safety of plant-based products, a number of information is required, among which the chemical composition of the plant-based product to be marketed in Europe. In order to assist food manufacturers and risk assessors, The European Food Safety Authority (EFSA) has been developing a database of botanicals reported to contain naturally occurring substances of possible concern for human health; the database also contains case reports of adverse effects following the oral intake of botanicals /botanical preparations. A systematic literature review was contracted out for the 2500 plants species contained in the database and composition / adverse effects information were extracted to be transferred to the EFSA database. This presentation will provide an overview of the methodology applied to build the EFSA database.

L128
PERSPECTIVES OF NON-TARGETED METABOLOMICS IN FOOD ANALYSIS

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In the newly created concept of "foodomics", non-targeted metabolomics play a key role to assess the "foodome" [1]. The respective platforms using high-resolution techniques target on assessing food quality comprehensively. Of the latter, one aspect is elucidating or confirming the authenticity of foods. This term generally is related to one or more of the following attributes: geographic origin, type of agricultural production, species and kind of raw materials, or certain process qualities such as sustainability or ecologic foot print. Suitable platforms such as Fourier transform ion cyclotron mass spectrometry (FT/ICR-MS) or multidimensional NMR allow a more detailed insight e.g. into complex food-related processes such as the Maillard reaction [2].
Apart from authenticity, non-targeted metabolomics may also open new avenues into safety evaluation of foods and food components. New developments in the field of mycotoxin to describe the "mycobolome" will be presented [1].

[1] Rychlik M, Kanawati B, Schmitt-Kopplin P (2017) Foodomics as a Promising Tool to Investigate the Mycobolome, Trends in Analytical Chemistry, doi.org:10.1016/j.trac.2017.05.006
[2] Hemmler D, Roullier-Gall C, Marshall JW, Rychlik M, Taylor AJ, Schmitt-Kopplin P (2017) Evolution of Complex Maillard Chemical Reactions, Resolved in Time, Scientific Reports 7: Article number: 3227, doi:10.1038/s41598-017-03691-z

Keywords: *foodomics, mycotoxins, Maillard reaction*

L129
NON-INVASIVE HIGH-THROUGHPUT FOOD VOLATILOMICS BY DIRECT INJECTION MASS SPECTROMETRY: PROFILING AND BIOPROCESS MONITORING

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The volatilome, considered as all volatile metabolites and volatile organic and inorganic compounds released by a system possibly also during and after processing, plays a relevant role in food science and technology: it is, e.g., important for crops ecology and physiology, it drives fruit changes during ripening and storage and controls to a large extent the way we perceive food before (odor), during (aroma) and after (aftertaste) consumption. Moreover, being spontaneously and continuously released, volatile compounds provide a non-invasive and rapid tool for the control of agriproducts and the real-time monitoring of biological and technological processes.
An efficient volatilomics approach, however, must rely on i) high sensitivity and large dynamic range because volatile compounds can produce biological or sensory effects at different, possibly very low, concentrations and ii) fast and non-invasive measurements both to allow the screening of large sample sets and the monitoring of rapid processes. These issues can be efficiently addressed by different Direct Injection Mass Spectrometry (DIMS) methods developed for volatile compound analysis, Proton Transfer Reaction Mass Spectrometry (PTR-MS) in particular. The lack of specificity of these techniques, as compared with chromatographic ones, is compensated by other features: they are very fast, non-invasive and provide high sensitivity even without sample pretreatment.
This contribution, after a short description of a prototypical DIMS set-up based on PTR-MS developed for agroindustrial applications, aims at pointing out DIMS pros and cons in food volatilomics by describing few selected applications investigated at the Volatile Compound Facility at FEM. Firstly, PTR-MS profiling of crops and dairy products has been used for sample sets exploration and to set classification or calibration models that link food volatilome with sensory or genomics allowing, for instance the efficient identification of quantitative trait loci related to fruit volatile compounds, the setting of instrumental models of sensory quality which should make "sensomic" studies realistic and the identification of typicality markers. Secondly, a fully automated system for the monitoring of volatile compounds released during biological or technological processes has been developed and used to investigate microbiological processes as bread leavening, lactic and alcoholic fermentation and spoilage during storage. Recent developments which should increase specificity (coupling with fastGC) and sensitivity (ion focusing in the drift tube) of PTR-MS based methods without compromising its positive features are also described. To date the simultaneous monitoring of most VOCs released by agroindustrial products can be achieved non-invasively, with high sensitivity (sub ppt) and with a thoughput of more than 300 samples a day.

Keywords: *PTR-ToF-MS, PTR-MS, volatilome, food, high-throughput*

Acknowledgement: *The support of the Autonomous Province of Trento is acknowledged (ADP 2017).*

L130*
MARKER DISCOVERY IN VOLATOLOMICS BASED ON SYSTEMATIC ALIGNMENT OF GC-MS SIGNALS: APPLICATION TO FOOD AUTHENTICATION

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This work focused on an application of volatolomics to the authentication of three walnut oils differing by their extraction process but with very similar composition. Therefore, the objective of the present study was to assess a systematic and untargeted approach to reveal Volatile Organic Compounds (VOCs) markers for oil authentication. One hundred and seventeen oils from the three different oil groups were analyzed by Dynamic HeadSpace Purge & Trap - Gas Chromatograhy - Mass Spectrometry (DHS P&T-GC-MS) during an analytical campaign of four months. The principal requirement of the systematic approach is to correct data noise, especially temporal drifts. A hybrid alignment strategy combining a warping-based alignment followed by a local peak realignment was developed to address the important temporal drifts generated in the dataset [1]. COW was first used to correct important linear drifts without peak shape modification and then a customized algorithm was used to correct residual local non-linear shifts. The study consisted of four steps to assess the hybrid strategy for edible oil authentication. First, the efficiency of the hybrid alignment approach was demonstrated in terms of signal alignment and quality of oil discrimination. Second, the reliability of the corresponding discriminant markers was validated according to expert processing and literature data. Third, the potential of the systematic and untargeted approach to reveal markers difficult to find with a manual search by an expert was discussed. Finally, the proposed hybrid strategy was benchmarked to two other state-of-the-art alignment strategies (iCoshift and STW) in terms of alignment quality and ability to detect discriminant markers. The work finally discusses the adding-value of the present finding for applying volatolomics to food authentication.

[1] C. Deport, J. Ratel, J.L. Berdagué, E. Engel, Comprehensive combinatory standard correction: A calibration method for handling instrumental drifts of gas chromatography-mass spectrometry systems, J. Chromatogr. A. 1116 (2006) 248–258. doi:10.1016/j.chroma.2006.03.092.

Keywords: *volatolomics, alignment, authentication, gas chromatography-mass spectrometry, systematic marker discovery*

L131
ISOTOPIC AND MASS PROFILING DATA TO ESTABLISH (DIS)SIMILARITIES BETWEEN AGRICULTURAL PRODUCTION PRACTICES IN TOMATOES

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Consumer´s interest in organic foods is increasing and so is the need for robust analytical tools for their authentication. The EU organic food market is the second largest in the world behind the US. Organic farming in the EU is supported by EU law, Regulations (EC) No 834/2007 and 889/2008/EC [1,2] However, the lack of reliable markers to discriminate between organic and conventional products makes this market susceptible to foods labelled as "organic" that have, in fact, been produced conventionally.
To date, analysis of stable isotopes has proven them to be valuable indicators of agricultural practices, although a complete discrimination has not been found. However, the MS profile of a food sample can be regarded as an analytical signature of the food product and thus can help in discriminating between different practices, reflecting the impact of both endogenous and exogenous factors as well as the food's properties. But, the potential of these tools to assemble the data sets of crop/fertilizer correlation has not been extensively explored for application to organic vegetables.
Thus, the principal aim of this study was to investigate the potential of advanced technologies based on liquid chromatography-high resolution accurate mass spectrometry (LC-HRAMS) for the identification of biomarkers capable of distinguishing between organically and conventionally grown tomatoes in greenhouse under controlled agronomic conditions, and in a climatic region of leading EU production such as the Mediterranean. Nitrogen stable isotope ratios (IRMS) were also used as a valuable supporting tool for the authentication of organic production.
Promising results according to farming systems and significant differences in peak areas were observed for some bioactive components. In light of these preliminary results, it is possible to conclude that metabolomic fingerprinting/profiling of low molecular weight food components is a hopeful approach for the identification of potential food biomarkers to discriminate between organically and conventionally grown tomatoes. The combination of IRMS and HRMS techniques were useful analytical tools to supporting the authentication of tomato organic crops in this study.

[1] EC Regulation 834/2007. Council Regulation 834/2007/EC of 28 June 2007 on organic production and labelling of organic products. Official Journal of the European Union L189, 20.07.2007:1-23.
[2] EC Regulation 889/2008. Commission Regulation 889/2008/EC of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. Official Journal of the European Union L250, 18.9.2008:1-84.

Keywords: *organic food, biomarkers, HRAMS, IRMS*

Acknowledgement: *M.J. Martínez Bueno thanks the Marie Skłodowska-Curie Actions (MSCA) for the individual fellowships (H2020-MSCA-IF-2015_#707816_ORGANIC QUAL TRACERS).*

L132*
NMR-BASED METABOLOMICS TO UNDERSTAND THE ABSCISSION PHENOMENON OF OLIVES AND FOR THE CHARACTERIZATION OF WINES

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Metabolomics, is one of the latest 'omics' sciences and deals with the study of global changes in the entire metabolite set of certain cells, tissues and organisms. The application of metabolomics in food systems, named "foodomics", can be applied to all food system processes from farm to human, including food resource production, industrial food processing and food intake by humans. The metabolites in food matrices are affected by multiple factors such as botanical or geographical origins, cultivars, climates, storage conditions, biological and physiochemical processing. Therefore, the comprehensive and quantitative analysis of metabolites, can provides the detailed features of foods. Nuclear Magnetic Resonance (NMR) is one of the most suitable techniques to obtain "high-throughput" analytical data in metabolomics, and it gives a complete view of the foodstuff (e.g., olive oil, wine, fruit juice, honey) metabolites. The distinguished advantages of NMR are its nondestructive and noninvasive nature and its ability to detect quantitatively and reproducibly a broad range of substances with different chemical properties such as sugars, lipids, organic acids and many others. Multivariate and univariate statistical analyses not only define the differences among food matrices based on their NMR spectra, but also suggest candidate biomarkers contributing to the features of food. Regarding the application of NMR in food metabolomics, herein I will present two different studies. The first one aims to identify mechanisms responsible for olive fruitlet drop of the cv Frantoio, a widely cultivated Italian olive cultivar. *Olea europaea* is one of the most important and widespread fruit trees in Mediterranean basin. Olives are a product of great economic importance for EU (about 73% of the global olive oil production). The low yield (and the high costs of the grove management) makes olive growing as a less profitable cultivation if compared with other crops. Since no specific information is available to understand the molecular mechanism and metabolic processes inducing fruitlet abscission in olive tree, this project aims to use metabolomics as an essential step to find possible practical solutions in order to reduce the incidence of fruitlet shedding and increase the final yield. The second one is a research work related to applications of NMR spectroscopy in combination with multivariate statistical analysis for the characterization and authentication of Tuscan wines. In this study, we demonstrate that NMR metabolomics is a very powerful analytical tool to investigate the variability of the metabolic profile of wines, not only to assess the grape variety, vintage and geographical origin, but also to distinguish wines produced in terracotta jars or in steel barrels.

Keywords: *foodomics, NMR, metabolic characterization, fruitlet abscission, wine autentication*

Acknowledgement: *These studies were conducted in collaboration with Prof. P. Tonutti, PhD S. Brizzolara, A.M. Dourou from Scuola Superiore Sant'Anna (Pisa, Italy) and PhD T. Martellini and L. Sposato from Chemistry department of University of Florence (IT).*

L133*
ESTIMATION OF ORGANOLEPTIC ATTRIBUTES OF OLIVE OILS BY UNTARGETED GC-MS AND METABOLOMICS-BASED STATISTICAL APPROACH DURING QUALITY CLASSIFICATION

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Olive oil quality is a matter of concern for consumers and producers. It establishes the differences between low value products with poor attributes and products with outstanding features, and, consequently, it contributes to set oil prices. This classification, as established by Spanish legislation, is performed by testers who establish if an olive oil must be labelled as extra virgin, virgin or lampante (not recommended for consumption). This strategy is known as "PANEL TEST", which classifies the oils according to two main properties: defects and good attributes. According to the literature [1], [2], the organic compounds responsible of these attributes have been determined to be predominantly volatiles, including esters, ketones, aldehydes, alcohols, terpenes, phenols and their derivatives, with different concentrations and odour thresholds. Taking this into account, a more objective methodology, based on instrumental responses, could be presented as a cheaper and faster alternative approach to PANEL TESTs interesting for the olive oil industry, and could be also useful as a complementary tool to prevent fraud due to sample adulteration. In this work, the capabilities of purge and trap extraction followed by thermal desorption in combination with gas chromatography (GC) coupled to single quadrupole mass spectrometry (MS) have been tested for the determination of volatile components of olive oil. Our results, have revealed a great potential for the identification of defect-related components, as well as for olive oil classification according to their quality when using metabolomic-based approaches. The full MS spectrum acquisition has allowed the detection and identification of volatile organic compounds (VOCs) in olive oil samples, including flawless extra virgin, virgin and lampante samples with a wide range of organoleptic defects. The metabolomics strategy consisted of three different steps: a component detection from GC-MS data using PARADISe [3], a multivariate analysis using EZ-Info and the creation of the statistical models with combinations of responses for compounds with the greatest impact on oil characteristics. The developed procedure allowed to tentatively identify a great number of compounds responsible for each type of defect, as well as to create an improved classification methodology. This classification method was finally validated using blind samples, obtaining an accuracy in oil classification of 85 % taking the official established method, "PANEL TEST" as reference, with a 100% of extra samples correctly classified, and a small overlapping between virgin and lampante samples.

[1] G. Luna et al. *Food Chem.*, vol. 98, no. 2, pp. 243–252, 2006.
[2] C. Sales et al *Food Chem.*, vol. 216, pp 365-373, 2017.
[3] L. G. Johnsen, et al *J. Chromatogr. A*, vol. 1503, pp. 57–64, 2017.

Keywords: *olive oil, metabolomics, gas chromatography, mass spectrometry, purge and trap*
Acknowledgement: *The authors acknowledge the financial support of Generalitat Valenciana, as research group of excellence (PROMETEO II/2014/023) and Collaborative Research*

on Environment and Food-Safety (ISIC/2012/016). This work and C. Sales grant has been supported by Universitat Jaume I research promotion plans (P1-1B2013-70).
The investigation has been performed within the frame of scientific collaboration between the "Ministerio de Agricultura, Alimentación y Medio Ambiente", the "Consejería de Agricultura, Pesca y Desarrollo Rural de la Junta de Andalucía" and the "Interprofesional del Aceite de Oliva Español".

L134
MAY 2 APPLES A DAY REALLY KEEP THE DOCTOR AWAY? UNTARGETED METABOLOMICS BASED ON UHPLC-HRMS REVEALED FURTHER INSIGHT INTO CARDIOVASCULAR DISEASE RISK AND GUT HEALTH

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Apples are among the most frequently consumed fruits in the world. Several observational studies have shown that apple intake is associated with a reduced risk of cardiovascular disease. Polyphenols and fiber are considered the principal mediators for these effects but the detailed mechanisms are unclear. It has been shown that polymeric flavanols, proanthocyanidins, the highest polyphenolic class in apples, reach the colon almost intact where together with fiber they interact with the gut microbiota producing simple phenolic acids but also increasing the population of beneficial bacteria and compounds.
Thus, a randomized, controlled, crossover, dietary human intervention study was performed to explore the hypothesis that frequent apple consumption decreases cholesterol, improves vascular function and beneficially modifies the gut microbiota composition and activity. Volunteers, (23 women, 17 men) with a consumed either 2 apples/d (Renetta Canada) or a control sugar matched apple juice (without fibre and polyphenols) for 8 weeks separated by a 4 weeks washout period in a random order. Faecal, plasma and 24-h urine samples were collected. Fecal samples were further used for gut microbiota studies. On the one hand, 100µl of plasma were placed in a Sirocco protein precipitation plate (Waters, USA) with ISs and eluted. Then, samples were filtered, eluted, evaporated to dryness and reconstituted. On the other hand, 100µl of urine were loaded on the pressure-96 manifold (Waters, USA) together with ISs, filtered and diluted. Plasma and urine extracts were analyzed by a hybrid LTQ-Orbitrap (ThermoFisher). Raw files were converted to mzXML format and processed with XCMS. In order to identify the features showing a significant difference between the meals, two independent linear mixed models were fitted to each molecular feature. The comparison of the two models allowed the features significantly affected by the meal factor to be identified and *p values* were calculated. Discriminative markers were then compared with the monoisotopic molecular weight, isotopic patterns, molecular formula and MS/MS spectra of metabolites proposed by freely available databases, providing different levels of identification, from unambiguous to tentative identifications.
To sum up, the daily intake of 2 apples has shown that significantly decreased Enterobactericease population in healthy subjects. Urine and plasma metabolic profiles were different ($p < 0.05$) between the apple and the control juice identifying 8 biomarkers in blood and 54 in urine. Several polyphenol microbial metabolites, such as valerolactones, valeric and phenolic acids, were identified, indicating that microbial activity is crucial and a prerequisite for the

absorption of apple polyphenols, producing active metabolites with potential health benefits.

Keywords: *foodomics, untargeted metabolomics, UHPLC-HRMS, polyphenols, biological fluids*

Acknowledgement: *This study was financially supported by the ADP project, funded by the Autonomous Province of Trento (Italy), and by the Joint Action Biomarkers in Nutrition and Health, Project Food Biomarkers Alliance (FoodBall) of the Joint Programming Initiative a Healthy Diet for a Healthy Life (JPI HDHL) and the respective national funding organization, Ministry of Education, University and Research (MIUR). The study was given a favorable ethical opinion for conduct by the University of Reading Research Ethics Committee (UREC Project No. 13/22 (AVAG study), and is registered at clinicaltrials.gov as NCT01988389.*

L135
THE CHALLENGES TO THE INTEGRITY OF THE GLOBAL GOOD SUPPLY SYSTEM AND THE ROLE OF ANALYSIS IN MEETING THEM

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Our food system has become hugely complex and will continue to become more so. With the need to feed an ever growing world population the stresses on this system are becoming more and more apparent. The drive to produce more food and do it more cheaply but in a sustainable way is admirable in terms of giving the global population access to food. However, the integrity of how we produce this food must be maintained and protected. The authors definition of Food Integrity is based an adaptation of the 1996 World Food Summit definition of food security: *Food integrity is achieved when all people, at all times, have physical and economic access to food which is safe and authentic and those producing the food have their human rights protected.*
The role of food analysis has never been more critical in terms of protecting the integrity of what we eat. Some of the emerging technology platforms that will play a vital role will be outlined. Perhaps even more importantly the current analytical gaps we have and how the Fourth Industrial Revolution may play provide some of the solutions to fill them will be discussed.

Keywords: *food integrity, emerging technology platforms, food analysis*

L136
MATRIX COMPATIBLE COATINGS IN SPME: AN ENABLING TECHNOLOGY FACILITATING FULL AUTOMATION OF FOOD DETERMINATIONS AND DIRECT COUPLING TO MASS SPECTROMETRY

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The ever-increasing requirement for determination of contaminants along with studies of comprehensive metabolite networks in food samples parallel advancements in solid phase microextraction (SPME) technique. The availability of state-of-the-art analytical instrumentation offering higher sensitivity and specificity has contributed to an increased range of applications covered by the SPME technique. The presentation will summarise the most underlying aspects in SPME development addressing some of the challenges encountered in the analysis of food samples, with particular emphasis placed on complex sample analysis and rapid screening. In addition, the development of new morphologies or extracting materials and novel sampling configurations as well as approaches compatible with high throughput lab and/or on-site determinations will be outlined. The recent development of matrix compatible SPME coating lead to interesting features experienced during extraction, some of them not anticipated. They are not limited to elimination of fouling and saturation effects during direct SPME of complex samples, but also balance coverage property, enabling "via free form" clean extraction of small molecules widely varying in physical properties leading to some interesting applications. For example, on-site sampling, in-vivo metabolomics, and rapid screening via direct coupling of sample preparation to mass spectrometry were facilitated by this development. To serve as a guide to potential opportunities for continued innovation in SPME food applications, special emphasis will be placed on the evolution of on-site and in vivo SPME techniques and their feasibility for both targeted determination of organic pollutants and biologically active compounds, as well as for global metabolite analysis.

[1] Trends in Analytical Chemistry 2015, 71, 236–248
[2] Anal. Chem. 2017, 89, 8021−8026
[3] Anal. Chem. 2017, 89, 3805−3809
[4] Anal. Chem. 2017, 89, 4046−4054
[5] Anal. Chem. 2017, 89, 7240−7248

Keywords: *complex sample analysis, rapid screening, matrix compatible coatings, SPME*

Acknowledgement: *The work was supported by Industrial Research Chair sponsored by Natural Sciences and Engineering Research Council of Canada and Canada Research Chair programs.*

L137
FIELD-DEPLOYABLE LASER-INDUCED-BREAKDOWN-SPECTROSCOPY FOR MULTIPLEXED DETECTION OF FOOD CONTAMINANTS USING RARE-EARTH LABELED ANTIBODIES EMBEDDED IN A LATERAL FLOW STRIP

J. Paul Robinson*[1], **Carmen Gondhalekar**[1], **Euiwon Bae**[1], **Bartek Rajwa**[1]

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Timely detection of food and feed contaminants is a biosecurity concern due to the significant health risks associated with outbreaks caused by accidental contamination and bioterrorism. In the event of an outbreak, the gold-standard for detection are mouse bioassays. While effective in detecting trace amounts of contaminants, these assays are lab-based and take days to perform – a strategy which is ineffective at providing rapid information for potentially wide-spread, fast-moving and health-harming outbreaks caused by unknown agents. Research efforts are therefore focused on developing field-deployable technologies for detecting one or a panel of pathogens in food/feed. Many of these technologies are based on fluorescent and colorimetric probes. However, these probes pose an inherent challenge: increasingly multiplexed assays require increasingly complex and large/non-portable detection instrumentation.
Our approach allows us to increase multiplexing capability, without compromising detection instrumentation simplicity and portability. We do so using rare-earth-metal probes which offer a higher dynamic range, less signal overlap and less background than organic fluorescent or colorimetric probes. Rare-earth metals conjugated to antibodies allow us to target and label the contaminant (in our study, these are Shiga, ricin and botulinum toxins). The toxins labeled with rare-earth-metal-conjugated antibodies are detected using laser-induced breakdown spectroscopy (LIBS). LIBS characterize the atomic composition of a sample based on the optical emissions produced by energizing the sample with a laser beam. Each metal-conjugated antibody produces its own unique set of emissions based on the identity of the metal. Regardless of how many metals are present in the sample, the excitation and detection instrumentation remain the same – a laser and two compact spectrometers that detect emissions between 214 and 630 nm.
Contributing to instrument portability is the assay format in which the rare-earth conjugated antibodies (detection antibodies) are employed. We are designing paper-based lateral flow strips to perform the bioassay, where the analyte and detection antibody are captured at a "test" region of the strip, and a "control" region ensures that the assay is working properly. Lateral flow strips are small, low-cost and easy-to-use, as proven by the widely successful pregnancy test. Identifying the detection antibody, and therefore the toxin, will be performed by analyzing the "test" line with LIBS. Based on the elemental composition of the line, the identity of the contaminant can be determined.

Keywords: *LIBS, portable toxin detection, toxins, rare-earth-labels, food borne pathogens*

Acknowledgement: *This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No.1935-42000-072-02G to the Center for Food Safety Engineering. 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. CG is a NSF Fellow (grant number DGE-1333468).*

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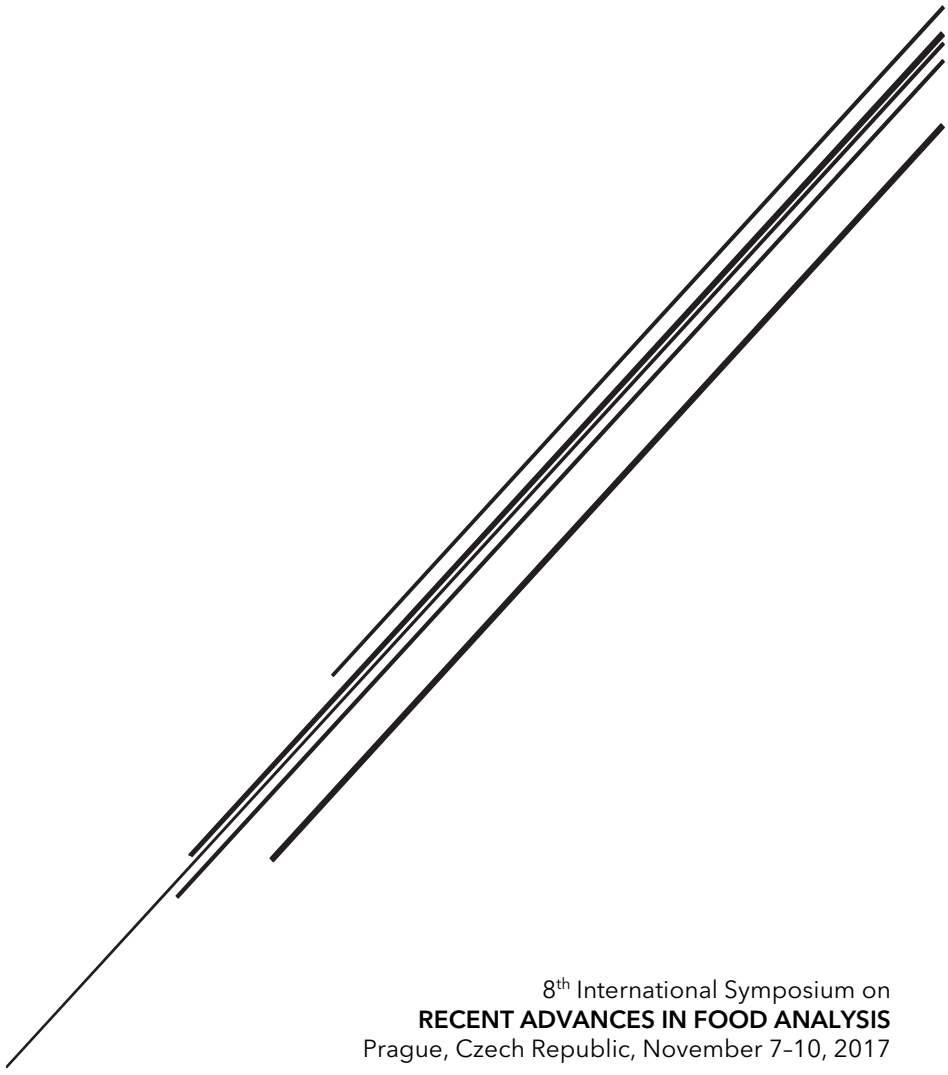

8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

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A1
MAJOR ALLERGENS IN COW MILK'S WHEY: CHEMICAL MODIFICATIONS INDUCED BY TECHNOLOGICAL TREATMENTS AND THEIR EFFECT ON ALLERGENICITY

Alessandra Gasparini*[1], **Sofie Buhler**[1], **Arnaldo Dossena**[1], **Stefano Sforza**[1], **Tullia Tedeschi**[1]

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Cow's milk is listed among the main food allergens: with fish, egg, crustacean/shellfish, nuts, peanuts, wheat and soy it is responsible for the 90% of food serious allergic reaction worldwide. The main milk allergens are caseins and the whey proteins α-lactalbumin and β-lactoglobulin. Milk proteins allergy is also the most prevalent allergy for young children with an incidence up to 2–7.5% of the population. One reason of this incidence might be the different protein composition between cow's and human milk, for instance β-lactoglobulin is not present in human milk while it is the main whey protein in cow's milk. Milk undergoes several technological treatments to guarantee its safety and stability for human consumption. Pasteurization is one of the most frequent thermal treatments, and it is performed at 72–75°C for 15–20s. However, Ultra High Temperature (UHT) treatment, performed at 135–150°C for 1-2 seconds, sterilizes food allowing the production of a milk with a shelf life longer than the pasteurized milk. The application of high temperatures might affect milk proteins inducing structural and chemical modifications. The major common modification is the Maillard reaction. This modification is promoted by the high temperatures and occurs between lactose, the main sugar present in milk, and lysine residues in proteins. In this context, it is interesting to investigate the effect of lysine lactosylation at a molecular level on proteins allergenicity. With this aim, a screening on different UHT and pasteurized milk samples was performed, studying in particular the modifications on α-lactalbumin and β-lactoglobulin. Wheys were isolated starting with removing manually the fat after centrifugation, then caseins were precipitated at pH 4.6 and the obtained wheys were filtrated. The analysis with UPLC–MS allowed the quantification of lactosylated whey proteins in the samples. After purification with semi-preparative HPLC, α-lactalbumin and β-lactoglobulin were digested with trypsin and chymotrypsin. The obtained peptides were analysed with LTQ–Orbitrap and UPLC–MS and lactosylated peptides were identified in the digested samples. Six lactosylated peptides were found in all the milk samples and compared with the known epitopes reported in literature. From this comparison it was possible to determine that some modified lysines are present in the epitopes. In order to study if the lactosylated lysines might affect the IgE-protein interaction, four peptides included in the epitopes were selected and synthesised using the standard Fmoc protocol on a rink amide resin, ending with the final acetylation of the N-terminal residue. In this presentation, synthetic strategies for obtaining the corresponding lactosylated derivatives will be showed and preliminary immunoblotting tests will be also discussed.

Keywords: *food allergy, milk whey, β-lactoglobulin, α-lactalbumin*

A2
SENSITIVE DETECTION OF ALLERGENS BY MASS SPECTROMETRY IN PROCESSED FOODSTUFFS

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Food allergy is a significant health problem. While, food industry and clinician are working on the management of cross-contaminations and treatments, respectively, laboratories must develop efficient analytical methods to ensure the detection of hidden allergens that can also cause severe adverse reactions.
Indeed, cross-contamination during food production is still common, the industry widely uses precautionary allergen labeling (PAL). To improve food labeling, reliable analytical methods are needed. Enzyme-linked immunosorbent assays (ELISAs) are the most widely used, but food processing can strongly alter their performance, leading to false negatives or at least to major underestimation of allergen contents. In particular, heat-processing denatures proteins and causes structural modifications liable to result in non-recognition of a target protein by conformational antibodies. DNA methods are more suitable for detecting processed allergens, but results cannot be compared, for lack of certified reference materials and DNA-to-protein content conversion factors. Mass spectrometry is now emerging as a promising alternative.
The present UHPLC–MS/MS-based method allows simultaneous, highly sensitive and specific detection of several allergens in food products, including processed ones. In this method, 10 allergens including milk, egg, soybean, peanut and tree nuts (hazelnut, walnut, almond, cashew, pecan nuts and pistachio) were incurred and processed in matrices. After a digestion and a purification (SPE C18), food allergen peptides were analyzed by UHPLC-MS/MS (Xevo TQS – Waters). We obtained a limit of quantification (LOQ), defined by a signal to noise ratio higher than 10, of: 0.5 mg of milk proteins, 2.5 mg of peanut, cashew, hazelnut and pistachio, 3 mg of egg proteins, and 5 mg of soy, almond, walnut and pecan proteins per kg of incurred foodstuffs.
To the best of our knowledge, this method allows the most sensitive currently available for the detection of allergens in several complex and processed matrices by UHPLC-MS/MS.

Acknowledgement: *We thank Waters Corporation, the Walloon Region (FirstDoCA project: Allermass convention 1217881) and the Technological Platform Mass Spectrometry Service (MaSUN, UNamur) for scientific support and for their financial contributions to this project.*

A3
SELECTION OF PEPTIDE BIOMARKERS BY HIGH RESOLUTION MASS SPECTROMETRY TO DEVELOP A REFERENCE METHOD ALLOWING THE QUANTIFICATION OF ALLERGENS IN PROCESSED FOOD PRODUCTS

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Food allergy is a rising global health problem. In order to protect the consumer, labelling of 14 ingredients that have the potential to cause an allergenic reaction is required by European legislation (directive 1169/2011/CE). Unfortunately, incidental presence of allergens in food is also possible due to cross-contamination and is not covered by legislation.
Even though detection methods with a good sensitivity and specificity have already been developed and are commercially available for a variety of food allergens, they are still subject to variability and inaccuracies mainly due to matrix effects (e.g. interference with other components, extraction efficiency, modification of targeted compounds during processing), sampling, non-uniform use of standards and units and the lack of internal standards.
To guarantee the efficiency of food control, the "Allersens" project aims to develop and validate a multi-allergen mass spectrometry based method (LC–MS/MS), targeting the 4 priority allergens (peanut, hazelnut, egg and milk) and being robust to food processing.
The first step of this project is to constitute a database of peptides that are specific for the allergens of interest, which will include information concerning peptide sequence, specificity, stability to food processing or known modification, and MS/MS parameters. This objective is based on an instrumental/experimental identification of these peptides by High Resolution Mass Spectrometry (HRMS).
Several reference materials, produced for the project, that are representative for real live food products and containing the allergens are used. Before samples could be analyzed by HRMS, an optimized sample preparation has been developed to extract, purify, and enzymatically digest the proteins in their constitutive peptides.
The final aim of this study is to develop an LC–MS/MS method as a golden standard that could serve as a reference for the more routinely applied detection methods.
Selection of peptide biomarkers will be presented for egg and peanut.

Keywords: *allergen, mass spectrometry, egg, peanut, HRMS*

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

A4
COMPARISON AND OPTIMIZATION OF THREE COMMERCIAL METHODS WITH A LC-MS/MS METHOD TO DETERMINE SULFITES IN FOOD

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As food additives, sulfiting agents are present in many food products and may cause an "allergic-like" reaction in a small group of sensitive people. Therefore, the U.S. FDA requires labeling of sulfites in food products if their concentration is higher than 10 mg/kg (ppm) measured as sulfur dioxide (SO_2). One method to determine sulfites in food is a recently developed LC–MS/MS method. Free and reversibly bound sulfite reacts with formaldehyde to form hydroxymethylsulfonate. This adduct is separated with a HILIC column, ionized with ESI in negative mode and detected by multiple reaction monitoring (MRM). It is a rapid, sensitive and selective method and shows recoveries ranging from 84 to 115 % in representative foods. In this study, the LC–MS/MS method has been compared with three commercial products that are marketed for rapid, field portable sulfite analyses. Ten samples have been measured with all four methods. One commercially available product is the Sulfite Assay Kit (Bioo Scientific) which is based on enzyme-reactions. When sulfite is oxidized to sulfate, hydrogen peroxide is formed which in turn oxidizes NADH to NAD^+. The NADH concentration can be measured with a spectral photometer (340 nm) which is proportional to the sulfite concentration present in the sample. Compared to the LC-MS/MS method, the kit is less sensitive and has high variability (between 22.2 and 124.2 % of the average value). It was found that the higher the sulfite concentration is the more reproducible the results. Another commercial option is the use of Quantofix® test strips (Sigma Aldrich). The strip is dipped in a sample extract and if sulfiting agents are present, the test field turns pink and can be compared with a colorimetric scale. The test strips in general show lower concentrations than the LC–MS/MS method. However, the detection limit of the strips is approximately 50 ppm SO_2, significantly higher than the 10 ppm regulatory labeling threshold. The third kit investigated was Spot Test strips (Spot Test Company). The blue spot is directly placed on a food sample and wetted with water or an aqueous food extract is transferred onto it. The color of the spot will decrease with increasing sulfite concentration. The spot density is measured with a densitometer and the sulfite concentration is calculated based on a standard curve. The results for the analytical standards and the samples were neither accurate nor reproducible. This study shows that the LC–MS/MS method is the most sensitive, selective and reproducible method by far. The Sulfite Assay Kit can be used to get an approximate sulfite concentration in a product. If the concentration is high enough, quantitative results can be reported. The Quantofix® test strips are an alternative for consumers to see if their product contains high sulfite concentrations but is not sensitive enough for products with less than 50 ppm SO_2. Moreover, the Spot Test strips show false positive and false negative results.

Keywords: *sulfites, LC-MS/MS*

A5
A MULTIDICIPLINARY APPROACH TO SELECT NATURALLY LOW-TOXICITY WHEAT GENOTYPES FOR CELIAC DISEASE PATIENTS

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Celiac disease is an autoimmune enteropathy triggered by the ingestion of gluten-containing grains in genetically susceptible individuals. The gluten-free diet is the only acceptable treatment to date, that often leads to a progressive clinical improvement in parallel with the healing of the intestinal mucosa. However, concerns have been recently raised about the consequences of a lifelong gluten-free dietary habit pointing out the need to select low toxic wheat genotypes and/or develop detoxification strategies.
Gluten proteins and particularly the gliadin fraction represent the main factor triggering celiac disease. Given the extremely high structural heterogeneity of gliadins, both the physico-chemical properties and the susceptibility to enzymatic treatment of gliadins can vary significantly among wheat genotypes (species, cultivars, breeding lines), influencing in parallel their immunogenicity [1–4]. Therefore, the proteomic characterization and the understanding of the correlation between gliadin polymorphism and relevant toxicity, assumes a particular significance in order to identify naturally low toxic wheat species and/or efficient detoxification technologies [5–6]. Several opposing results have been presented in the last two years on this topic, mainly due to strict dependence of the experimental evidences on the selected analytical approach.
In this communication, a collection of *Triticum turgidum var. durum* genotypes was systematically characterized in terms of gliadin profile by following a multiple approach based on genetic, chemical and immunological analysis. The information provided by immunoassay-based quantification (R5-ELISA), and protein profiling by means of different separation techniques, both liquid chromatography and gel electrophoresis, were combined to highlight gliadins polymorphism and to account for the selection of candidate genotypes with potential natural low toxicity. Such candidate low toxicity genotypes were further investigated by in-vitro simulated gastroduodenal digestion, LC-MS characterization of the resulting peptides pool and immunological t-cell assays for differential-toxicity assessment. T-cell lines and immortalized B lymphocytes were produced from five different celiac disease patients and healthy donors for statistical relevance of the immunological data. The results provided by such multi-disciplinary characterization will be presented and critically discussed.

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Acknowledgement: *The research was funded by "S. Wheat Pro. - Proteomic characterization of Selected durum Wheat cultivars for PROduction of low toxicity-food products towards celiac disease patients - SIR 2014 (RBSI14QQ1W)".*

A6
DETECTION OF GLUTEN ON SURFACES AND CLEANING-IN-PLACE WATERS USING THE R5 DIP STICK

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In 2015, the AOAC Gluten Expert Review Panel adopted the dip-stick RIDA®QUICK Gliadin as AOAC *Official Method℠* 2015.16 First Action for processed and non-processed food. Since the dip-stick is also suitable for direct swabbing procedures, we validated several surfaces and additionally cleaning-in-place (CIP) waters (with and without surfactants) as part of the AOAC *Performance Tested Method℠* process. For surfaces an area of 10 x 10 cm was swabbed with the dip-stick. Afterwards the stick was incubated for 5 min in buffer and read out visually for a positive or negative result. CIP waters were diluted with buffer before measurement. All surfaces (stainless steel, plastic, silicone rubber, and sealed ceramic) were tested at 0, 0.25, 0.5, 1.0, 2.0, and 4.0 µg/100 cm^2 PWG-gliadin with 20 replicates for each concentration. The testings were blind coded since contamination of surfaces on one hand and swabbing and testing on the other hand were performed separately by different analysts. Probability of detection (POD) curves were constructed and used to estimate an $LOD_{95\%}$ gliadin amount. The outcome was an $LOD_{95\%}$ amount between 1.6 and 3.0 µg/100 cm^2 gluten. For CIP waters, a commercial gluten material was used for spiking the solutions. Three chemically different cleansing reagents were analyzed blind coded at six different concentration levels again with 20 replicates per Level. 100% positive results were obtained for minimum gluten concentrations between 50 and 100 ng/ml gluten. If the CIP water does not contain cleansing reagents, the lowest detectable gluten level of was 10 ng/ml. This proves the excellent suitability of the RIDA®QUICK Gliadin not only for food analysis but also for surface and CIP water testing.

Keywords: *gluten, surfaces, CIP water, LFD, validation*

A7
LC-HRMS/MS FOR THE SIMULTANEOUS DETECTION AND QUANTIFICATION OF MULTIPLE ALLERGENS

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EU legislation requests the inclusion of allergenic ingredients on labels of packed foods. Therefore, there is a need of sensitive and quantitative analytical methods capable to determine allergenic contaminants to protect susceptible consumers. Targeted proteomics has emerged as promising technique thanks to its ability to quantify multiple proteins simultaneously and to its increased specificity and reproducibility compared to antibody-based and DNA-based methods.
In the present work, a bottom-up proteomics approach was used to detect multiple allergens (i.e., milk, soy, egg, and crustaceans) in animal-derived food matrices. Protein extracts from different allergens were digested using trypsin. Then, an untargeted survey experiment using liquid chromatography coupled to high resolution tandem mass spectrometry (LC–HRMS/MS) was performed to select proteotypic peptides from the investigated allergens. Subsequently, a targeted method based on parallel reaction monitoring (PRM) was developed to record the precursor-to-product ion transitions for each selected peptide. Two peptides for each monitored protein were included in the method.
Ten peptides were finally selected and simultaneously monitored giving limits of detection comprised between 0.5 and 2.5 µg/g in fortified matrix samples. Linearity of the response and repeatability were studied and verified. The method was finally tested analyzing certified reference material giving satisfactory results.

Keywords: *LC–HRMS, allergens*

Acknowledgement: *Authors thanks Italian Ministry of Health for financing (RC IZSVE 11/15 - CUP B22F16000260001).*

A8
DEVELOPMENT OF THE NEXT GENERATION OF SOY ALLERGEN IMMUNOASSAYS

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Soy allergy affects approximately 0.4% of children globally and is one of eight major food allergies. These eight represent nearly 90% of food allergies worldwide.
Existing immunoassay tests to detect soy in food often only respond to its native unprocessed state, yet most of the soy ingested is processed – e.g. soy sauce and soy milk. Underestimation of the concentration of processed soy may result in unintended soy allergen residues that could pose a risk for those affected by soy allergy. Immunoassays that are compatible with more aggressive extraction buffers and techniques could have potential to detect soy proteins in highly processed foods.
A range of samples was obtained that represented the different types of processed soy used in food. These samples were extracted using three different buffers at three different temperatures (ambient temperature, 37°C, 60°C). The extraction efficiency is expressed as percentage extracted protein (determined using the bicinchoninic acid (BCA) assay) in total protein (determined by Kjeldahl analysis). In this study, three antibodies were used: two raised against native soybean and one raised against a commercially available processed soy sample. After antibody purification, the binding characteristics were tested by immunoblotting. Finally, the antibody reactivity against native and processed soy products was assessed using an ELISA format.
Protein extractability was increased by addition of a reducing agent and a chaotrope, in combination with heating up to 60°C and sonication. This buffer reduced the difference in extraction efficiency between native and processed soy. Specificity testing showed, that each of the two anti-native soy antibodies had one major recognition site, while the anti-processed soy antibody bound to acidic and basic subunit of glycinin. The inhibition ELISA showed little to no differentiation between unprocessed and processed soy and could successfully distinguish between soy and non-soy samples. Next steps will be further optimization and validation including assessment of matrix effects.

Keywords: *allergen, immunoassay, antibody, soy, processed*

A9
RAPID DETECTION OF NUT ALLERGENS USING A PLANAR-WAVEGUIDE BASED BIOSENSOR

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It has been estimated that food allergies affect between 2% and 3% of adults in the industrialised world (Sicherer and Sampson 2014; Tetens 2012) and upwards of 10% in young children. Undeclared food allergens can cause a significant health risk to susceptible individuals whereby over 180 allergenic compounds have been identified. Due to insecurities in food supply chains, the unpredictable nature of an allergenic response and the variation of dose required to produce an adverse effect, food allergens need to be effectively controlled through informative labelling and rapid novice user manageable detection systems to ensure public safety.
This study plans to provide a proof-of-concept for a rapid planar-wave guide based biosensor for the detection of nut allergens within foods samples. The cartridge system and SnapEsi reader from MBio Diagnostics has previously shown feasibility for the detection of multiple compounds in multiple fields of study (Lochhead et al. 2011; McGrath et al. 2013; McNamee et al. 2014). Incorporating advanced nano-spotting technologies, in-house produced nut standards are printed on the nanoarray assay system for development of a competitive assay for the rapid detection of nuts. Rapid extraction techniques have been developed and incorporated into the assay design enabling the detection of nuts from sample to result in under 15 minutes, without the use of laboratory equipment enabling field deployment.
The sensitivity of the peanut, pine nut, and almond assays was determined from the midpoint of the curve (IC_{50}) to be 389 ng/ml, 110 ng/ml and 270 ng/ml respectively. Cross-reactivity was not observed between any of these nut assays against other treenut targets at 50 ng/ml (<1%). Walnut, Hazelnut, Cahsew, Macadamia and Pecan nut assays are under evaluation.
The MBio Diagnostics system coupled with a rapid extraction procedure offers the potential to be a rapid, portable detection system for nut allergens in food. The nano-array structured of the biosensing platform allows for the integration of other allergen target proteins into the one multiplexed test.

Keywords: *treenut, multiplex allergens, fluorescence biosensor*

A10
SIMULTANEOUS DETECTION OF FOOD ALLERGENS WITH A BEAD -SUSPENSION FLOW CYTOMETRY IMMUNOASSAY

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Food allergy prevalence has increased over the last decades up to 7%. Efficient monitoring of allergens in food is required to ensure safety of consumers facing this health problem. European allergen labeling legislation in food covers 14 ingredients (Regulation EU N°1169/2011): egg, milk, peanut, tree nuts, cereals containing gluten, soybeans, crustaceans, fish, mollusks, celery, mustard, sesame seeds, lupin and sulphites.
Most of current analytical methods for food allergens are based on immunological reactions (ELISA and immunostrips) or PCR. Classical immunoassays are generally only suitable for the detection of one single allergen at a time, or are limited to few targets. In contrast, multiplexed PCR tests are available, but for some major allergens like milk or egg, this technique lacks of sufficient performances.
Flow Cytometry bead-based Immunoassays (FCIA) present all the advantages of immunological tests (sensitivity, specificity, high-throughput), additionally providing a great multiplexing capacity. Individually encoded fluorescent microparticles are used as solid support to build up immunoassays directed to different targets, which can be combined in a final test to broaden the compounds to be detected in a single analysis. The aim of this work was to develop a multiplex FCIA for the simultaneous analysis of four major food allergens.
A five-plex sandwich assay has been developed for milk (β-lactoglobulin and casein), egg, peanut and soy. A universal buffer--based extraction method (multi-allergen and multi--matrix) has been evaluated, and the performances of the assay have been tested in diverse food matrices spiked with a mixture of the allergens: speculoos, cookies, ice--cream, tomato sauce, spices (cumin and paprika) and chocolate. The five allergens were detected at 10 $\mu g\ g^{-1}$ (protein content) in all the matrices, with very satisfactory recovery rates, which were comprised between 81% and 96% for β-lactoglobulin, 62% and 103% for casein, 95% and 145% for egg, 80% and 101% for peanut, and 48% and 120% for soy.
The results herein presented point out the potential of Flow Cytometry Immunoassays for multi--allergen determination in food, and the interest of their application in routine quality control by food industries and official entities. Further work is ongoing to expand the application of the test to other allergens and matrices.

Keywords: *flow cytometry immunoassay, food allergens, multi-allergen, multi-matrix, detection*

Acknowledgement: *This work received funding from the Belgian Walloon Region (CWALity Programme).*

A11
SENSITIVE AND SPECIFIC ALLERGEN SCREENING ANALYSIS USING LC-MS/MS

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Allergens in food can result in severe or fatal reactions. At present, there is no known cure for an allergenic reaction and the only thing a person can do is to avoid the potential cause eg nuts, milk etc. Food allergens are of increasing interest due to food allergy recalls that have doubled in recent years. To help safeguard consumers from food related allergies, warning labels on packaging and in restaurants are a must these days to allow people to make a decision on what they eat. However, food testing is also important and as a result, it is vital to have a robust and specific analytical method to reliably identify and quantitate allergens that may be present. This work presents data from a method that has been highly characterized and verified to determine several different types of allergens using a tryptic digest and LC–MS/MS analysis to measure allergen peptides with a high degree of flexibility, specificity but also with high sensitivity. Samples were trypticly digested and the resulting extracts analyzed using LC–MS/MS. Peptides that were identified to be associated with a specific allergen were measured using electrospray ionization and scheduled MRM data acquisition. In this presentation a comparison between traditional methods and the LC–MS/MS will be described. Important considerations for method development will be discussed. The ability of the method to identify allergens and to quantify them in food samples will be presented and discussed. The method results from several different food matrices will be presented to demonstrate the potential of this method.

Keywords: *allergens, peptides, scheduled MRM, high sensitivity, LC-MS/MS*

A12
HOW TO ANALYZE THE MILK OF THE FUTURE?

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Total world milk production (83% of milk produced is cow's milk) is expected to continue its growth in the next 10 years, by 1.8% per annum.
Bovine milk coming from animals with different genetic profiles can have different impact on consumers in terms of gut issues and general intolerance.
It is reported that a genetic selection of cows is currently taking place not only in Europe but also in the rest of the world.
Although not proven yet, people start thinking that "A2 Milk" causes less health issues than "common A1 Milk"; according to this, A2 milk is now more expensive than "common A1 Milk" and, more and more milk producers are preferring A2 bovines in an increasing trend.
Mérieux NutriSciences and Padova University developed a LC/HRMS method to selectively distinguish "A2 milk" from "common A1 Milk".
Starting from the knowledge of different cattle genotypes (DNA).
Considering possible variants of β-casein (PROTEINS).
Correlating it with proteotypic final biomarkers (PEPTIDES).

84 scientific papers constitute the bibliographical section part of Research Work of the Master Thesis on Pharmaceutical Biotechnologies (done in Mérieux NutriSciences R&D Department) entitled "DEVELOPMENT OF A LC-HRMS METHOD TO DETECT A1 AND A2 VARIANTS OF β-CASEIN IN BOVINE MILK" that will be discussed in Padova Univesity (October 2017).

Keywords: *A2 milk, mass spectrometry, allergens, LC-HRMS, casein*

A13
DIVERSITY OF SEED PROTEINS, INCLUDING ALLERGENS, IN COMMON BUCKWHEAT

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Common buckwheat (*Fagopyrum esculentum* Moench) contains abundant proteins, minerals and vitamins, leading to this plant being attributed high nutritional value. However, for some individuals, several proteins in common buckwheat can cause severe allergic symptoms and anaphylaxis; therefore, research on these proteins is important. Because common buckwheat is a self-incompatible plant, the genetic background differs among individuals within the same cultivar and among seeds within the same individual. These differences are latent in a population, leading to difficulty in understanding the diversity of proteins that are present in this plant. In this study, we investigated the diversity of proteins in common buckwheat by analysing the self-compatible strains developed in recent years, which revealed various genetic differences, in addition to self-incompatible strains.
Common buckwheat seeds were powdered and suspended in buckwheat protein extraction buffer (0.086 M NaCl, 0.033 M $NaHCO_3$) containing protease inhibitors or Laemmli sample buffer (Bio-Rad Laboratories, Inc.), and proteins were extracted at 4°C overnight. The extracted protein solutions were then centrifuged and filtered. The proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions; the separated proteins were visualised using Coomassie Brilliant Blue staining, and the level of expression of Fag e 2, a common buckwheat allergen, was analysed using western blotting with specific rabbit polyclonal antibody.
In the self-incompatible strains, the SDS-PAGE pattern and the level of expression of Fag e 2 in the proteins from mass of seeds were similar among some strains. However, the SDS-PAGE pattern and the expression of Fag e 2 in the proteins extracted from each seed of the self-incompatible strains differed. This indicates that the difference between seeds is equalised within a strain, and as a result, the difference between strains is not detected. In contrast, in self-compatible strains, the SDS-PAGE pattern and the level of expression of Fag e 2 in the proteins extracted from each seed differed among the strains. Furthermore, the range of the level of expression of Fag e 2 in self-compatible strains was broad. This shows that there is diversity by genetic differentiation in the proteins of the self-compatible strains. In conclusion, we showed the possibility of breeding new varieties of common buckwheat with not only different allergenicity, but also with different quality.

Keywords: *buckwheat, protein allergen, anaphylaxis*

Acknowledgement: *This work was partially supported by "JSPS KAKENHI Grant Numbers JP16H02530" and "Science and technology research promotion program for agriculture, forestry, fisheries and food industry 28003A".*

A14
DEVELOPMENT AND EVALUATION OF DIFFERENT DNA AMPLIFICATION APPROACHES BASED ON LOOP-MEDIATED ISOTHERMAL AMPLIFICATION AND REAL-TIME PCR FOR THE DETECTION GLUTEN-CONTAINING CEREALS IN FOOD SAMPLES

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The treatment of gluten-related disorders, including celiac disease (CD), wheat allergy (WA) and non-celiac gluten sensitivity (NCGS), is based on a lifelong, and strict, gluten-free diet. Thus, reliable and sensitive methods are required to detect the presence of any intentional or unintentional gluten contamination. Traditional approaches rely on the detection of these proteins based on specific antibodies, but recent approaches go for an indirect route detecting the DNA that indicates the presence of cereals with gluten content.
DNA-based assays have proven to be useful tools by targeting DNA as a marker for the presence of the offending ingredient [1,4], both for the detection of undeclared ingredients in food products and for the evaluation of cleaning procedures for industrial equipment. The use of DNA targets is interesting for methods requiring a high sensitivity since DNA is not affected by the variability of the phenotype, they present a high thermal stability and DNA amplification methods, such as PCR and qPCR, allow the amplification of the number of copies of the initial DNA target. Moreover, they have the advantage of allowing the detection of individual species [2], or the simultaneous detection of a collective allergen group [3] depending on the designed primers and the chosen sequence, which gives an additional flexibility to this approach.
In the current study two different DNA amplification techniques, quantitative loop-mediated isothermal amplification (qLAMP) and real-time PCR (qPCR), were evaluated for their capacity to detect and quantify gluten-containing cereals in different types of food samples. Furthermore, two detection chemistries were tested for qPCR, hydrolysis probe and an intercalating dye, and a commercial master mix along with an in-house made mixture were evaluated for the qLAMP. In this sense, correct specificity was obtained with all approaches, being both qLAMP approaches the fastest assays, but not suitable for seeds. Therefore, qPCR proved more sensitive than the qLAMP when analyzed pure DNA as well as spiked samples, for this particular application.

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Keywords: *qPCR, qLAMP, alpha2-gliadin, Gluten*

Acknowledgement: *This work was supported by the project Nanotechnology Based Functional Solutions (NORTE-01-0145-FEDER-000019), supported by Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and by NANOIMMUNOTECH S.L. in the framework of the project Smart Factory for Safe Foods (SF4SF) supported by FEDER Innterconecta 2015.*

A15
RAPID, DNA-BASED METHOD DEVELOPMENT FOR DETECTION OF SOY CONTENT IN FOOD SAMPLES

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Soy is widely used by the food industry in many different products for its functionality, such as texture improvement, viscosity building, protein filler, water and oil management, and gelling properties. The widespread use of soy by the food industry leads to a high risk of cross-contamination of food products not containing soy as an ingredient. Nevertheless, since soybean is also considered as a potentially allergenic ingredient, its presence should be declared on the label of foodstuffs, regardless of the added amount (Directive 2007/68/EC, 2007/68/EC). Accordingly, sensitive analytical methods are important to determine the presence of soy contamination in order to protect sensitized persons.

In the present study, we developed a detection method for soybeans with high specificity and sensitivity using PCR. A fast and reliable diagnostic system has been developed for the detection of soy content in food product. This qualitative test is based on a recombinase polymerase amplification which can be performed on the "field", *in situ*, where it may be necessary to determine soy content in food products at once. The soy detection based on RPA method, which is an isothermal (constant temperature is around 38°C) amplification utilising strand displacing DNA polymerase and single strand binding recombinase. The required equipments for the procedure are pipettes, a portable homogenizer and a portable thermostat. DNA amplification is carried out at a constant temperature, and the detection is based on antibody reaction. Among RPA applications with different probes, we tested Twist Amp nfo kit, which can be used with endpoint detection cost effectively. Endpoint detection was performed using lateral flow strip with easy yes/no indicator. As for sample preparation we were also seeking for minimal hands-on requirements. The detection limit is one copy of the target sequence in 1 µl reaction volume. The test can be used for uncovering falsification of local brands on the spot within a very short (25–45 min) period of time. The applicability of the newly developed method was tested on several food samples.

The present approach can be adopted for the detection of other food ingredients or allergens if the species-specific target DNA sequence is known, e.g. in case of almond, celery, walnut.

A16
DISCRIMINATION BETWEEN GLUTEN AND GLUTEN FREE CEREAL FLOURS BY UV-VIS SPECTROSCOPY COUPLED WITH MULTIVARIATE ANALYSIS

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Nowadays, there is a constant need for developing fast and sensitive methods for characterization of various food products. UV-VIS spectroscopy has proven to be quite efficient for analyses of various foodstuffs such as beer, wine, oils, meat, fish, etc. Worldwide, more than a million people are affected with celiac disease and this number is constantly increasing. Consequently, presence of gluten in different food products has become a very important issue. Absorption spectra of 50 gluten containing (wheat, spelt, barley, oat and rye) and 33 gluten free (buckwheat, corn and rise) cereal flours were collected on UV-VIS spectrophotometer 2600 Shimadzu in a spectral range from 225 nm to 800 nm. All flour samples were purchased in market and measured without any sample preparation. Principal Component Analysis (PCA) was used to explore if there are significant differences between abovementioned flour types. Partial Least Squares Discriminant Analysis (PLS-DA) classification model was able to classify flour samples based on presence of gluten with success rate of 98.5%. Based on given results we can conclude that UV-VIS spectroscopy coupled with adequate multivariate analysis has great potential for the fast, sensitive and non- destructive determination of gluten in various cereal flours.

Keywords: *cereal flours, UV-VIS spectroscopy, gluten, authenticity*

ALLERGENS

A17
VALIDATION OF A NEW RAPID LATERAL FLOW TEST FOR SIMULTANEOUS DETECTION OF CASEIN AND B-LACTOGLOBULIN IN FOOD

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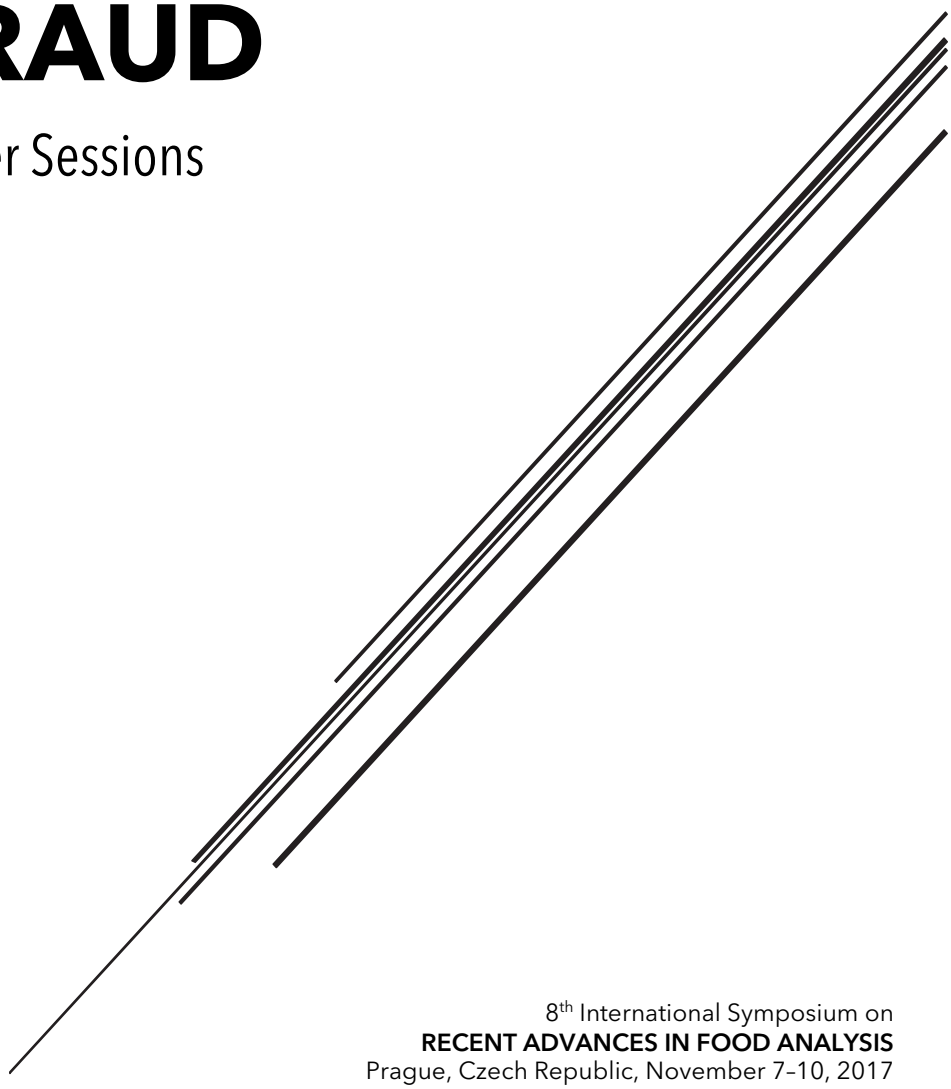
Milk is one of the main allergenic ingredients included in food labeling regulations from different countries due to the high prevalence of allergy, especially in childhood. However, milk is extensively used as an ingredient in food industry thanks to its valuable technological properties. As a consequence, milk is widely spread in facilities becoming an important source of hidden allergens through cross-contact. Other milk by-products such as whey or caseinate are also widely used as food ingredients, so their labeling is also mandatory. β-lactoglobulin is the main representative among the whey proteins in terms of allergenicity and abundance. On the other hand, caseins are major proteins in milk and in addition they are more resistant to denaturation by thermal processing than whey proteins. Therefore, the selection of only a unique target (casein or β-lactoglobulin) to analyze the presence of milk allergens in food products is insufficient when the source of milk proteins in a food or ingredient is unknown.
In this work we present a new test based on LFIA (lateral flow immunocromatography) that combines the detection of both proteins in one rapid test. When the sample is negative to milk proteins, only one blue line is developed over the stick (control line), indicating the test has run correctly. Whether the sample is positive, one or two red lines appear, depending if the sample contains β-lactoglobulin, casein or both. The performance of this new test was validated according to AOAC guidelines for validation of qualitative methods. POD (Probability of Detection) was determined by analyzing at least six different levels of each specific target as well as with UHT milk. Results of POD evaluation demonstrated that a level as low as 0.1 ppm of β-lactoglobulin or 1 ppm of caseinate could be detected (POD=1.00). Besides of the specific milk proteins, sensitivity was also evaluated on UHT milk to assess the limit of detection with a thermal processed product that can be used as ingredient. Levels of 0.1% of milk could be detected (POD=1.00), even mixed with matrices such as wine, liquor, orange juice and soy drinks. Specificity analysis revealed no cross-reactivity with a panel of 30 different food ingredients. Robustness was also evaluated by introducing reasonable changes in the procedure and determining the effect on the results. The new method has demonstrated to be a rapid and suitable tool to detect milk proteins in processed food, ingredients, food-contact surfaces and rinsing water from CIP processes.

Keywords: *antibiotic residues, on-site analysis, food safety, automatic system*

Acknowledgement: *This work has been supported by the program RETOS-COLABORACION 2015 from the Mº de Economía y Competitividad (Spanish Government), RTC-2015-3818-2.*

AUTHENTICITY, TRACEABILITY, FRAUD

Poster Sessions



8th International Symposium on
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AUTHENTICITY, TRACEABILITY, FRAUD

B1
EXCESSIVE HEAT LOAD OF EXTENDED SHELF LIFE (ESL) MILK AND CREAM CAUSED BY (UN)INTENTIONAL OVER-PROCESSING?

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The recent trend towards a longer keeping ability of pasteurized milk, without the negative flavour change normally associated with ultra-high temperature (UHT) treatment, has resulted in the development of extended shelf life (ESL) milk and other ESL dairy products. However, heating causes a significant loss of organoleptic and nutritional quality (e.g., cooked flavour; vitamin destruction). Therefore, different *Time-Temperature Integrators* (TTIs) have been used to evaluate the heat load of ESL milk products.
The objective of this study was to investigate the actual heat load of liquid milk (*n*=200) and whipping cream (*n*=58) samples at retail in Austria, either raw, pasteurized, *ESL* or *UHT*, respectively. Based on the existing FIF/IDF standards, improved RP-HPLC methods were firstly developed for the analysis of furosine and acid-soluble ß-Lg in liquid milk using a Symmetry™ 300 C_{18} column (3.5 µm, 2.1×150 mm) (Waters). The established RP-HPLC method enabled the separation of whey proteins within 21 minutes and was used for determination of acid-soluble ß-Lg. Furosine was analyzed by ion-pair chromatography RP-HPLC within 7 minutes. The optimized HPLC method for the analysis of ß-lactoglobulin was then successfully transferred to UHPLC equipped with an Acquity UPLC BEH300 C_4 (1.7 µm, 2.1×100 mm) column. Net separation time was cut down to 1.7 min.
Referring to liquid milk, half of the analyzed samples designated as ESL milk showed acid-soluble ß-Lg contents lower than 1.800 mg/L milk, which had been proposed as threshold level in Austria. Most of these ESL milk samples with excessive heat-load had a surprisingly low amount of native, ß-lactoglobulin (< 500 mg/L) and a high furosine content (> 40 mg/100g protein), which was almost comparable to that of UHT milk. As ESL milk has shown a dramatic increase in Austria recently, and has been widely accepted in many other European countries (e.g., Germany) in the meantime, the nutritional and organoleptic quality of this new category of liquid milk urgently needs to be controlled in the future.
Concerning whipping cream, ß-Lg concentrations were very low in all samples and were definitely not appropriate to differentiate between pasteurized/ESL/UHT cream samples. In contrast, furosine levels increased with higher heat load of cream in the logical order pasteurized < ESL << UHT, and allowed a significant discrimination of heat load of these products. Thus, furosine content of 70 mg/100g protein was suggested as an upper heating limit for pasteurized cream, whereas 100 mg/100g protein could be accepted as obligatory limit for tolerable heat load of ESL cream.
In conclusion, acid-soluble ß-Lg is most suitable for heat load evaluation of liquid milk, whereas furosine proved to be a reliable indicator to assess the heat load of whipping cream. Thus, both methods are appropriate to check so-called "ESL" dairy products for possible excessive heat load that does definitely not meet consumers' expectations!

Keywords: *heat load evaluation, ESL milk and cream, furosine, acid-soluble ß-lactoglobulin, UHPLC*

B2
CHARACTERIZATION AND DISCRIMINATION OF WHEATS AND FLOURS BY DART-HRMS

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In Italian food market durum wheat are used to produce pasta while from soft wheat are produced bread, biscuits and others bakery products. Due to favorable climate conditions, Italy is a strong producer of many different varieties of wheat, and the most of traditional Italian products are made of wheat durum flours.
Authenticity represents an important food quality parameter not only for food producers or regulatory bodies, but also for consumers; the most common fraudulent procedure is the addition of soft wheat flour to durum wheat flours.
The following experimental study aims to test the effectiveness of an analytical unconventional non-targeted screening method for a characterization of durum and soft wheat varieties, and identification of small amount of soft wheat in durum wheat flours.
Ten cultivars of wheat are used coming from six varieties (Triticum *aestivum, T. aestivum turgidum, T. aestivum Durum, T. aestivum monococcum, T. aestivum dicoccum, T. aestivum spelta)*; moreover one durum and one soft commercial wheat flour. Mixture at different percentage of durum and soft wheat flours were analyzed too.
The analyses are made with a high resolution mass spectrometer with a DART (Direct Analysis in Real Time) ion source and an Orbitrap mass analyzer, with a resolution of 70000 FWHM. Mass spectra of hydrophobic and hydrophilic fractions of samples were acquired in triplicate in both positive and negative ionization mode. The use of an atmospheric pressure ionization method allows to do the analysis directly on the sample with a minimum preparation and with the advantage that is 10-20 times faster than typical chromatography applications.
The use of the high resolution mass spectrometer allows to get thousands of signals which every m/z corresponds to one or more homologous compound (included isotopes, fragments or adducts), to obtain a complete fingerprint of samples.
All experimental data were processed with MetaboAnalyst 3.0, a web application which provides many MS data treatment and normalization procedures. MetaboAnalyst was used to perform to characterize wheats and their identification, using the mainly discriminating signals. In addiction the chemometric treatment applied to all data set obtained from the analysis of mixture of durum and soft wheat flours allows to detect low percentage of one flour to the other, the resulting procedure seem to be a tool rapid and reliable to prevent flours frauds.

Keywords: *DART-HRMS, fraud, wheat, flour*

B3
APPLICATION OF VOLATILE COMPOUNDS PROFILES FOR THE ASSESSMENT OF APPLES GROWN IN THE SYSTEMS OF ECOLOGICAL AND INTEGRATED PRODUCTION

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The project focuses on the determination of the volatile substances by the method SPME-GS-MS in apple varieties Angold, Florina, Gloster, Goldstar, Idared, Melodie, Ontario, Rubín, Šampion and Topaz grown in organic and integrated production systems, the quantification of differences in the profiles of volatile substances depending on the variety, the year and the conditions of cultivation. Knowledge about selected volatile substances as secondary metabolites associated with agro-technical production conditions represent significant points in verifying the authenticity of different varieties of apples of organic and integrated production. At the same time, the identified characteristics contribute to discover the findings that can be used in other scientific disciplines and to the identification of varieties that have favorable bio-properties in several aspects, so it is worthwhile to cultivate them.

Keywords: *apple, bio organic, integrated SPME*

Acknowledgement: *I would like to thank for the financial support from the specific university research (MSMT No 20-SVV/2017).*

B4
AUTHENTICATION OF BOTANICAL ORIGIN OF RAPE HONEY BASED ON VOLATILE COMPOUND PROFILES

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Quality assessment of honey is often related to its botanical origin. The project focuses on the characterisation of rape honey, mainly on determination and identification of volatile compounds for authentication of its botanical origin. Head space solid phase microextraction coupled to gas chromatography mass spectrometry (SPME GC/MS) was employed in analysis of 28 samples of rape honey (their botanical origin was proved by melissopalynology) and 28 samples of multiflower honey, which were collected in period from 2014 to 2016 in the Czech Republic. In the first stage of the study, the expected key compounds (dimethyl disulphide, 3-methylpentanoic acid, benzaldehyde, benzyl alcohol, benzene acetaldehyde, phenylethyl alcohol, 2-methoxy-4-vinyl-phenol, beta-damascene) were monitored and the results were compared with literature data. In the second stage, the volatile compound profiles were classified by multidimensional statistical analysis.

Keywords: *rape, honey, origin, volatiles*

Acknowledgement: *Financial support from specific university research (MSMT No 20-SVV/2017).*

B5
ROADMAP FOR SELECTING A REDUCED SNP PANEL FOR SPECIES IDENTIFICATION AMONG MUSSELS FROM MYTILUS GENUS

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Smooth shell mussels from *Mytilidae* family are one of the most cultivated and marketed bivalves appreciated as a wholesome and tasty food source in many countries. The international seafood trade has adopted standards and regulations intended to ensure food quality, safety and authenticity along the food chain "from ocean to plate". European regulation regarding labelling of fishery products (EU.1379/2013 2013), states that information about commercial and scientific name of the species must be included on the label. But administrative traceability systems (physical labelling, information recording and automatic data treatment) are not flawless and require validation through analytical procedures. DNA analysis is recognized as an ideal food traceability tool, molecular methods developed for species identification (SI) based on Single Nucleotide Polymorphisms (SNPs) coupled with allocation algorithms can be used to verify administrative traceability systems. Here, we present a roadmap for selecting SNP loci with high information content to perform SI among mussels from *Mytilus* genus. *Mytilus* SNP were obtained from two sources: a) 49 identified from three European taxa by data mining in EST libraries (Zbawicka *et al*. 2012, 2014) and b) 90 SNP discovered in Chilean mussels by RADseq (Araneda *et al*. 2016). These SNPs were genotyped in individuals previously identified with the nuclear PCR-RFLP marker ME 15/16, that targets the adhesive protein gene described as diagnostic for these taxa (Santaclara *et al*. 2006). The SNPs from the first source were genotyped in individuals classified as *M. trossulus, M. edulis, M. galloprovincialis* and *M. chilensis,* and the SNP from the second source in individuals from the last three species. In each resulting dataset, we applied two criteria to select the most informative loci: F_{st} outliers (95% CI) and the Minimum Allele Frequency (MAF) at four levels (MAF>0.4, >0.3, >0.2 and >0.1), resulting in five reduced SNP panels by dataset. To test the performance of these panels, we conducted reallocation tests using the leave-one-out procedure. The highest performance for both data sources was obtained using all loci (99.7 and 94.0% of individuals correctly re assigned to species, respectively). The best criteria for selecting loci were a) MAF>0.1 with 25 loci reallocating correctly the 99.4% of individuals and b) MAF>0.1 and F_{st} outliers with 47 and 27 loci respectively, that correctly reallocated 94.0% of individuals. Moreover, combining information from both criteria (MAF and F_{st} outliers) allowed the creation of minimal panels: a) 3 SNP and b) 8–14 SNP that had similar performance re-allocating individuals to species, a) 96.8–98.7% and b) 93.0–93.5% respectively. On the basis of these results, we recommend the use of F_{st} outliers and MAF criteria to create reduced SNP panels for species identification among the *Mytilidae* family.

Keywords: *traceability, mytilus, species identification, snp, assignment*

Acknowledgement: *FONDEF Grant-ID16I10013*

B6
IDENTIFYING WINE APPELLATION VIA POLYPHENOLIC SCREENING OF NIAGARA PENINSULA ONTARIO CANADA WINES

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Whether scientifically valid or not, the concept of terroir and its effect on flavour is universally accepted in the wine world [1]. A wine's taste and value is influenced by where it comes from. The Niagara Peninsula appellation of Ontario, Canada is an up and coming cold climate wine producing region. We wanted to characterize the polyphenolic compounds found in Ontario wines to see if we could group wines produced in the appellation by their geographic location. Additionally, we included wines from other geographic locations in Ontario and beyond.
Wine samples were analyzed on an accurate mass SCIEX X500R QTOF with an integrated liquid chromatography system. The 25 wine samples were acquired in both positive and negative TOF MS/IDA/MS/MS mode. The LC/MS data was then processed in MarkerView 1.3 statistical software and a PCA analysis was done.
The Niagara white wines formed a very distinct group when compared to the other white wines from other parts of Canada, Europe, and South America. There was good group separation between Niagara Chardonnay and British Columbia Chardonnay as well as Canadian and French Riesling.
The Pinot Noir analysis highlighted some of the differences in the appellation of Niagara Ontario with the wines grouping by sub-appellation. Here we see 5 distinct groups for wines. We also start to see some division in the Lake Erie Shore Niagara 20 Mile Bench wines.
The ability to confirm, with a very high degree of accuracy, where a wine comes from is a powerful tool in discouraging wine adulteration and fraud. Future work will include analyzing wines from distinct vineyards within the appellation to determine if a more discrete grouping of the wines can be achieved. This appears to be the first attempt at analyzing wines by LC/MS and PCA for this geographic region/wine appellation.

[1] How subtle is the "Terroir" effect? Chemistry-related signatures of two "Climats de Bourgogne" C. Roullier-Gall et al. PLoS ONE 9(5)

Keywords: *wine, polyphenols, PCA, mass spectrometry, Ontario, VQA*

B7
A MULTIPURPOSE RAMAN PLATFORM FOR SURFACE AND SUBSURFACE FOOD SAFETY AND QUALITY INSPECTION

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Both surface and subsurface food inspection is important since interesting safety and quality attributes can be at different sample locations. This study presents a multipurpose line-scan Raman sensing platform for food safety and quality research. The platform can be configured for Raman chemical imaging (RCI) mode for surface inspection and spatially offset Raman spectroscopy (SORS) mode for subsurface inspection. In the RCI mode, macro-scale Raman imaging was realized using a 785 nm line laser up to 24 cm long with a push-broom method. In the SORS mode, a 785 nm point laser was used and a complete set of SORS data was collected in an offset range of 0–36 mm with a spatial interval of 0.07 mm using one CCD exposure. The RCI and SORS modes share a common detection module including a dispersive imaging spectrograph and a CCD camera, covering a Raman shift wavenumber range from 100 to 2900 cm^{-1}. An orange carrot slice and milk powders adulterated with two nitrogen-rich chemicals (i.e., melamine and urea) were used to test high-spatial-resolution (0.07 mm/pixel) and large-field-of-view (230 mm wide) settings of the RCI mode for food surface evaluation. Fluorescence-corrected images at selected Raman peak wavenumbers were used to generate chemical images to show Raman-active analytes on the whole sample surfaces (e.g., carotenoids over the carrot cross section and adulterants mixed in the milk powder). Also, a layered sample, which was created by placing a 1 mm thick plastic sheet cut from original container on top of cane sugar, was used to test the SORS mode for subsurface food evaluation. Raman spectra from the plastic sheet and the cane sugar were successfully resolved using a self-modeling mixture analysis algorithm, demonstrating the potential of the technique for authenticating foods and ingredients through packaging. The line-scan Raman imaging and spectroscopy platform provides a new tool for surface and subsurface inspection for food safety and quality applications.

Keywords: *Raman spectroscopy, chemical imaging, lasers, subsurface inspection, food safety*

B8
HAHSUS - MANUKA HONEY AUTHENTICATION BY HS-SPME-GC/MS AND UHPLC-PDA-MS/MS COMBINED WITH STATISTICS

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Manuka honey is one of the most adulterated monofloral honeys in the world since it is the major medical grade honey currently approved for clinical application, especially for wound healing. The antibacterial activity of manuka honey is mainly caused by methylglyoxal (MGO), aside of other as yet unknown compounds. This has led to more so called manuka honey being sold on the market than actually produced. For this reason the blending and adulteration of manuka honey has come into focus everywhere. Therefore, the New Zealand Government and the UMFHA have requested robust and clear parameters for the identification of genuine manuka honey.
In our study, more than 150 honey samples from monofloral manuka, kanuka, and other New Zealand honeys supplied by the UMFHA were characterized by SPE-UHPLC-PDA-MS/MS and HS-SPME-GC/MS. A classification system named HAHSUS (Honey Authentication by HS-SPME-GC/MS and UHPLC-PDA-MS/MS combined with Statistics) was developed which is capable of differentiating and classifying manuka honey from other New Zealand honeys, especially from the pollen-identical kanuka honey. It is also possible to estimate the percentage of manuka honey in manuka-kanuka honey mixtures.
The HAHSUS method will be presented.

Keywords: *manuka honey, marker, food fraud, authentication, statistics*

Acknowledgement: *We would like to thank our research group, especially our diploma students S. Fiedler, L. Ispiryan, K. Recklies, and M. Weigel.*

B9
FIGHTING THE FOOD FRAUD: DETECTING VEGETABLE OILS IN MILK PRODUCTS

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The adulteration of food is of a primary importance for all the actors involved in the food chain, from the producers to the consumers. The adulteration frequently involves the replacements of one or more expensive ingredients with cheaper substitutes. The growing interest in the food authenticity requires reliable and suitable verification methods to prevent deliberate or accidental mislabeling. One of the sectors more involved in food frauds is the cheese and milk product area where the milk fat, for economical or rheological reasons, could be partially substituted with cheaper vegetable oils.
To detect vegetable oils added to milk fat sterols analysis, dehydroxylated sterols analysis, fatty acids profile and triacylglycerols (TAGs) analysis were used.
22 cheese and milk product samples were analyzed, either from retails or ethnic markets. GC-FID was used to determine sterols, dehydroxylated sterols and fatty acids profile. A DART source coupled with an ion trap detector was used to determine the lipid mass profile of the samples.
The reference method ISO 12078:2006 allows to detect the presence of vegetable oils in milk fat through the analysis of phytosterols, mainly β-sitosterol, campesterol and stigmasterol. Opposite, the milk fat has cholesterol as the main sterol (97-98%) with little amount of Δ 7- cholesterol and 24 metylencholesterol only. Fatty acids analysis has to be accompanied with statistical analysis and could act as screening method. The lipid profile obtained with DART-MS analysis could highlight the long chain fatty acid TAGs of vegetable oils that are not present in milk fat. This analysis could be a screening method too. Among the samples analyzed, cheese and spreadable products, two of them revealed the presence of vegetable oils.

Acknowledgement: *This work was supported by the Italian Health Ministry Project n. PRC2014-014T.*

B10
RAPID DETECTION OF HERB AND SPICE ADULTERATION

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High end food commodities such as Herbs and Spices are susceptible to fraudulent activity as they command a premium price for retail. It is estimated that the global herb and spice market is worth in the region of US$4 billion. Supply chains in this global marketplace are complex and vulnerable to fraud from criminals dealing in economically motivated adulteration. It is therefore in the best interests of stakeholders throughout the supply chain to detect and deter this kind of activity. Screening techniques to detect adulteration are being developed using the spectroscopic techniques, Fourier Transform Infra-Red (FTIR), and Near Infra-Red (NIR) combined with chemometric modelling. These techniques are gaining prominence due to their ease of use, rapidity and minimal sample preparation with potential to be used by stakeholders in the field. Typically, the raw spectral data of a range of samples along with possible known adulterants are collected, pre-processed used with chemometric algorithms to convert it into qualitative models which can be used to determine if a sample has been adulterated. Recent developments for analysis of Herbs and Spices, regarded as high risk from fraud, will be presented.

B11
GRAPE VARIETY AUTHENTICATION OF CHINESE RED AND WHITE WINES USING 1H NMR-BASED FINGERPRINTING

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In China's wine industry numerous economical-driven fraud incidents have been revealed in the past, e.g. most often cases of false declaration of grape varieties, which damaged the reputation of the Chinese wine market. Therefore, the feasibility of discriminating grape varieties of Chinese red and white wines was investigated using ^{1}H NMR spectroscopy in combination with a multivariate statistical procedure consisting of two steps: Principal Component Analysis (PCA) plus Linear Discriminant Analysis (LDA). Three grape varieties of red wines (Cabernet Sauvignon, Rose Honey, Cabernet Gernischt) and white wines (Ugni Blanc, Long Yan, Chardonnay) were examined. The combination of PCA and LDA yielded in a sufficient discrimination of the examined grape varieties. The validity of the PCA/LDA model was confirmed by internal leave-one-out cross validation (LOOCV) as well as by external repeated double random cross validation (RDRCV). LOOCV and RDRCV led to average correct classification rates of 82% and 83% for red wine varieties, respectively, and 94% and 90% for white wine varieties, respectively. The results demonstrate that ^{1}H NMR spectroscopy combined with multivariate analysis is an effective tool for verifying the authenticity of Chinese wines. The data collection methods and the demonstrated approaches for data evaluation proposed in this study are considered to be essential to provide a solid backbone for the establishment of a Chinese wine database.

Keywords: *1H NMR spectroscopy, multivariate analysis, PCA, LDA, wine authentication*

B12
PAPRIKA AUTHENTICATION USING FTIR SPECTROSCOPY AND ONE-CLASS MODELLING – IS THIS THE KEY TO DETECTING ADULTERATION?

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Spices have been used since ancient times to flavor foods and beverages. Just as long dishonest practices have been going on in the supply chain and the motivation for spice fraud is still the same as it was in ancient times - that is financial gain. Because they are high-price commodities and consumption is increasing world-wide spices represent an attractive category for fraudsters. Therefore, it is not surprising that spices are among the top notifications in the European Rapid Alert System for Food and Feed (RASFF). The big challenge in the detection of adulteration is that only substances that are being investigated are usually found, though, especially unknown or unforeseen adulterants can be overlooked. That is the reason why a lot of effort has been put into the development of food fingerprinting techniques.
The basic idea of the fingerprinting approach is to combine non-targeted analysis using a spectroscopic or spectrometric method and multivariate statistical data analysis. The aim is to capture as many features or compounds as technically possible within one measurement and therefore to provide a comprehensive insight into the composition of food matrices. The subsequent chemometric analysis of the spectral information enables the differentiation of samples due to e.g. the addition of adulterants.
In this study, the fingerprinting approach using Fourier transform infrared (FTIR) spectroscopy and soft independent modelling of class analogy (SIMCA) was tested for the purpose of determining deviations from typical product characteristics, particularly the addition of unknown adulterants. For this, a representative set of paprika powders was investigated to determine the data space of non-adulterated samples and define the critical limits for further classification. The performance of the established one-class model for adulteration detection was tested by predicting artificially spiked samples with common adulterants (gum arabic, lead chromate, lead oxide, polyvinyl chloride, silicon dioxide, Sudan I and Sudan IV). Further, the influence of preprocessing methods on the model performance was investigated. The results of the study demonstrate the potential of FTIR spectroscopy and one-class modelling for a broad anomaly testing.

Keywords: *food fraud, adulteration, authenticity testing, food fingerprinting, non-targeted analysis*

Acknowledgement: *This research was executed in the framework of the EU project SPICED (Grant Agreement: 312631) with the financial support from the 7th Framework Programme of the European Union.*

B13
GEOGRAPHICAL DIFFERENTIATION OF GRAIN MAIZE USING SPECTROSCOPIC METHODS

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Grain maize is an important part of animal nutrition across different species and types of feed material. It is also a raw material to different production lines resulting in a multitude of food products. In addition maize is grown and traded globally, qualifying it to an adequate demonstrator matrix for investigating authenticity questions. Verification of the geographical origin might be of particular interest in ban situations, when for example feed material of a particular area might become a subject of fraudulent declarations to circumvent strict import rules. Here non-targeted methods that do not focus on one or more known analytes, in combination with multivariate data analysis can be used to discriminate samples by their geographical origin. In the present study the differentiation of unprocessed grain maize according to the geographical origin was investigated. For this, numerous grain maize samples of known origin from all over the world have been collected and analyzed using non-targeted methods. Nuclear Magnetic Resonance (NMR) spectroscopy and attenuated total reflection Fourier Transform Infrared (FT-IR) spectroscopy were applied to both powdered samples as well as extracted oil fractions. Subsequently, multivariate methods were tested to build classification models for three spatial levels, namely, within Germany, European countries and worldwide. Primary results of this study are presented herein.

Keywords: *feed, non-targeted analysis, NMR, FTIR, geographical origin*

B14
LEAVING THE LABORATORY BEHIND: RAPID IN-FIELD FOOD AUTHENTICITY SCREENING USING HANDHELD SPECTROSCOPY

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Food fraud is an extremely topical and important issue. It is estimated to cost the world economy $US49b per year. The National Food Crime Unit estimates that it costs British families £1.17b per year. The British Retail Consortium suggest 1 in 10 consignments of imported basmati rice has been adulterated, whilst our recent work on oregano fraud showed 25% of retail product, on UK shelves, was adulterated and this pattern is similar globally.
Economic gain is often the goal. Authentic products are substituted with (or diluted by) inferior/ cheaper products, as was exemplified by the 2013 European horsemeat scandal. However, there are also health implications, for example the 2008 adulteration of Chinese infant formula, with melamine, which saw over 300,000 babies hospitalised with multiple mortalities.
Conventional methods used to determine food authenticity are laboratory based, require skilled operators, are expensive or time consuming and can take days or even weeks to complete. Meanwhile the product passes along the supply chain and in many cases reaches the supermarket shelf or consumer tables before results are reported. New and better ways of checking the authenticity of foods and their ingredients are required. Methods capable of rapid detection anywhere, anytime, in the food supply chain are what industry are demanding. This can be achieved through the use of handheld spectroscopic analysis in conjunction with chemometric modelling.
Our poster presents our latest research in developing spectral databases and chemometric models, on handheld portable spectroscopic instruments, to enable stakeholders to determine food authenticity in high risk commodities.
This research will cause a paradigm shift in food fraud detection by taking authenticity testing out of the laboratory setup and putting it in the hands of end users who can test on-site anywhere, anytime, in the supply chain and get immediate results right at their fingertips.

Keywords: *handheld spectroscopy, chemometric models, non-targeted analysis, food authenticity*

Acknowledgement: *This research has been funded through the Invest NI POC programme. Project Number PoC 615: The future of food authenticity: Rapid in-field detection.*

B15
DETERMINATION OF CARBOHYDRATES IN HONEY

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Honey is a complex mixture of sugars produced in nature by honeybees. The sugar composition of honey varies and is mainly dependent on its floral source. While the majority of honey's carbohydrates are fructose and glucose (85-95%) the remaining carbohydrates, a mixture of di-, tri-, and several larger oligosaccharides, and of analytical interest. Thes low concentration carbohydreate are useful for the determination of floral origin and can help determine authenticity.
We developed a High Performance Anion Exchange chromatography with Pulsed Amperomteric Detection (HPAE-PAD) method to measure and quantify the mono-, di-, and trisaccharides in honey. Separation of honey sugars was achieved on the recently introduced Dionex CarboPac PA210-4µm column, which provides fast, high-resolution separations for most mono- through tetrasaccharides using a KOH or NaOH mobile phase. Prior HPAE-PAD methods for honey carbohydrate analysis required sodium acetate-containing mobile phases. With only hydroxide required in the mobile phase the instrument is able to automatically prepare the mobile phase, thereby greating improving method reproducibility. Because detection was by PAD, no sample derivatization was required. This method resolved 15 honey sugars in 45 min, injection to injection. PAD is sensitive thus allowed the determination of low concentration carbohydrates in honey, while at the same time detecting the high concentrations of the major components, glucose and fructose. These properties allowed us to show differences in a collection of honey samples and show that HPAE-PAD profiling could detect a 10-20% addition of industrial sugar syrups (adulteration) to honey.

Keywords: *honey, adulteration, carbohydrate, profile, food fraud*

B16
FOODAUTHENT: COLLECTION, ANALYSIS AND UTILIZATION OF ANALYTICAL FOOD FINGERPRINTS

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In addition to completely traceable food and feed, an increasingly globalized trade, along with worldwide commodity chains, requires harmonized and valid analytical methods to ensure the quality, safety and authenticity of food and feed. Accordingly, non-targeted analysis, in which spectroscopic methods such as Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared Spectroscopy (FT-IR) are often used, is becoming a method of increased scientific interest. These analytical techniques are based on the acquisition of a chemical fingerprint of the respective food or feed and thus allow a comprehensive characterization of the product's composition. By chemometric analysis of the obtained data various authentication challenges can be investigated, inter alia the verification of geographical origin or identification of deviations from the expected product composition.
However, the routine use of these methods is currently restricted to certain products (e.g. juice, wine and honey) often in conjunction with commercial solutions. This is due to the absence of prerequisites, such as:
- standardized protocols for sample analyses,
- concepts of validation for fingerprinting methods,
- uniform data formats,
- shared databases with reference values and respective product-accompanying metadata.

These aspects are addressed in the national funded project *FoodAuthent*. Here in, a system for the collection, analysis and utilization of analytical food fingerprints will be developed. In context of food fingerprinting approaches, the primary objective of *FoodAuthent* is to promote the next steps towards a harmonized analysis aiming a perspectively implementation into routine analysis and official control.
Within the 3-year project period, two issues (i) FoodFraud-Detect (detection of food adulteration) and (ii) FoodRegio-Detect (proof of the geographical origin of food) will be especially focused, whereas three exemplary selected food matrices (hard cheese, seed oil and spirit of viticultural origin) will be analyzed by means of fingerprinting methods.
With regard to selected issues, first results on the development of a fingerprinting method of hard cheese using FT-IR-spectroscopy will be presented.

Keywords: *non-targeted analysis, chemometrics, authentication of food, geographical origin*

Acknowledgement: *The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.*

B17
PROGRESS TOWARDS STANDARDIZATION OF NON-TARGETED METHODS

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Non-targeted analytical approaches are upcoming and potential tools in authentication processes aiming at a comprehensive characterization of food and feed matrices. In particular, investigations concerning the origin or variety as well as the detection of often unknown adulterations are possible. A lot of effort has been directed into the development of non-targeted analytical approaches, particularly by feasibility studies, whereas the use of these methods in routine analysis is currently limited to a few products, e.g. juice and wine. The reason for this is the lack of important prerequisites, such as
- strategies for method validation, considering both chemical analysis and statistical data evaluation, and quality assurance measures,
- reliable databases of representative samples, and
- uniform data exchange formats for jointly usable databases.
Nonetheless, in the recent past, these gaps were recognized by the scientific community and activities initiated towards potential solutions, e.g.:
- First validation concepts and guidance documents have been developed for non-targeted analysis and multivariate data evaluation (incl. sampling, analysis and validation).
- Ongoing research projects (e.g. FoodIntegrity and FoodAuthent) started to investigate and bundle options for harmonization of non-targeted analytical approaches.
- In the field of metabolomics activities are to be mentioned which might be transferred to the authenticity domain, e.g. the COSMOS project developed policies to ensure that metabolomics data are encoded in open standards, supported by open-source data management tools and disseminated in open-access databases.
- Accreditation bodies have acknowledged first approaches based on non-targeted testing methods.
- Interlaboratory comparisons (in particular for NMR spectroscopy) have been organized and conducted, according to internationally agreed procedures.
Thus, progress towards standardization of non-targeted methods develops step-by-step. Some of the major international efforts will be picked up and

Keywords: *food authenticity, fingerprinting, validation, harmonization*

Acknowledgement:
FoodIntegrity: The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.
FoodAuthent: The project is supported by funds of the Federal Ministry of Food and Agriculture (BMEL) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme.

B18
FAST ANALYSIS OF NATURAL AND ARTIFICIAL VANILLA FLAVORINGS - COMPUTER ASSISTED DEVELOPMENT OF A ROBUST, FAST AND SENSITIVE UHPLC METHOD

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Vanilla is one of the most important flavors worldwide and is widely used in foods, beverages and perfumes [1]. Natural vanilla extract contains up to several hundred substances with vanillin, vanillic acid, 4-hydroxybenzoic acid and 4-hydroxybenzaldehyd as the major components [2]. Due to the continuously increasing demand and the resulting high costs of natural vanilla extracts, artificial flavorings are often used instead. Vanillin can be obtained through various methods like chemical synthesis and biotransformation [3][4]. These artificial flavors can contain synthetic vanillin, ethyl vanillin, eugenol, guaiacol, vanillin mandelic acid and others.
As authenticity criteria for vanilla, the ratios of the major components vanillin, 4-hydroxybenzaldehyde, vanillic acid and 4-hydroxybenzoic acid are frequently used. In order to monitor the composition and therefore quality of vanilla flavors contained in food, an analytical method needs to enable individual quantification of any of these ingredients as well as possible ingredients like the precursors from the synthesis of vanillin.
This poster shows the development and optimization of a rapid UHPLC screening test for the separation and quantification of eleven natural and artificial vanilla flavoring substances as well as some precursors for the quality control of vanilla products using an automated method scouting / method optimization workflow.

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Keywords: *vanilla, vanillin, artificial flavorings, UHPLC screening, method scouting*

B19
DETERMINATION OF MILK FAT CONTENT IN CONFECTIONARY PRODUCTS

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Confectionery industry in Serbia offers a various range of confectionery products, which are following the current world brands. Annually, over 140 000 tons of various chocolate products, candies and biscuits are produced in Serbia. In order to prevent the emergence of less quality products, a quality assurance of confectionery products is carried out by the manufacturers and accredited laboratories. It is essential to note that Serbian national regulations distinguish plain chocolate from milk chocolate, plain biscuit from biscuit with butter, plain caramel candies from caramel candies with milk, and these products require a certain minimum of milk fat content.
The milk fat content is analyzed in SP Laboratory, accredited by the ISO / IEC 17025 standard, which checks the quality of confectionery products.
The method for determining the milk fat content is accredited since 2009 in the SP Laboratory and is based on the determination of semimicro value of the total lower fat acids and semimicro butyric acid value. A semimicro value of residue is acquired from their difference which is indicating the presence of coconut fat that can be found in total fat in addition to milk fat.
In the period from 2014 to 2016, more than 450 samples of milk chocolate, biscuit with butter and caramel candies with milk were analyzed in the SP Laboratory. Therefore, between 91–93% satisfy the prescribed minimum content of milk fat, 4–7% of the samples were close to the minimum quality limit, while only 2–3% of the product did not satisfy the defined minimum content of milk fat.
Based on the obtained results of the controlled samples in the SP Laboratory over the past three years, it can be concluded that the producers of confectionery products in Serbia respect the criteria defined by the national regulations. Appropriate quality control of the confectionery products ensures the authenticity of the products.

Keywords: *milk fat, quality control*

B20
RECLAIMING PARASITES FROM FISH ACCORDING TO EU REGULATION 1276/2011: CAN HISTOLOGY DISCLAIM POTENTIAL FRAUDS ON FREEZING TREATMENT?

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Selling fish products as fresh when they have actually been frozen-thawed is a common fraudulent practice and fish intended for raw consumption must be previously frozen according to EU Regulations, in order to protect consumers from potential parasites infection; thus, besides being a commercial fraud, non compliance with this rule also represents a sanitary fraud. At IZSPLV in 2009 a histological method to distinguish fresh fish from fish frozen at -20°C for 24 hours (as preventive treatment included in EC Regulation 853/2004) was set up, with the following performances assessed on 35 species: sensitivity 90.70% (C.I95%. 82.49–95.9%), specificity 92.59% (C.I.95% 75.71–99.09%). In the present study the histological method's reliability in distinguishing between fresh fish and fish frozen at -35°C for 15 hours according to EU Regulation 1276/2011, was evaluated.
To achieve this goal, an experimental study was designed on 3 of the most common fish species present on the Italian market: *Sparus aurata*, *Salmo salar* and *tuna fish*. 118 samples, never subjected before to temperatures below zero, were purchased and divided into 2 groups of reference samples: group A (n=59), composed by fresh samples (stored at 0-4°C); group B (n=59), experimentally frozen at -35°C for 15 hours in the blast chiller IF51M (SAGI Spa), then thawed at 0-4°C. The temperature of the cell was continuously monitored, as well as the core temperature of the product. After, respectively, refrigeration and freezing/dethawing, all samples were fixed in 10% neutral buffered formalin for 24 hours and routinely processed. Paraffin embedded blocks were cut on a microtome into 3±2 µm sections and stained with haematoxylin and eosin (HE). Two histopathologists examined the slide preparations by optical microscopy at increasing magnification of the objectives (4X, 10X, 20X, 40X) and classified them as frozen-thawed or fresh, according to the standard operating procedure criteria in use at IZSPLV. Frozen-thawed samples (positive) were identified when optically empty vacuoles of various dimensions, caused by ice crystals, were observed in the cytoplasm of muscle cells; fresh (negative) samples were identified when these microscopic changes were absent. Sensitivity and specificity of the histological method were calculated, with corresponding 95% confidence intervals; Cohen's kappa was calculated as a measure of agreement.
All samples were correctly identified. Statistical analysis showed 100% (CI95%: 94–100) sensitivity and 98% (CI95%: 91–100) specificity for the histological method in distinguishing between the 2 groups of samples. Cohen's kappa was 1, indicating optimal agreement between the readers.
In conclusion, the histological method confirms its reliability in distinguishing between fresh and frozen-thawed fish, even when a preventive treatment at -35°C for 15 hours is applied.

Keywords: *fish, histology, fresh, frozen-dethawed*

B21
DEVELOPMENT AND VALIDATION OF A DNA BARCODING METHOD FOR THE SIMULTANEOUS IDENTIFICATION AND DIFFERENTIATION OF 15 MAMMALIAN AND 11 POULTRY SPECIES IN FOOD

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Since the European horse-meat scandal in 2013, identification and differentiation of animal species in raw meat and meat products play an increasing role. One of the most common frauds worldwide is substitution of high priced meat species by low-cost ones. In addition, meat products are prone to be adulterated by mixing different animal species without declaration. Thus, for food control authorities it is essential to prevent debasement of food and to guarantee correct information for customers to favor foods over others for religious, ethical or health reasons.
Verification of food authenticity is based on the analysis of proteins or nucleic acids. In general, DNA based methods are more applicable to raw, processed and heat-treated food products because DNA is a relatively stable molecule that is found in all organism and shows species-specific differences in sequence. Most DNA assays for meat species identification rely on the use of polymerase chain reaction (PCR). In the past years numerous real time PCR methods have already been developed for the identification of domestic and game animals. However, several single- or multiplex PCR-setups are needed for accurate analysis of even only one food-sample. The implementation of "next generation sequencing (NGS) technologies", especially the massively parallel sequencing of amplicons, which is based on the analysis of species-specific differences in short DNA sequences, offers new possibilities in food research. So-called DNA barcoding has been shown to accurately identify a wide variety of species in individual foodstuffs or in mixtures. Furthermore, short artificial oligonucleotides flanking the specific sequences allow for pooling and simultaneous analysis of many samples within one mixture (indexing).
In this study we focus on development and validation of a DNA barcoding method to amplify a target region of the mitochondrial 16S rDNA gene of 26 animal species commonly consumed in Austria. Indexed and pooled libraries were sequenced on the MiSeq® platform from Illumina and analyzed in an automated amplicon analysis workflow implemented on the Galaxy platform to identify the respective animal species by comparing with reference sequences (NCBI database). A duplex PCR assay was developed for the amplification of DNA from 15 mammalian species (primer sequences from literature) and 11 poultry species (using a novel primer pair). The applicability and reliability of the method was investigated by analyzing individual DNA extracts from muscle meat, DNA extract mixtures and extracts from model sausages. The results suggest that all meat species included in this study can be identified down to 0.1% even in 96 indexed and pooled samples simultaneously in one run on the MiSeq®.
Before introduction to routine this high-throughput, cost effective technology, which is not limited to these 26 species, needs further in-house validation in sensitivity, recovery and accuracy.

Keywords: *food authenticity, species identification, amplicon sequencing*

Acknowledgement: *This work is supported by the Austrian Agency for Health and Food Safety (AGES).*

B22
STABLE ISOTOPE COMPOSITIONS OF POLISH CIDERS

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Stable isotope analyses have been useful tool for food authenticity control. Important limitation of the application isotopic method for food authenticity control is a lack of database of stable isotope composition for different origin food. Stable Isotope Laboratory of Institute of Nuclear Chemistry and Technology from many years carry out a study of isotopic composition of food for elaboration and implementation new IRMS methods and database for some food from polish market. Our recent study carried out concern the intramolecular isotopic distribution pattern between the ciders components.
Cider is the popular drink produced by apple juice fermentation. Main chemical components of cider are: water, ethanol, sugars, flavors and CO_2. The subject of the study is determination of stable isotope composition of water, ethanol and CO_2 included in ciders.
Samples of commercial ciders were purchased from Polish market. We examined their isotopic composition by the use IRMS methods. Our study concerns the isotopic composition of: $\delta^{13}C$ ‰ in ethanol, oxygen $\delta^{18}O$ in water and carbon $\delta^{13}C$ in CO_2. The isotopic compositions of water and CO_2 were determined using Gasbench (ThermoQuest, Bremen, Germany) connected in continuous flow mode to DELTA^{plus} (FinniganMat) mass spectrometer. The isotopic compositions of ethanol were performed using the same mass spectrometer with Elemental Analyzer Flash EA1112 (ThermoQuest, Italy). Every sample was measured sixth time for carbon and oxygen isotopic composition. The standard deviations of the values obtained from measurements was 0.2 ‰ for $\delta^{13}C$ (ethanol and CO_2) and 0.15 ‰ for $\delta^{18}O$ (water).
In conclusion, the work will be continued for the bigger population of commercial products (ciders). The final product of the study will be the database of stable isotopic compositions for all basic components of ciders of different origin. By the use the database, the isotopic method for origin control of ciders by stable isotopes will be implemented for everyday practice. Finally, on the base of the study the correlations between a carbon isotopic composition $\delta^{13}C$ in CO_2 and C_2H_5OH and $\delta^{18}O$ in water are elaborated. The results of our study for commercial samples of ciders will be presented and discussed.

Keywords: *cider, stable isotope, food control*

Acknowledgement: *The work has been supported by the statutory activity of the Institute of Nuclear Chemistry and Technology.*

B23
ISOTOPE FINGERPRINTS: ORIGIN OF TEQUILA WITH GC COUPLED WITH ISOTOPE RATIO MS

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The blue agave (*Agave tequilana* Weber var. Azul) is a native plant of the Jalisco region in Mexico and is an important economic product that, by law, is the only one allowed to be used in the production of tequila. Globally, tequila is a popular alcoholic beverage, which has led to increasing demand and thus production, with a subsequent increase in export value to the Mexican economy. This provides for an opportunity of economically motivated fraud either by adulteration and mislabeling of original tequila or production of fake tequila.
Gas chromatography/isotope ratio mass spectrometry provides a powerful tool for determining carbon, oxygen and hydrogen isotope fingerprints in beverages and food. Thermo Scientific™ TRACE™ 1310 GC coupled with Thermo Scientific™ GC IsoLink II™, Thermo Scientific™ ConFlo IV™ Universal Interface and a Thermo Scientific™ DELTA V™ isotope ratio mass spectrometer offers a solution for identifying the purity and adulteration of products.
Biosynthesis of organic molecules in *A. tequilana* requires water that comes principally from rainfall. Therefore, oxygen isotope fingerprint of the *A. tequilana* plant, and local sugars used in mixed tequilas, is primarily given by the rainfall water in those regions and can provide a geographical tool for origin. Here we report carbon and oxygen isotope fingerprints from commercial tequila, sugar cane and the *A. tequilana* plant. Coupled $\delta^{13}C$ and $\delta^{18}O$ values of ethanol allow differing the original branded mixed tequila from *A. tequilana* and sources of sugar (corn and cane). This indicates that mixed tequila can be clearly differentiated from pure tequila, which derives 100% from *A. tequilana*. In addition, it also shows the difference between *A. tequilana*, original mixed tequila and sugar sources, meaning that adulterated and mislabeled tequila can be differentiated from original tequila and original source ingredients.

Keywords: *isotope, ratio, IRMS, chromatography, tequila*

B24
ISOTOPE FINGERPRINTS: AUTHENTICATION OF HONEY BY LC COUPLED WITH ISOTOPE RATIO MS

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Honey is considered as a value-added food of natural origin, yet it is simply structured regarding its composition, which makes it prone to economically motivated adulteration by addition of sugars of other sources. Testing for adulteration can be done using various methods, including melissopalynological pattern analysis, sensory analysis, amino acid profile analysis, and others. The limit of detection of these methods is generally in the double-digit percent range, depending on the type of sugars added. The introduction of bulk ^{13}C isotope analysis by White and Doner in 1978 was a major step towards establishing better methods sensitivity. But carefully selected mixtures of sugars can mimic both, the bulk ^{13}C composition and the sugar profile of the natural product.
Compound specific isotope analysis can refine the authenticity fingerprints of honey. The methodology based on the chromatographic separation of the carbohydrates and carbohydrate fractions and the subsequent isotopic analysis by coupling LC with isotope ratio mass spectrometry (IRMS). Carbohydrates eluting from the ion exchange column are chemically oxidized to carbon dioxide within the aqueous mobile phase using the Thermo Scientific™ LC IsoLink™ interface. Dissolved CO_2 is removed from the liquid phase and entrained into a stream of helium. The individual CO_2 peaks in helium - which correspond one to one with the peaks of the individual compounds - are subsequently dried and then admitted to the IRMS system. The individual $^{13}C/^{12}C$ isotope ratios are determined by the Delta V mass spectrometer for the following carbohydrates and carbohydrate fractions: i) glucose, ii) fructose, iii) disaccharides, iv) trisaccharides, and v) oligosaccharides.
The $\delta^{13}C$ of bulk honey is determined by analyzing pure honey samples in a Thermo Scientific Flash 2000 elemental analyzer coupled to a Delta V mass spectrometer and a ConFlo IV referencing device (all Thermo Fisher Scientific, Bremen, Germany). Samples are prepared by encapsulation in tin foil and introduced into the elemental analyzer without additional treatment.
The protein fraction is prepared by Na_2WO_4 precipitation from aqueous sample solution, dried and analyzed using the configuration described above.
The resulting maximum difference between the following values is being calculated: a) bulk $\delta^{13}C$, b) protein $\delta^{13}C$, and c) the individual carbohydrates and fractions (i –iv, above). A large difference in the values (on the order of 1‰ or greater) might indicate adulteration and requires further investigation. This work describes a multi-parametric methodology, looking at both, bulk and compound specific $\delta^{13}C$, deducing isotope fingerprints and identifying adulteration.

Keywords: *isotope, ratio, IRMS, HPLC, honey*

B25
VALIDATION OF A RAPID LATERAL FLOW METHOD FOR THE DETECTION OF COW MILK IN WATER BUFFALO, SHEEP OR GOAT MILK

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Adulteration of milk from sheep, goat or water buffalo with cow milk has been a widespread practice due to the higher cost of milk from those species. In addition, milk production is highly seasonal in some of such species, which causes a deficit in some periods of the year. Cheeses produced with milk other than cow milk have their own organoleptic characteristics that make them different and for which consumers are willing to pay a higher price. In this context, many varieties of cheeses are covered by the Protected Designation of Origin (PDO) system to ensure the quality and genuineness of these products. In order to ensure the correct identification and labeling of these products, it becomes necessary that the whole production chain is involved in the quality and traceability of the product.
Methods for milk adulteration detection based on lateral flow immunoassay (LFIA) are an effective tool to be used in self-control in dairies and to verify the quality of milk previously to collection in farms. LFIA tests for the detection of cow milk in sheep and goat milk as well as for the detection of goat milk in sheep milk were firstly developed more than a decade ago but up to date there is yet no rapid test that allows the detection of cow milk in buffalo milk. In this work we present the first rapid test based on LFIA to detect cow milk in buffalo, sheep or goat milk. The performance of the method was validated according to internationally AOAC guidelines for qualitative methods.
No false-positive result was found after analyzing 146 negative samples (51 of buffalo milk, 52 of sheep milk and 43 of goat milk) from individual animals. The lowest level of adulteration with a Probability of detection (POD) of 1.00 (confidence interval between 0.90 and 1.00) was found at 0.5% of cow milk. This level is below the current EU allowed level of adulteration, set at 1%, and at the level of the limit of detection recommended for routine methods (Commission Regulation (EC) No 273/2008). The test showed no significant hook effect, giving positive results even up to 100% of cow's milk. The robustness of the method was also assessed. Thus, variation in the time of assay, volume of the analysis buffer and different batches of the test were evaluated in order to detect any effect on the false-positive rate or on the limit of detection of the test. The effect of environmental or compositional factors (such as high level of fat, protein and somatic cell counts) that could affect the fluidic of the assay was also evaluated.
The new rapid test to detect cow milk in milk from buffalo, goat or sheep milk has demonstrated to be a suitable tool to control the milk quality in routine analysis. This kind of tests is very easy to use and it can be performed by untrained staff during milk collection at the farm or upon arrival at dairies.

Keywords: *milk adulteration, authentication, lateral flow test, validation, LFIA*

Acknowledgement: *This work has been supported by the financial support of International projects of technological cooperation (EUREKA), E!5810, obtained from CDTI, IDI-2011/0413, (Spanish Government).*

B26
FEED FRAUD: EXPERIENCE WITH THE DETECTION OF FEED FRAUD IN THE CZECH REPUBLIC

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European legislation does not currently contain any uniform legal definition of the term „feed fraud". Regulation (EU) 767/2009 mentions feed fraud in general terms: Feed may only be placed on the market and used if: (a) it is safe; and (b) it does not have a direct adverse effect on the environment or animal welfare. In addition feed business operators placing feed on the market shall ensure that the feed: (a) is sound, genuine, unadulterated, fit for its purpose and of merchantable quality; and (b) is labelled, packaged and presented in accordance with the provisions laid down in this Regulation and other applicable Community legislation.
Generally feed fraud is understood as deliberate fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser´s knowledge and with financial gain as a motivation. Feed fraud can mean not only harm to the consumer but it can also be significant risk for safety of people and animals. Therefore, considerable attention shall be paid to controlling feed fraud.
Central Institute for Supervising and Testing in Agriculture (CISTA) carries out official controls of feedingstuffs and raw materials for feedingstuffs production in the Czech Republic. Since 2016 CISTA has been designated as a member of Administrative Assistance and Cooperation system (AAC). AAC allows to share information among EU Member States about serious findings from official inspections on fraud, forgery, smuggling and deception, related to food and feed with cross-border implications.
CISTA has detected several cases of feed fraud. Controlling feed fraud requires different approach from feed safety and feed quality controls. Problem in the detection of adulaterations is the fact that the composition of the feed complies with the declaration and unknown adulterants can be overlooked. Several different methods are used to detect feed fraud in CISTA laboratory, ranging from classical wet-chemical procedures, microscopic method, commodity expert´s evaluation to modern instrumental techniques.
Several examples of detecting feed fraud are presented to illustrate analytical approach in CISTA laboratory.

[1] REGULATION (EC) No 767/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC
The EU Food Fraud Network and the System for Administrative Assistance and Food Fraud, Annual Report 2016, European Commission

Keywords: *feed fraud, analytical approach*

B27
AUTHENTICATION OF MILK AND DAIRY PRODUCTS USING LC–MS/MS – TARGET PROTEOMIC ANALYSIS APPROACH

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Food adulteration is a growing problem worldwide. One of the major issues in dairy industry is the adulteration of sheep´s and goat´s milk with more available and less expensive cow´s milk. The fraudulent blending of undeclared cow´s milk into dairy products not only yields illegal profits of manufactures, in addition it might have a serious consequence for people allergic to cow´s milk proteins.
Considering protection of consumers´ interests and manufacturers from dairy products adulteration, a method based on mass spectrometric identification of milk proteins was developed for the purposes of the official control. This method, with a target proteomic analysis approach, is founded on MRM detection of unique peptides in tryptic digest of protein extracts via LC–MS/MS (QqQ). Unique peptides from major milk protein - casein were selected by means of Skyline software and UniProt database. The method was optimized for identifying of different animal species of milk (cow´s, goat´s and sheep´s) in cheese with detection limit of 1 % of added/non-declared milk.

Keywords: *proteomics, cheese, fraud, mass spectrometry*

B28
TACKLING FRAUD IN SEAFOOD GLOBAL SUPPLY CHAINS: INNOVATIONS IN DETECTION USING RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS)

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The increasing number of reports regarding food fraud scandals has brought food authenticity and safety to the attention of regulators, industry and consumers worldwide. Seafood products are one of the most internationally traded food commodities and feature second among commodities that are most vulnerable to food fraud. As per Article 35 of the EC regulation 1379/2013, the commercial designation of the species, production method and geographical origin constitute the "traceability" of the product and must be disclosed to the consumer. Ambient mass spectrometry (AMS) methods have overcome several intrinsic constraints of traditional mass spectrometric analysis schemes, allowing in situ, real-time analysis of a wide variety of samples. REIMS has been used for the analysis of human tissue during surgery and has shown to be capable of the identification of various tissue types based on lipid fingerprinting. In this study, we present an effective, near real time method to identify seafood speciation, geographical origin, catch and production method authentication using REIMS. Raw data were acquired using a Medimass REIMS and/or Waters REIMS source coupled with a Waters Xevo G2-XS QTof mass spectrometer operated in single stage MS, negative and sensitivity mode. All specimens were sampled using a monopolar handpiece that was equipped with a smoke evacuation line that was mounted on the atmospheric interface of the mass spectrometer. Full spectral information was recalibrated, normalised, baseline subtracted and binned up to 0.1 *m/z* bin size. The resulting data was subjected to multivariate analysis including principal component analysis (PCA) followed by a linear discriminant analysis (LDA) using a non-commercial prototype software developed by Waters and Live ID software enabling real-time classification of samples.
Over 4000 spectra were obtained from five different authenticated species of white fish; cod, coley, haddock, pollock, and whiting to generate the speciation and haddock catch method chemometric models. Similarly, spectra were acquired for three commercially important species of shrimps; tiger prawn (*Penaeus monodon*), king prawn (*Litopenaeus vannamei*) and Argentinian red shrimp (*Pleoticus muelleri*). Among tiger Prawns, wild caught prawns from Madagascar; and farmed prawns from Vietnam were studied. Farmed king prawns from three different geographical origins; Thailand, Vietnam and Honduras were also studied. All spectral data were acquired between m/z 200-1200 and both PCA and LDA score plots built using m/z 600-950 identified clear signs of discrimination based on species, geographical origin, catch and production method. Validation of the PCA–LDA white fish speciation models carried out with another batch of fish samples resulted in a 98.99% correct classification rate. REIMS technology could provide a paradigm shift across authenticity applications by providing real-time, reliable, and simple method for the analysis of food products.

Keywords: *REIMS, near-instantaneous profiling, fish speciation, geographic origin, catch method*

Acknowledgement: *The BBSRC (BB/M0503162/1) and Waters Corporation for their funding and continuous support throughout this work.*

B29
DETECTION AND QUANTIFICATION OF TMA AS INDICATOR OF FRESHNESS RATE IN FISHERY PRODUCTS BY DIRECT SAMPLE ANALYSIS WITH HIGH RESOLUTION MASS SPECTROMETRY

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Fish rate freshness is an important parameter in the field of commercial and sanitary frauds and, among food frauds with possible health implications, the supply of fish in poor condition proposed as fresh plays an important role. Different techniques, as physical, microbiological or chemical methods, have been developed for the assessment of the fish spoilage. The latter are based on research and evaluation of substances, for example biogenic amines (BAs), a group of alkaline compounds stable against heat and acid conditions.
The present study describes the development of a method able to detect and quantify trimetylammine (TMA) by means of Direct Sampling Analysis (DSA) coupled with a High Resolution Mass Spectrometer with a Time of Flight detector (AxION2-TOF). We investigated three different seafood, Sea bream (*Sparus aurata*), Octopus (*Octopus vulgaris*) and Red mullet (*Mullus barbatus*), coming fresh from the wholesale fish market of Ligurian Sea (FAC), and from local supermarkets of Torino (FS).
Three samples of each species of FAC were fortified with TMA from 5 to 30 mg/100 g of fishery products; samples were then extracted and analysed to create a calibration curve used to quantify the level of TMA in all fishery products. Extraction consisted in mixing 5 g of homogenized muscle with 10 ml of a solution of methanol and water (50:50); samples were vortexed for 5 minutes and centrifuged for 1 minute at 2000 x g. The supernatant resulting from each extraction was used for APCI–MS–TOF analysis in positive mode.
Samples of FAC were extracted from day 0 to 20 and then acquired in the same session while samples of FS were extracted and analysed on the day of purchase. The peaks areas of $[TMA+H]^+$ of fishery products fortified in a range of 5–30 mg/100 g were used to build a reference curve. The peaks area registered for the three species were similar and therefore combined to create a unique calibration curve for TMA in the matrix. It was linear within the range of 5–20 mg/100g, with a regression coefficient higher than 0.99.
For each fishery products, TMA has been quantified by using the calibration curves and around day 7 the level of TMA reached 20mg/100g in all the FAC species investigated; in FS samples TMA overcame the 20 mg/100g in the 10% of the examined red mullet and in 20% of sea bream and octopus. The results obtained suggest this method as innovative, simple, rapid and useful for routine screening analysis and could be applied to screen also other type of fishery products in order to detect food fraud, especially in species that visually do not show evident signs of degradation.

Keywords: *TMA, fish freshness, mass spectrometry, food fraud*

Acknowledgement: *This work was founded by the Italian Ministry of Health (grant no. IZS PLV 01/14 RC). The authors are grateful to dr. G. Fazio for his help in collecting samples at the fish market of Ligurian Sea.*

B30
AUTHENTICITY CONTROL OF BEVERAGES AND WATER IN FOOD WITH GASBENCH II SYSTEM USING ISOTOPE FINGERPRINTS

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In this presentation the application of stable isotope fingerprints in beverage and water in food is explored. Data are shown that show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints in beverages and the technology used is also provided. It can easily be adapted to water in food (e.g. meat).
The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect whether the labeled product is authentic or if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.
Detecting food and beverage fraud can be achieved using stable isotope measurements with the isotope equilibration technique (Method OIV-MA-AS2-12, EU regulation no. 822/97). This because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable standardized technique in food and beverage fraud prevention and food safety. For wine in the EU isotope signals are stored in the EU-WineDatabase or other national databases, e.g. >1400 ΔO^{18} of water in European wines, national databases).

Keywords: *isotope fingerprints, beverage and water, method OIV-MA-AS2-12 EU regulation no. 822/97, authenticity, isotope equilibration technique*

B31
EA-IRMS: TRACING THE GEOGRAPHICAL ORIGIN OF ROASTED AND GREEN COFFEE USING ISOTOPE FINGERPRINTS

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Coffee is one of the most popular beverages worldwide, sourced from different geographical regions and exported through a commercial chain that usually involves several intermediates. To ensure that coffee beans come from labelled locations, laboratories need an analytical solution, enabling to discriminate geographical origin, with a special emphasis on the country of origin.
Roasted and green coffee beans have a fingerprint, a unique chemical signature that allows them to be identified: isotope fingerprints of carbon, nitrogen, sulfur, hydrogen and oxygen have been reliably used for origin, authenticity and product label claim verification.
In this poster, we report isotope measurements from green and roasted coffee beans measured using the Thermo Scientific™ EA IsoLink™ IRMS System. These data illustrate how isotope fingerprints can determine the origin of coffee beans. Consequently, it is evident that isotope fingerprint approach helps support legislation on food integrity and labelling (EC Reg. No. 1169/2011) and product geographical indication/origin (EC Reg. No. 510/2006) and therefore, protect consumers and brands.

Keywords: *green coffee, roasted coffee, isotope fingerprints, hydrogen, labeling*

B32
FOOD AND BEVERAGE FRAUD PREVENTION USING ISOTOPE FINGERPRINTS

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In this presentation the application of stable isotope fingerprints in food and beverage fraud detection is explored. Data are shown that show how stable isotopes offer conclusive answers on questions associated with origin, adulteration and correct labeling of food and beverage products. An overview of the interpretation of isotope fingerprints and the technology used is also provided.
The food and beverage industry suffers from fraudulent activities that include incorrect labeling of products and adulteration, which has a significant impact on food and beverage safety, brand names and reputation and the market economy. Preventing food and beverage fraud is a key challenge that requires a reliable, cost-effective analytical process that can detect whether the labeled product is authentic or if it has been changed after the final manufacturing process, or alternatively if it has been independently produced, using alternative ingredients, but labeled as an original product.
Detecting food and beverage fraud can be achieved using stable isotope measurements because stable isotopes can differentiate between food and beverage samples which otherwise share identical chemical composition: this is called the isotope fingerprint. Using the isotope fingerprint of food and beverage products is a reliable technique in food and beverage fraud prevention and food safety.

Keywords: *food fraud, isotopes, beverage fraud*

B33
HOW TO ASSURE SAFETY, QUALITY AND AUTHENTICITY IN THE FRUIT JUICES?

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The fruit juices are considered within the 10 most susceptible food products to be adulterated [1]. The complicated supply chain also contributes to increase the risks on the safety, quality and authenticity. In a global market, fruit juices are produced in America, Asia, Africa or Europe and shipped in bulk to the bottlers all over the world.
The Voluntary Control System (VCS) implemented by the industry as a self-control tool has been proved as a very helpful tool to minimize the risks and to protect the juice producing sector from unjustified attacks. The system has been successfully working for more than 40 years. The VCS system is based on different activities such as on-site audits plus the drawing of running production samples and delivery counter samples for safety, quality and authenticity analysis. Those analyses are a combination of traditional and state-of-the-art methods (DNA, NMR profiling fingerprinting, etc.).
With such controls the number of typical frauds such as over dilutions, mixing with cheaper raw materials or adding no authorized substances [2] are not so frequently found in the markets and, if found, the VCS immediately initiates corrective measures, that can be scaled up to court cases.
The VCS also focuses in the safety aspects coming from the first step in the chain, the agriculture. For that reason regular control of amounts of heavy metals on the soil and pesticides used on the fields are done. The result is that juice producing industry is included in a rapid alarm system (FRAPP: Fruit Risk Assessment Program for Pesticides) and can take measures before the identified products can get into the final juices to be consumed by consumers.
The last activity of the VCS has been to close the existing gap regarding the bulk transport of the juices. The proper cleaning of the tankers has been identified as a risk that was not satisfactorily solved up to now. With the inclusion of the tank cleaning stations and transporter into the VCS, the supply chain is safer than ever with more than 80% of the worldwide production controlled.

[1] Moore, J. Spink J and Lipkus M. Development and Application of a Database of Food Ingredient Fraud and Economically Motivated Adulteration from 1980 to 210. Journal of Food Science, 2012 Volume 77 Number 4. P R118-R126.
[2] Johnson, R. "Food fraud and economically motivated adulteration in food and food ingredients" Congressional Research Service Report (2014).

Keywords: *authenticity, food fraud, fruit juices*

Acknowledgement: *SGF International e.V.*

B34
GENETIC IDENTIFICATION OF CITRUS DEPRESSA AND CITRUS MADURENSIS BASED ON THE CHLOROPLAST DNA

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Citrus depressa Hayata, called shiikuwasha in Japan, is most popular sour fruit in Okinawa Island, Japan. Shiikuwasha contains high concentrations of polymethoxylated flavones in its peel, one of the most important health-promoting components of citrus. The market for shiikuwasha products has grown rapidly as a result of increasingly health-conscious consumers and price increase, challenging shiikuwasha farmers to live up to with product demand. As a result, shiikuwasha juice adulterated with calamondin (*Citrus madurensis* LOUR.) juice as an alternative ingredient. The discrimination method which already assumed the ingredient which is characteristic of calamondin, 3′,5′-di-C-b-glucopyranosyl phloretin, polymethoxylated flavones and g-terpinene and index as a detection technology to guarantee the distribution of the pure shiikuwasha product was developed. However, the identification method with a more highly-detailed by using DNA is strongly demanded. The purpose of this study was to analyze the region of chloroplast DNA of shiikuwasha and calamondin using the single nucleotide polymorphisms (SNPs) for identification.
Isolation of total DNAs was performed using DNeasy Plant Mini Kit. We amplified *trn*L-*trn*F and *trn*T-*trn*L region as total DNAs which we extracted from pericarp by the polymerase chain reaction (PCR). SNPs at the *trn*L-*trn*F and *trn*T-*trn*L intergenic region of the chloroplast DNA was confirmed in shiikuwasha and calamondin. DNA marker to detect species-specific SNP found by alignment, the 3′ end of the forward primer designed the primer corresponding to the base substitution site. We performed the PCR of all samples using these primers which we designed.
We designed the primer to detect species specific SNP found by *trn*L-*trn*F and *trn*T-*trn*L region and confirmed the specificity. As a result, it was shown that shiikuwasha and calamondin were identifiable by using two primers.

Keywords: *DNA markers, PCR, SNP, Shiikuwahsa, Calamondin*

B35
AUTHENTICITY ASSESSMENT OF APPLE AND STRAWBERRY KEY AROMA COMPOUNDS WITH GAS CHROMATOGRAPHY-COMBUSTION-ISOTOPE RATIO MASS SPECTROMETRY (GC-C-IRMS) ANALYSIS

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Presented work relates to the field of determining the authenticity of the raw materials such as natural flavourings. Natural aromas are becoming extensively used in many industrial food products due to consumer demand for natural products. While natural raw materials are becoming more expensive, there is also increasing pressure on prices and pronounced quality. Thus, many natural materials show the need for control of authenticity, as they are commonly being falsified in terms of dilution, mixing, or incorrect declaration of origin of natural resources. A dynamic headspace solid-phase microextraction (SPME) methodology used with gas chromatography-mass spectrometry (GC-MS) and gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) analysis was developed for a compound identification and stable isotope analysis that can be used to differentiate between natural and synthetic aroma compounds of various aroma compounds in apple and strawberry self-produced, commercial distillates samples, and also several other essential oils.
Development and optimization of working parameters that influence the SPME method efficiency, for both apple and strawberry active aroma compounds were done. Results show that successful extraction of different aroma compounds requires different extraction conditions. Once optimised, SPME technique obtains highly reproducible results from low concentrations of analytes with short analysis time.
GC-MS was used for the identification of aroma compounds based on their mass spectra comparison with NIST spectral library and also by the combination of retention indices. The dual analysis gives strong evidence for compound identity. The results show a difference in the presence or absence of certain aromatic components as well as the relationships between them.
Further, when applying stable isotope analysis results in GC-C-IRMS analysis, peak identification was done by comparison to GC-MS and by second confirmation with authentic standards. In GC-C-IRMS analysis our main aim was to determine the $\delta^{13}C$ values of aromatic components, which significantly affect the perception of flavour. When $\delta^{13}C$ values of aroma compounds from several commercial essential oils and distillates, labelled as natural, were compared to existing literature, several possible falsifications were identified.
Our first results show that SPME is an appropriate tool for volatile extraction and further identification with GC-MS and characterization of aroma compounds with GC-C-IRMS for apple and strawberry distillates, and other essential oils. To gain more confidence in the interpretation of measured values, an extensive database is required, which is under construction and is going to be upgraded on a yearly basis.

Keywords: *flavour compounds, authenticity, stable isotopes, SPME, GC-C-IRMS*

Acknowledgement: *Research is implemented in the framework of Smart Specialization Program: Food for Future.*

B36
A NOVEL LATERAL FLOW METHOD FOR THE QUANTIFICATION OF COW MILK

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Introduction
Higher priced milk is commonly and fraudulently adulterated with lower priced cow milk, used in direct human consumption or in cheese manufacturing. In addition, cow's milk allergy is one of the most common food allergies. To date, the percentage of cow milk in other species milk has been detected by lateral flow rapid tests and quantified by ELISA with a total procedure time of at least 10 and 90 minutes respectively.
Aim
The aim of this work was the combination of the quantification capability and the immunochromatography swiftness into a lateral flow device that detects and quantifies bovine milk in other species milk.
Methods
An indicative lateral flow strip consisted of a nitrocellulose membrane with a high affinity monoclonal primary antibody test line and an anti-species control line, a conjugate pad with a colloidal gold conjugated secondary monoclonal antibody and an absorbent pad. Monoclonal antibodies against bovine IgG are produced after mice immunization, preparation of hybridomas, clones screening and protein G purification. The colloidal gold nanoparticles (40nm) were produced by reduction of chloroauric acid and the conjugate with the secondary antibody was carried out by pH-modulated absorption. The milk sample was diluted five times with a running buffer before strip immersion and the percentage of bovine milk was determined by a novel quantification system using a high technology scanning device.
Results
This lateral flow strip requires one drop of milk and the procedure total duration is 10 minutes. The combination of the high-affinity antibody pair and the advanced quantification system, contributed to a high range standard curve. According to scanning device findings, the type of graph was close to ELISA method levels.
Conclusion
This lateral flow device constitutes an extremely valuable tool in the rapid quantification of cow milk in other species milk, since it combines a fast protocol with the significance of the results.

Keywords: *lateral-flow, milk fraud, milk adulteration*

B37
PLANT METABOLOMICS - MAXIMIZING METABOLOME COVERAGE BY OPTIMIZING MOBILE PHASE ADDITIVES FOR NON-TARGETED MASS SPECTROMETRY IN POSITIVE AND NEGATIVE ELECTROSPRAY IONIZATION MODE

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Non-targeted metabolomics applications are employed in various fields; in the food sector they enable the proof of authenticity parameters like geographical origin, organic cultivation, biological or chemical identity. Currently, one of the most widespread analytical platforms for non-targeted metabolomics approaches is liquid chromatography connected with an electrospray ionization quadrupole-time-of-flight-mass spectrometer (LC-qToF-ESI-MS(/MS)). The number of metabolites in a single plant ranges from 5.000 to 25.000 different compounds. Moreover, the total number of metabolites in the plant kingdom is estimated at 200.000 including various other compounds. Due to their high diversity, it is not possible to detect all metabolites by a single analytical method. The higher the resolution and performance of a technology, the more molecules can be registered. This is particularly important for non-targeted metabolomics studies free from hypothesis, as they offer a way to detect simultaneously many other possible metabolites and therefore increase the likelihood of identifying all relevant compounds.
To enhance ionization and MS-sensitivity as well as liquid chromatography separation, different organic solvents and mobile phase additives are applied. The selection of a suitable additive depends on the metabolites that should be detected, mobile and stationary phase as well as ionization mode. Therefore, in this study different mobile phase additives were tested in order to improve electrospray ionization process and to detect as many metabolites as possible with high peak intensities in positive and negative ionization mode. Influences of modifiers were examined for nonpolar and polar compounds, as optimal conditions are not always the same.
The results demonstrate, that no single solvent modifier was appropriate to give the best performances, but an extensive validation can improve significantly data quality and hence also information content. Furthermore, a skillful combination of different modifiers for the various ionization modes is able to extend the analytic spectrum that can be detected.

[1] Marina Creydt and Markus Fischer. "Plant Metabolomics - Maximizing Metabolome Coverage by Optimizing Mobile Phase Additives for Non-Targeted Mass Spectrometry in Positive and Negative Electrospray Ionization Mode." Analytical Chemistry, Just Accepted Manuscript (2017/08/29 2017).

B38
SHRIMP FRAUD: EVALUATING CHARACTERISTIC METABOLOMIC FEATURES FOR AUTHENTICATION BY HIGH AND UNIT MASS RESOLUTION SPECTROMETRY

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Unfortunately, fishery products occupy the second highest ranking position among commodities that are at most risk of food fraud. Among these, marine shrimps and prawns, especially the penaeid shrimps, accounts for more than 17% of the global seafood consumption. As per Article 35 of the EC regulation 1379/2013, the mandatory information to be provided to consumers for all categories of fishery and aquaculture products includes the commercial designation of the species, production method and geographical origin. DNA based assays are established methods for determination of fish species identity whereas stable isotope based assays have been the methods of choice for provenance determination. However, high resolution mass spectrometry based food metabolomics holds potential to test all these authentication issues in a single analytical platform. Authentic samples were collected either directly from aquaculture farms or through local supermarket chains. Six species of shrimps namely Tiger Prawn (*Penaeus monodon*), King Prawn (*Litopenaeus vannamei*), Indian Prawn (*Fenneropenaeus indicus*), Pink Speckled Shrimp (*Metapenaeus monoceros*), Argentinian Red Shrimp (*Pleoticus muelleri*) and Red Prawn (*Solenocera crassicornis*) were included in the study. Among Tiger Prawns, wild caught prawns from India and Madagascar; and farmed prawns from Vietnam were sources as were farmed King Prawns were from India, Thailand, Vietnam and Honduras.
Methanolic extracts of freeze-dried samples were analysed in full scan positive and negative ionisation mode using a Waters Acquity I-Class UPLC coupled to a Xevo G2SQTof. Chromatographic alignment, peak picking and feature identification of the acquired data were carried out on Progenesis QI (Nonlinear Dynamics) software followed by multivariate analysis in SIMCA-P (Umetrics) software. OPLS-DA chemometric models could discriminate between species, geographical origin and production method. The R^2 and Q^2 values for all the models were >0.9. Further, characteristic metabolomic features for each species were determined by group wise comparison of the data. These features were evaluated in a targeted, triple quadrupole LC-MS/MS method and at least one exclusive biomarker for each species was selected. Subsequently, a confirmatory LC-MS/MS (Waters Xevo TQ-S) method for authentication of species was developed. An OPLS-DA model built using the characteristic features of Tiger Prawns and King Prawns was shown to discriminate between the geographical origins of these species. The data generated gives a strong indication that the routine testing for species authenticity as well as the geographical origin of the prawns can be achieved using triple quadrupole LC-MS/MS.

Keywords: *authentication, HRMS, LC-MS/MS*

B39
GENETIC PROFILING: BOTANICAL ORIGIN OF TRUFFLES

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The botanical origin of food is of high priority for consumers and manufacturing companies that are prepared to accept higher prices for the valuable characteristics from certain species. As a consequence, indicating the agricultural commodities can have significant influence on purchasing decisions and thus offers a special motivation for food fraud.
High-quality foodstuffs with a high importance of species are truffles are subterranean Ascomycete fungi living in a symbiotic relationship with the roots of particular trees. There are many different varieties of edible truffle: the Summer or Burgundy truffle (*Tuber aestivum/ uncinatum*) and the Périgord truffle (*T. melanosporum*) are appreciated for their unique taste patterns. The most expensive edible truffle is the white Alba truffle (*T. magnatum*) followed by the Périgord truffle with a price of 1.000-2.000 €/kg.
Asian black truffles (*T. indicum, T. sinense, T. himalayense*) are morphologically very similar to the Burgundy truffle and the Périgord truffle, however, they are less valuable in terms of their flavour profile. Due to their lower price (100-200 €/kg) Asian black truffles are often used for the "dilution" of high priced European truffle species.
The DNA sequence is best suited to tackle the biological origin. This is due to the fact, that DNA is present in every cell and generally not affected by external influences like weather or soil composition over a long period of time.
This poster presents fingerprinting studies of different truffle varieties using microsatellite (inter simple sequence repeats (ISSR)- and RAPD (random amplified polymorphic DNA)-markers.

Keywords: *food fraud, truffle, genomics*

B40
PROFILING STEROLS IN OLIVE OIL FOR QUALITY AND AUTHENTICITY USING SUPPORTED LIQUID EXTRACTION (SLE), SOLID PHASE EXTRACTION (SPE) AND GC-FID

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The determination of cis/trans fatty acid methyl esters (FAMEs) is a nice solution for some label claim and grading requirements in Olive Oil. As its popularity continues to increase, due to reported health effects, and culinary uses, further profiling is necessary for a quantitative authenticity test, especially with different organoleptic regional notes. Profiling sterols by GC can help quantify these otherwise subjective, but important consumer properties, while assisting from regional adulteration/label claim fraud.
Several official methods for detecting adulterants and determining olive oil quality have been established in order to guarantee authenticity and safety. One such method is the determination of sterol concentrations in olive oil. A sterol profile provides an important criterion for distinguishing virgin olive oil from refined oil as well as a fingerprint for the detection of several seed oil adulterants and even geographical origin. Solid Supported-liquid extraction (SLE) using a Strata DE (diatomaceous earth) column followed by solid-phase extraction (SPE) cleanup with an activated Strata Si-1 cartridge was used to determine the concentration sterols, erythrodiol and uvaol in olive oil. Sterol profiles obtained with this method confirmed the classification of adulterated and certified extra virgin olive oils according to International Olive Oil Council (IOC) criteria. Method accuracy was assessed from the absolute recoveries of brassicasterol (82%), campesterol (75%), stigmasterol (77%), β-sitosterol (100%), erythrodiol (134%) and uvaol (118%). Compared to the official IOC procedure, which uses liquid-liquid extraction and thin-layer chromatography, the SLE-SPE modification is less time-consuming and labor-intensive, avoids the use cumbersome glassware and large amounts of solvent, and allowed up to 16 samples to be extracted in parallel.

B41
STUDY OF ISOTOPIC AND ELEMENTAL COMPOSITION OF MILK AND DAIRY PRODUCTS

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The different composition of cow fodders (natural and processed) and different composition of drink water (mainly tap water) are a reason of seasonal and regional variation of isotopic and trace elements composition of fresh milk and finally the dairy products. The stable isotope composition of milk is affected by environmental conditions like a climate, geographical region, season and anthropogenic: methods of milk production (organic and commercial) with the feeding strategy used.
The aim of this study was to demonstrate the differences in regional and seasonal variations of stable isotopes and trace elements composition of milk. The samples of fresh and commercial milk were gained from main regions of milk production in Poland and measured by the use the isotope ratio mass spectrometer. The measurements of stable isotope composition (carbon, hydrogen, oxygen, nitrogen and sulfur) of food provide a very sensitive method for control of their origin and authenticity. The mass spectrometry technique (IRMS) is the main method for measurement of the stable isotopes composition in food and beverages. The DELTA$_{plus}$ mass spectrometer connected with an H/Device instrument was used to determine hydrogen isotope ratio. Oxygen isotope ratio was determined in water samples on a Gasbench instrument connected with a mass spectrometer. For the determination of C, N, and S in solid materials the Elemental Analyzer coupled with a mass spectrometer was used. The measurements of trace elements concentration were performed, after acid microwave digestion, by the use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for selected dairy product samples.
Finally, application of the multielement (isotope and trace element concentrations) method for origin control of dairy products requires still additional research on a larger population of dairy product samples, from different regions, farms (organic and commercial) and seasons. After the construction of the big database for isotopes and trace elements, the method (database) could be used as a standard for origin and authenticity control of dairy products for selected (tested) regions.

Keywords: *milk origin, control authenticity, stable isotopes*

Acknowledgement: *This work was supported by the Polish Ministry of Science and Higher Education under grant W79/FAO/IEAE/2016.*

B42
RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS) IN THE FIGHT AGAINST BEEF ADULTERATION

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The European horse meat scandal of 2013 and subsequent Elliott Review of food networks in the UK highlighted the extent of food fraud by adulteration and substitution in global food production. In developing methods for detecting food fraud, speed is as important as specificity and sensitivity. The Institute for Global Food Security at Queen's University Belfast in collaboration with Nestlé and Waters is pioneering use of Waters' Rapid Evaporative Ionisation Mass Spectrometry (REIMS) technology coupled to a high frequency current electrosurgery iKnife as a tool for real-time analysis of the adulteration, speciation and geographical origin of foods. The iKnife causes localised heating of a sample, producing a vapour rich in molecular information which is sampled directly by a Waters Xevo G2-XS Quadrupole Time-of-Flight Mass Spectrometer. Spectral data is used to build multivariate models which classify an unknown sample in seconds. No sample preparation is required.
A model has been built to detect adulterating non-muscle tissues in minced beef. Authenticated beef meat, bovine offal and other waste tissues obtained from processing industry partners were assessed for characteristic mass spectra by iKnife sampling. Beef and adulterants were minced and blended (1-20%). Patties (20g) were prepared by hand and cut 12-15 times by iKnife in 30W AutoCut mode, each cut taking 3-4 secs. A PLS-Discriminant Analysis (PLS-DA) model was built with >100 different beef patties and selected adulterants (3000 spectra, m/z 600-950 range). The predictive power of the model is determined by analysis of blended patties under the same conditions. A single iKnife cut is typically analysed and classified several times to ensure that even a small pocket of adulterating tissue yields a characteristic spectrum classified as a non-beef tissue within a few seconds of cutting. Preliminary validation shows detection of adulteration at approximately 10% in minced beef: sufficient to screen for economically viable meat adulteration. Detection capability depends on the tissue present and degree of adulterant mincing.
The horse meat scandal demonstrated the economic benefits of substituting processed beef with a cheaper protein. Other potential meats for such fraud include pork, lamb and goat. A minced meat speciation PLS-DA model has been developed using iKnife REIMS. Beef patties blended with authenticated samples of each meat type were cut by iKnife as above. Preliminary validation demonstrates detection of beef substituted with 10% or less meat from other species.
Analysis by ambient REIMS technology is much faster than PCR or protein marker techniques. Whilst REIMS cannot match their sensitivity, it may provide a rapid screening method in advance of a more sensitive confirmatory analysis. The next challenge is to interface REIMS with a more portable mass spectrometer for use in the processing or retail setting to provide true real-time screening of processed meat products.

Keywords: *rapid evaporative ionisation mass spectrometry, food authenticity, beef adulteration, bovine offal, meat speciation*

B43
CHARACTERIZATION OF GRAPE VARIETIES INDIGENOUS TO CYPRUS, AND AUTHENTICATION OF CYPRIOT WINES

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This study has been conducted to perform separation of botanical origin based on the phenolics composition of the wines. Cyprus has been a wine producer country since about 4000 B.C, thus there are several indigenous varieties, among them Maratheftiko, mavro (red) and Xinisteri (white) varieties. The characterization of the grapes of the indigenous cultivars as well authentication of the wines from the indigenous and tranditional varieties (cabernet Sauvignon) will promote the cypriot wines as a constituent of the Mediterranean diet, regarding the positive effect of antioxidants on human health. Red wine has beneficial effects on human health attributed mainly to polyphenols components. Polyphenols of red wine reduce the formation of free radicals, help in the prevention of oxidative damage, increase vasodilation and have a kidney-protective effect. They also exhibit anti-microbial properties.
Characterization of the phenolic content and the antioxidant capacity of the skin and seeds of grapes from Maratheftiko and Xynisteri was conducted by the Folin–Ciocalteu and the DPPH method in skins and seeds extracts. The Maratheftiko variety presented a higher antioxidant capacity, in both the skins and the seeds, when compared to the Xynisteri variety. In addition, the oligomeric-monomeric proanthocyanidins and polymeric tannins were quantified in the seeds extracts by the BSA method. The polymeric tannins in seeds were in higher concentrations than the oligomeric-monomeric proanthocyanidins in both varieties. Also, the profile of the anthocyanins in Maratheftiko variety in skins was recorded using HPLC showing the malvidin as the main anthocyanin followed in smaller amounts by peonidin, petounidin, delphinidin and finally cyanidin.
In addition, the effect of the botanical origin on the profile of the wine phenolics was investigated. The monovariental wines of three vine cultivars, Maratheftiko, Mavro, and Cabernet Sauvignon were analyzed for Hydrobenzoic acids, Hydrocinnamic Acids, Flavonoles, Flavan-3-oles and Anthocyanins composition by a HPLC-PDA system. A multi-varied statistical analysis was applied on data using the SPSS software package. It is noteworthy that the factor which distinguishes Mavro wines from the other two wine varieties is the concentration of Malvidin, consisting a varietal indicator for wines of Mavro variety. Moreover, the average amount of the concentration of the Hydroxycinnanic acids and Flavan-3-oles significantly differentiates wines of Mavro from those of Maratheftiko and Cabernet Sauvignon varieties. The concentration of total Flavonols significantly differentiates wines of Maratheftiko from those of Mavro and Cabernet Sauvignon varieties. Additional, the concentration of Hydroxybenzoic acids differentiates wines between all the three varieties Mavro, Maratheftiko and Cabernet Sauvignon from each other.

Keywords: *cypriot wines, marathefiko, xinisteri, polyphenols, authenticity*

B44
AUTHENTICATION / CONTENT VERIFICATION OF SEA BUCKTHORN IN HERBAL TEAS BY METABOLOMIC FINGERPRINTING STRATEGY USING U-HPLC-HRMS ANALYSIS

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Herbal teas are very often used especially due to their therapeutic effect or as a prevention of health problems. Some herbs can be expensive for herbal tea production. The popularity and the financial aspect may lead to fraudulent practices from manufacturers resulting in deception of their consumers. Therefore, it is important to control the authenticity of those products.
The main object of this study was sea buckthorn, whose fruits and even leaves are very nutritious, containing a number of vitamins, carotenoids, organic acids etc. The composition of herbal tea is very complex and almost never only one plant (component) is used, but it is a mixture. For instance, rose hip is very popular and also relatively cheap ingredient, which is commonly added into the mixture of herbal teas. In some cases, when some producer is not fair to customers, the declared content of sea buckthorn does not correspond to reality. For this reason, an effective tool for controlling the declaration of sea buckthorn content in the herbal teas is needed.
Nowadays, the trend in authentication is untargeted analysis. The main advantage is to obtain more characteristics in a single measurement. The most suitable strategy for this purpose is metabolomic fingerprinting, for which many different analytical techniques (methodologies) exist. In our case ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (U-HPLC-HRMS/MS) was used for acquiring metabolomic fingerprints. Using this technique an analytical procedure for detecting/verifying content of sea buckthorn in herbal teas has been developed. Several authentic samples of sea buckthorn were analysed. Data from fingerprints were statistically processed and based on these analyses several markers for sea buckthorn identification has been found. Four compounds (two in positive and two in negative ionization) were identified as possible markers for authentication of sea buckthorn in tea. Their tentative identification was performed on the basis of accurate mass, summary formula and fragmentation ions compared with available databases. One of those compounds was identified as hippophae cerebroside, a compound specific for sea buckthorn. Others are probably triterpenoids with coumarin-derived group and triacylglycerol. Thus, from the original non-target analysis, the procedure could be simplified to target the analysis on these markers. Finally, we used those markers for semi-quantification of sea buckthorn in 11 herbal teas purchased in stores. In the most cases the declared content of sea buckthorn was in accordance with the measured value but one sample was suspected of adulteration.

Keywords: *authenticity, sea buckthorn, metabolomic fingerprinting*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This work was also supported by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272).*

B45
THE AUTHENTICATION OF POPPY SEEDS BY U-HPLC–HRMS/MS

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Poppy (*Papaver somniferum* L.) is a globally grown crop. Some of its varieties are used in the food industry, the others in the pharmaceutical industry. In Central European countries, especially in the Czech Republic, the poppy seeds are favorite ingredients for the production of a range of bakery products, such as poppy seed fillings for cakes or other desserts, and for the production of poppy seed oils. On the other hand, technical poppy varieties are predominantly grown in most other countries. These "technical" varieties are characterized by high levels of opium alkaloids and they are used to prepare pharmaceuticals and often are abused for illegal drug production.
In recent years, the poppy fraud issue has been emerged due to the mixing (or complete substitution) of high-quality poppy seeds for the seeds of cheap technical varieties, primarily used for pharmaceutical purposes and not suitable for food. The content and ratio of individual opium alkaloids is dependent mainly on the cultivar of poppy but also on the geographical origin. Nowadays, it is possible to determine the quantities of individual opium alkaloids in a poppy seed sample by different analytical procedures, often routinely used. However, a reliable procedure for the authentication of geographical origin of poppy is still unavailable.
Therefore, the submitted study deals with the authentication of geographical origin of poppy seeds, based on the metabolomic fingerprinting employing ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry (U-HPLC–HRMS/MS). For this purpose, samples originating from China, Australia, Turkey, Spain, Germany, Slovakia, Austria and the Czech Republic were analyzed by U-HPLC–HRMS/MS in order to characterize poppy seeds originating from different countries.

Keywords: *poppy, authenticity, U-HPLC–HRMS/MS, PCA*

Acknowledgement: *This study was supported by National Agency for Agriculture Research (Project no. NAZV-QK1720263).*

B46
VARIETAL AUTHENTICATION OF CZECH AND MORAVIAN WINES BASED ON THE PHENOLICS CONTENT

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The adulteration of food, beverages and dietary supplements is a growing problem worldwide. Wine is one of the most popular and, due to its great financial value and the relatively large amount produced, also one of the most common alcoholic beverages subject to fraud and mislabeling. Wine is a complex mixture of hundreds of chemical compounds. Their pattern and concentrations can be influenced by many factors (grape variety, ripeness, climate, wine growing area, winemaking process, vintage year etc.). Because of their influence on colour, taste, body, bitterness and astringency, polyphenols play the key role among the chemical compounds present in wine. In addition, there is also extensive information available about the beneficial effects of polyphenolic compounds on human health. Wine can be adulterated in many ways (dilution with water, colouring, falsification of geographical origin etc.). In the last few years, there has been growing interest in developing analytical methods for wine authentication. The European Parliament has identified wine as one of the commodities, for which authentication method should be developed. However, while the strategy for identification of wine geographic origin is available, a reliable procedure for the authentication of a wine variety is still missing.
In this study, applicability of profiling of phenolic compounds using ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (U-HPLC-HRMS/MS) for wine authentication according to the grape variety has been investigated. In total, 41 red wine samples (varieties Pinot noir, Saint Laurent, Portugieser Blau) and 44 white wine samples (varieties Riesling, Pinot gris, Roter Traminer) were analyzed. Samples were extracted with ethyl-acetate and acidified water and then analyzed using the above mentioned analytical platform. Subsequently, after automated data mining and alignment procedures, principal component analysis (PCA) was applied, which provided an initial overview of the distribution of the samples. To enhance separation of the samples based on their variety, statistically insignificant ions were removed. In addition, a partial-least squares discriminant analysis (PLS-DA) was performed and a statistical model was obtained and validated. The quality of the models was assessed according to their recognition and prediction abilities. The most promising model, which reached 94.3 % in recognition ability and 79.7 % in prediction ability, was obtained for white wines based on records in positive ionization mode. Finally, characteristic markers contributing the most to the separation of the varieties were identified; they belonged mainly to the groups of cinnamic acid derivatives, stilbene derivatives and flavonoids.

Keywords: *wine variety, authentication, profiling, U-HPLC-HRMS/MS, phenolics*

Acknowledgement: *This work was supported by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272), by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by specific university research (MSMT No 20-SVV/2017).*

B47
APPLICATION OF SUPERCRITICAL FLUID CHROMATOGRAPHY FOR AUTHENTICATION OF HEMP OILS

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Hemp is a very unique plant rich in biologically active compounds which has a great potential in medicine (therapy of cancer, epilepsy, etc.) and in food industry (hemp products). In the recent decade, the interest of hemp products has been increasingly growing. One of the popular hemp products are cold pressed hemp oils, appreciated mainly due to its favorable content of unsaturated fatty acids and other biologically active compounds, especially cannabinoids. The production of hemp oil is very expensive because the yield of cold pressed hemp oil is relative low. In the recent years, cases of hemp oils adulteration (mixing with cheaper oils) were recorded therefore are necessary to develop appropriate analytical method for authentication the hemp oils.
"Omics" strategy is used for authentication of the hemp oils. The main objective is to identify characteristic markers for the hemp oils and to create a statistical model based on that which could reveal hemp oils adulteration with cheaper oils. The liquid chromatography with high resolution mass spectrometry (LC-HRMS) is the most widely used technique for "omics" analysis but this technique is not good for samples with high content of lipids because retention of triacylglycerols is high (time of analysis is long). Compared to that, supercritical fluid chromatography (SFC) is for these samples satisfactory, thanks to the early elution of triacylglycerols.
In our study a novel strategy was developed and validated employing supercritical fluid chromatography coupled with high resolution mass spectrometry (SFC-HRMS) for authentication of hemp oils. Characteristic markers for hemp oils were identified and developed and validated method was used for the authenticity of hemp oils on the Czech market.

Keywords: *hemp oil, SFC-HRMS, authenticity, Cannabis*

Acknowledgement: *Financial support from specific university research (MSMT No 20-SVV/2017).*

B48
A NOVEL APPROACH TO ASSESS QUALITY AND AUTHENTICITY OF SCOTCH WHISKY BASED ON GC-HRMS

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The engagement with fraudsters and detection of adulterated food and beverages is continuous and becoming increasingly more sophisticated. Scotch whisky´s popularity and reputation has unfortunately led unscrupulous traders to try to take unfair advantage by selling their products as Scotch whisky when they are actually not Scotch whisky. To detect fraudulent practices and document quality parameters, a number of laboratory tests based on various principles including chromatography and spectroscopy has been developed. In most cases, the analytical methods are based on targeted screening strategies. In our study, to isolate and pre-concentrate as much as possible volatile and semi-volatile compounds, ethyl-acetate extraction was used. In the next step, non-target approach, fingerprinting of whisky components based on gas chromatography coupled with tandem mass spectrometry (Q-TOF mass analyzer) was employed. The data obtained by analysis of a unique set of 180 authentic whisky samples (differing in region of origin, maturation in various cask and age) provided by the Scotch Whisky Research Institute were assessed by advanced chemometric methods. Principal component analysis (PCA), partial least squares discriminant analysis (PLS–DA) and orthogonal PLS–DA were applied for classification model construction. Very good separation according maturation casks (bourbon, sherry, red, white and port wine) was achieved, significant markers most contributing to the clustering were identified (e.g. diethyl tartrate or vanillin acetate).
Selected markers enabled separation not only between samples aged in ´wine´ and/or ´bourbon´ casks and even the samples of Highland origin.
In the next phase, 20 fake samples and 24 ´unknown´ samples provided again by Scotch Whisky Research Institute were analyzed and the data processed in the same way. Employing the chemometric model developed for this purpose, marker compounds enabling distinguishing fake samples were found. Based on their mass spectra, several food additives (e.g. triacetine - E 1518) were identified. These ´new´ markers might be added on the target analytes list for a routine control.

Acknowledgement: *This work was supported by the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and by the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613688.*

B49
THE POTENTIAL OF FT-RAMAN SPECTROSCOPY, CORROBORATED WITH CHEMOMETRICS, IN THE DISCRIMINATION OF ROMANIAN WINES

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Most of the published works, related to wine characterizations by mean of vibrational spectroscopies, are especially refereeing either to the determination of main chemical compounds, like: sugars, acidity and alcohol or to the study of fermentation process. There are a small number of studies which proposed the discrimination of wine, with respect to their geographical origin, variety and vintage, by using a combination of visible-near or mid-infrared spectroscopy associated with chemometric methods. In wine industry, infrared spectroscopy (IR) is successfully used for different analysis purpose while, Raman spectroscopy potential was not extensively explored in this field, despite its advantages in the characterization of water containing samples, regarding to the relatively weak water bending mode in the fingerprint range. Thus, only a few reports investigating the applications of Raman spectroscopy in wines characterization and differentiation have been published so far.
The present study aims the discrimination of 65 Romanian white wine samples by mean of FT-Raman spectroscopy, particularly based on the anti-Stokes spectral range of the Raman spectra, in corroboration with chemometrics. In order to achieve this goal, the choose sample set was formed by four wines varieties, coming from five consecutive vintages 2012-2016, produced among three different Romanian regions (Transylvania, Muntenia and Moldova). The best differentiation that was obtained among wine samples was related to variety; in this case the anti-Stokes region of Raman spectra being a very powerful tool. Thus, the four investigated varieties (Sauvignon Blanc, Chardonnay, Riesling and Pinot Gris) were individually discriminated, with respect to the other sorts, in a percent of 100% in initial classification while in cross-validation procedure a percent of 98.5% or better was obtained.

Keywords: *FT-Raman; wine discrimination chemometry*

Acknowledgement: *This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P2-2.1-PED-187/2017, within PNCDI III.*

B50
SERS APPLICATIONS IN WHITE WINES DIFFERENTIATION

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The interface between the silver nanoparticles (AgNPs) obtained by the classical wet method and the white wines has been explored using the surface enhanced Raman scattering technique (SERS), aiming to assess the technique capability for providing direct spectral biomarkers of wines. Regardless of the wine color, their normal Raman scattering excited with visible laser lines showed strong fluorescence signal superimposed with the main bands attributable to the alcohol and water, which drastically hamper wines components detection or wines discrimination without additional chemometry.
In this work, two wine varieties, Feteasca Regala and Sauvignon Blanc, produced during five consecutive years in three different Romanian regions (Transylvania, Banat and Moldova) were compared. From all above mentioned vintages, one sample of Feteasca Regala and another one of Sauvignon Blanc, belonging to each geographical area, were collected.
The strongest FT-SERS band observed at 232 cm^{-1} which is typical for the SERS systems containing Ag-O binding sites, confirm the adsorption of the anthocyanin species via the O atom from the skeletal ring. Differences in the intensities of these bands could be tentatively related with the concentrations of the tartaric and malic acids in wines along with the anthocyanins. Their co-adsorption on the AgNPs in each wine variety is strongly related to their variable content.

Keywords: *SERS, wine, anthocyanins*

Acknowledgement: *This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P2-2.1-PED-187/2017, within PNCDI III.*

B51
FRACTIONATION OF BLACK SOLDIER FLY BIOMOLECULES FOR FEED/FOOD OR TECHNOLOGICAL APPLICATIONS

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Currently there is a need to find alternative protein sources for feed and food applications, induced by the increasing world population, as well as rising meat consumption per capita in developing nations. Beside conventional protein sources, novel protein sources, derived from diverse food waste, food by-products and other residual biomasses, have been recently proposed in EU as an alternative to animal-derived proteins. Anyway, the diversity of these sources represents a technological problem for processing. Insects may represent a possible solution since some of them naturally develop in organic wastes, incorporating the nutrients into their bodies, reducing the amount of waste material in the process and generating a more homogeneous and valuable biomass. Moreover, in comparison with other livestock, insects require less feed, land and produce less greenhouse gas emissions for their growth. Beside proteins, also chitin and fats, can be obtained from insects. In order to use protein fractions as supplement in the feed and food sector, anyway, it is necessary to develop optimal extraction method that allows to preserve their quality. Alternative non-food uses are currently explored, for example bioplastics production.
In the present work, the chemical composition of black soldier fly larvae was determined, and different fractionation methods were explored, with the aim to recover pure protein, fat ad chitin fractions. Fat fraction was easily recovered with solvent extraction. The separation of protein and chitin fractions was achieved first with a single step total protein extraction, using alkali. A different milder extraction method was also tested, following the stepwise method proposed by Osborne (1907). Both methods allowed to obtain a good recovery of the protein fraction (about 90%), but only in the second one the extracted proteins preserved the integrity of their structure, as determined by performing SDS-PAGE analyses and o-phthaldialdehyde tests to assess the degree of hydrolysis. As an alternative to the previous two chemical processes, proteolytic enzymes were also used for the solubilisation of the protein fraction and its separation from chitin. Among the enzymes tested, Alcalase resulted in a higher degree of nitrogen solubilisation (60 %) and lower degree of hydrolysis in comparison to other enzymes.

Keywords: *chemical extraction, protein, chitin, enzymatic assisted extraction, black soldier fly*

Acknowledgement: *This study is partially part of ValoriBio (Valorization of organic waste using insects to obtain biomaterials for agricultural purposes) project and was funded by Regione Emilia-Romagna through POR FESR 2014/2020 - Axis 1 Research and Innovation.*

B52
DETERMINATION OF PROTEINS AND PEPTIDES RESPONSIBLE FOR ADVERSE REACTIONS TO WHEAT: STRATEGIES FOR MITIGATION

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Celiac disease (CD) is a chronic autoimmune enteropathy triggered, in genetically predisposed individuals, by exposure to peptides deriving from incomplete digestion of gluten. The amount of these peptides after digestion depends on several factors, mainly wheat genotype and cultivation region. Following methods previously developed in our group [1], gluten peptides generated by digestion can be quantified by UPLC/ESI-MS in different wheat varieties, or in the same varieties cultivated in different regions.
Together with CD, wheat is also responsible for IgE-mediated allergic reactions, and allergens are found among gluten, albumins, and globulins. CM3, one of the wheat alpha-amylase inhibitor subunits, is one of the major wheat allergens, being implicated in atopic dermatitis, baker´s asthma and intestinal inflammation. CM3 content is also not constant in wheat, but mostly depends on cultivation region. Indeed, also in this case a method for its quantification in different wheat samples, based on enzymatic treatment and subsequent UPLC/ESI-MS analysis using an isotopically labelled internal standard, has been developed by our group [2]. To prevent the onset of CD, and to reduce allergic reactions, it would be of interest to select wheat with a low content of CM3 and/or a low content, after digestion, of gluten-related CD peptides. Possible strategies for mitigation include: a) selection of varieties; b) selection of cultivation area; c) germination. In this presentation, a durum wheat collection made of 6 genotypes (Duilio, Dylan, Claudio, Iride, Simeto, and Svevo) cultivated in 4 Italian regions (Emilia Romagna, Marche, Apulia, and Sicily), were investigated for their content of CD-related peptides, after *in vitro* gastrointestinal digestion. In this way, the best combination variety/cultivation region was identified to have wheat samples producing the lowest possible amount of CD-related gluten peptides after digestion. In a different approach, one of this variety was germinated in a lab incubator at 25°C, lyophilized, digested and analysed by UPLC/ESI-MS to assess the effect of 6 days of germination on the amount of CD-related gluten peptides after ingestion. Germination, actually, induced a very important reduction, after simulated gastrointestinal digestion, in the generation of CD-related gluten peptides. Likewise, CM3 content was found to be markedly reduced by germination. In conclusion, appropriate selection of varieties and cultivation area as well as appropriate technologies, such germination, might indeed reduce the impact of wheat in the onset of CD and in the appearance of wheat allergies, possibly exerting a preventive effect able to contain adverse reactions to wheat.

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Keywords: *celiac disease, wheat allergy, germination, LC/MS, simulated gastrointestinal digestion*
Acknowledgement: *The present work has been done in the framework of the project "Smart Wheat", funded by Emilia Romagna Region (Italy), POR FESR 2014-2020, Action 1.2.2.*

B53
AUTHENTICATION OF MEAT SPECIES USING PROTEIN ELECTROPHORESIS AND PCR

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The reliable species identification of bulk meats, particularly after butchering, dicing or mincing, has been a considerable problem faced by food analysts for many years. Species identification is even more problematical in processed and heated products such as smoked and cooked sausages, canned and cooked meats. During last years, there have been various examples of the substitution of an expensive meat species with meat from a cheaper species. However, identification of species origin is relevant not only for economical, but also for religious or public health concerning reasons. Numerous analytical methods that rely on protein analysis have been developed for species identification in meat products (e.g., electrophoresis techniques, liquid chromatography and immunoassays). However, as presence and characteristics of proteins depend on cell types, and most proteins are heat-labile, techniques based on DNA rather than on protein are preferable at present.
Nevertheless, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), urea-PAGE and isoelectric focusing were applied to the meat reference samples. As long as raw meat samples of single species were concerned, these protein electrophoresis techniques proved to be rapid, convenient and cost-effective screening tools to confirm the species origin of doubtful meat samples.
In order to test the specificity of primer-pairs recently reported to be specific for a certain animal species, authentic raw meat samples of various relevant species (e.g., cattle, buffalo, sheep, goat, pig, wild boar, horse, rabbit, red deer, reindeer, roe deer, kangaroo; chicken, turkey, goose, duck, ostrich, pheasant, quail, pigeon) were analyzed by species-specific polymerase chain reaction (PCR). Mostly, PCR reaction (e.g., annealing temperature) had to be optimized in order to get a specific amplicon. However, in some cases, primers were not specific but showed false-positive results even under optimized analytical conditions.
The effect of heat treatment of meat (75 to 133°C) on the efficiency of the PCR amplification was tested using several primer-pairs specific for bovine meat. Depending on the length of the resulting amplicon, species identification using species-specific PCR was successful even after heat treatment of meat.
Recently, the discrimination between domestic pig and wild boar meat has become a major challenge for analysts and food control authorities. Several primer-pairs published by different research groups and restriction enzymes were tested to assess their specificity under optimized reaction conditions. From the data obtained, it can be concluded that PCR-RFLP methods based on the selected D-loop region and cytochrome b gene are definitely not suitable for reliable differentiation. However, an accurate discrimination between wild boar and domestic pig meat was achieved by using primers targeting the NR6A1 gene, and subsequent RFLP analysis using *Msp* I as a restriction enzyme.

Keywords: *meat, species identification, protein electrophoresis, PCR*

B54
DETECTION OF COFFEE ADULTERATION USING THE MONOSACCHARIDES PROFILE BY CAPILLARY ELECTROPHORESIS-TANDEM MASS SPECTROMETRY

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Coffee is one of the most consumed beverages in the world, and it is important to the economies of countries involved in its production and exportation. For that reason, coffee adulteration represents a problem of economic order specially when adulterated with roasted materials, which are difficult to visually detect in samples of roasted ground coffee [1]. The detection of coffee adulteration with soybean and corn by capillary electrophoresis-tandem mass spectrometry was accomplished by evaluating the monosaccharides profile obtained after acid hydrolysis of the samples. The acid hydrolysis using H_2SO_4 increases the ionic strength of the sample impairing the electrophoretic separation. Therefore, $Ba(OH)_2$ was used to both neutralize the medium and reduce the content of sulfate by precipitation of $BaSO_4$. The best separation of nine monosaccharides (fucose, galactose, arabinose, glucose, rhamnose, xylose, mannose, fructose, and ribose) and inositol, as internal standard, was obtained in 0.5 mol·L^{-1} triethylamine, pH 12.3. The monosaccharides are separated as anionic species at this pH. The proposed method is simple, fast (< 12.0 min), presents linear calibration curves (r^2=0.995), and relative standard deviation for replicate injections lower than 5%. The LOQ values for all monosaccharides were lower than 0.01 mmol·L^{-1}. Principal component analysis (PCA) was used to evaluate interrelationships between the monosaccharide profile and the coffee adulteration with different contents of soybean or corn, or both. Fucose, galactose, arabinose, glucose, sucrose, rhamnose, xylose, mannose, fructose, and ribose were quantified, and differences between adulterated and genuine coffee could be detected. Commercial samples of roasted ground coffee available in the Brazilian supermarkets were analyzed verifying the specific monosaccharides profile in these samples with the corresponding reference samples and around 29% of them can be considered adulterated.

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Keywords: *coffee adulteration, monosaccharides profile, CE-MS/MS, corn, soybean*

Acknowledgement: *Agilent Technologies and FAPESP*

B55
A NOVEL TETRAPLEX REAL-TIME PCR ASSAY FOR THE SIMULTANEOUS QUANTIFICATION OF ROE DEER, RED DEER, FALLOW DEER AND SIKA DEER

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As factory farming is associated with quality and safety issues, e.g. protection of animal welfare and the presence of antibiotic residues in meat, consumers increasingly prefer organic and sustainable food as well as meat derived from animals kept in species-appropriate husbandries. Meat from game animals has always been appreciated for its characteristic flavor and tenderness. Nowadays, it is even more popular because of its high content of anti-inflammatory omega-3 fatty acids and the fact that it lacks residues of drugs like growth hormones and antibiotics. But even though deer farming has started all over the world, there is still a limited supply of game meat.
Food producers might be tempted to substitute more expensive meat from game animals by cheaper meat from domesticated animals like pig in order to increase their economic profits. According to the Codex Alimentarius Austriacus, 38% of the meat content in a "game" sausage has to originate from game animals. Therefore, food control authorities not only need to identify but also to quantify game animal species in food.
So far, our working group has developed a duplex real-time PCR method for the simultaneous determination of the content of roe deer and the sum of red deer, fallow deer and sika deer. But the increasing trend to market deer under their species specific names instead of the comprehensive term "deer" makes the availability of species specific methods necessary. For example, if a product is specifically declared to contain fallow deer, meat from fallow deer must not be substituted by meat derived from e.g. red deer.
The aim of this work was to combine species specific methods for roe deer, red deer, fallow deer and sika deer to a multiplex assay in order to save analysis time and costs. In comparison to the duplex assay, the tetraplex assay additionally enables the quantification of the individual deer species. This multiplex assay was validated and tested for its applicability to identify and quantify game animal contents in routine food analysis. For DNA mixtures and model game sausages in which the content of the target animal species was > 5%, recoveries between 70% and 158% were obtained. Furthermore, the assay's applicability for food analysis was tested by analyzing commercially available game products.

Keywords: *game meat, food fraud, quantification, multiplex real-time PCR, authenticity*

B56
DEVELOPMENT OF DUCK WELFARE EVALUATION SYSTEM IN THAILAND: WELFARE STANDARD TO MEAT AUTHENTICITY AND QUALITY

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Authenticity and quality of meat products are key features used to verify labelling compliance, the respectable trades of high product qualities and the consumer's expectation. An animal welfare protocol currently is one of significant goals in animal production industries for proving of high qualities of animal lives, animal handlings as well as veterinary profession efficiency. Thailand is a large poultry production country. To assure poultry welfare as one key of all structures, declaration of meat product authenticity and quality is crucial. Duck is the target species in this study. A duck welfare evaluation protocol for Thailand was designed based on the guideline of OIE Terrestrial Animal Health Code and the RSPCA Duck Welfare Standard 2015. The five-freedom rules and the welfare standard of duckling handling, health monitoring, catching system and transportation system were developed and examined in four independent groups of at least one hundred ducks a time along the production cycle. The development of scoring systems and the stress response (duck corticosterone) were justified in selected fifteen samples to the real practice by multiple trials and adjustments accordingly. These were considered the first evaluation prototype of the duck welfare evaluation system in Thailand.

Keywords: *duck welfare, evaluation system, Thailand, meat authenticity and quality*

Acknowledgement: *This research is supported by Thailand Research Fund no. RDG5720053, 2014-2017. The author thanks Mr. Suksan Chamsing, Mr. Somyod Kankhunthod and Ms Montisha Suwansong for technical supports.*

B57
A NOVEL, HIGHLY ROBUST LC-IRMS SOLUTION FOR HIGH-THROUGHPUT DETERMINATION OF HONEY ADULTERATION

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Honey is a high value commodity, whose quality is defined both by its botanical and geographical origin. This generates a strong consumer demand for certain, premium-priced products, which have become the target for adulterations. A useful tool to detect the addition of sugar to honey products is based on the well documented difference in δ13C values between C3 (natural honey) and C4 (added sugar) plants. LC-IRMS has the unrivaled advantage of the simultaneous determination of δ13C values from glucose and fructose, allowing the detection of more sophisticated honey adulteration.
In this presentation, a new technology is presented that hyphenates LC with IRMS in a novel way. Unlike the existing LC-inlet solution via chemical oxidation, the new inlet system is based on high-temperature combustion. Experimental data show outstanding accuracy and precision combined with an unrivaled robustness. Thus, it is the perfect choice for routine, high-throughput, compound-specific LC-IRMS analysis of honey to detect sophisticated adulteration processes.

Keywords: *LC-IRMS, adulteration, honey, sugar, IRMS*

B58
MINIATURIZED DEVICES FOR SAMPLE PREPARATION PROVIDING HIGHLY EFFICIENT DNA EXTRACTION FROM OLIVE OIL

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Olive oil is considered among the top 10 products that are most at risk of food fraud [3], one of the most frequently reported fraudulent practices, is the addition of cheaper oils obtained from plants other than olive. Several vegetable oils have been reported as adulterants of olive oil, due to their lower prices [1]. DNA is a very interesting target molecule for olive oil authenticity studies due to the high stability and durability of DNA when compared to other target molecules, moreover sequences are independent from geographical, climatic or agronomical factors. However, a critical step in the case of complex and highly processed food matrixes, is the DNA extraction and purification. This step should ensure an efficient recovery of nucleic acids. Although several methods had been described for the isolation of DNA from biological samples, the cumbersome matrices of olive oil samples constitute a significant challenge [2]. Additionally, some compounds that inhibit enzymatic activity might be present, and affect subsequent steps such as DNA amplification.
At INL we are working on the development of tailored, miniaturized, automatized and faster analytical approaches, including sample preparation devices, designed to perform accurate, reliable and sensitive analysis from complex matrixes such as food products. Microscale solid phase extraction (μSPE) is used for on-chip DNA extraction and purification, being possible to put in contact a higher volume of initial binding material with the solid phase [4] and recover the DNA in a lower volume during the elution phase. This feature allows to concentrate the DNA when minute amounts are present in the sample such as for olive oil.
We have developed a washable and reusable device containing a commercial disposable silica membrane, which is the solid phase for the DNA capture. A protocol has been optimized for improved DNA yield, and tested with olive oil samples. The efficiency of the DNA purification approach was determined by estimating the DNA yield, and the purity of the DNA extract was evaluated by the ratios of the absorbance at 260:280 / 260:230. The obtained results have shown that the achieved DNA yield is around 4 times higher than with commercial DNA extraction kits, with acceptable making this method a very interesting sample preparation approach for DNA based analysis in this and other samples with minute DNA content.

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Keywords: *olive oil, DNA analysis, miniaturized devices, microscale solid phase extraction (μSPE), sample preparation*

Acknowledgement: *This work was supported by the project Nanotechnology Based Functional Solutions (NORTE-01-0145-*

FEDER-000019), supported by Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and by the project NANOeaters (0181_NANOEATERS_1_e), supported by the European Regional Development Fund (ERDP) under the EP - INTERREG V A España Portugal (POCTEP).

B59
"REIMS WITH CHEMOMETRICS" A TOOL ADAPTED FOR THE IDENTIFICATION AND AUTHENTICATION OF A PDO STATUS PRODUCT "BEURRE D'ARDENNE"– A CONCRETE EXAMPLE!

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Belgium is well known for its many traditional food products, such as chocolate, beer, cheese and French fries. Some of these are labeled conferring a status to them and making them likely to be copied.
A first approach of *Rapid Evaporative Ionization Mass Spectrometry* (REIMS) was carried out to evaluate its potential to authenticate a PDO product, the "butter of the Ardennes". The royal decree of December 18, 1984 describes the criteria to be respected to produce butter under this label. REIMS uses high resolution mass spectrometry to analyze in real time vapor produced by a surgical knife (iknife) cutting the sample. The differentiation of tissues is especially based on the composition in lipids of this vapor. The transposition of this technology towards the butter analysis seemed logical.
For this study, the REIMS source was coupled to the Xevo G2-XS QTOF (Waters Corporation) operated in sensitivity mode. The vaporization time was of approximatively 4 seconds and negative ionisation mode was used. The masses were tuned from 50 to 1200 amu. Palmitic acid was used as external standard of mass correction and subtraction of background noise was applied.
During a first series of tests, fat of various origins (vegetable oils, margarines, butters) were analyzed by means of the REIMS source. The analysis of the results by PCA led to a complete differentiation of the types of fat. The more specific analysis of the butter samples showed than it was possible to distinguish "industrial" butters from PDO butters.
On the basis of these preliminary results, a new test was built by integrating the principles of the spectroscopic analyses: constitution of a database (analyzes of a significant number of samples of various nature - industrials, PDO, low-fat content, churn, NIZO proceeded-, n=170), development of a model (chemometrics aspects) and validation of the model by the analysis of a set of independent samples, representative of the database.
A model based on PCA (used for data dimensionality reduction) and LDA for supervised classification was generated. The multivariate analysis of the spectral range limited from 600 to 1200 amu (phospholipids zone) of the 170 samples shows that PCA/LDA model allows a better separation of PDO butters than a single step PCA model.
The last phase of the study concerned the model validation by means of analysis of independent test butters. The results obtained with this set shows a rate of correct assignment of 85% (34 samples out of 40).
These first results show the potential for REIMS in the field of product authentication. The choice of another multivariate model and the spectral zone frome 50 to 600 amu could improve the performances of discrimination. The information obtained with REIMS should not however be put in direct relationship with the criteria defined in the specifications of PDO. But, it will be possible to find new criteria well leading to the same conclusion.

B60
FINGERPRINTING AND IDENTIFICATION OF BEER BY COMPREHENSIVE HPLC OLIGOSACCHARIDE PROFILING AND PRINCIPAL COMPONENT ANALYSIS (PCA)

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Beer represents a significant commodity of the globalized food industry. With the growing acceptance of beer culture, there is also a need for accessible analytical approaches for profiling of beer substances to allow for its classification, identification and protection of origin.
HPLC utilizing an ion exclusion principle (IEX) offers an efficient approach for analysis of mono- and oligo-saccharide contents in beer. In this work we present a utility of IEX HPLC on sulfonated low-crosslinked polystyrene phases in Ag+ form to create unique fingerprints based on resolution of 16 maltooligosaccharides in untreated beer samples. We evaluate factors influencing the results from sample preparation, peak reproducibility and column stability to data evaluation. We then present creation of a comprehensive beer fingerprint database containing over 100 profiles and employment of hierarchical clustering on principal component analysis data (PCA). Finally, we demonstrate characterization and identification of multiple cases of unknown beer samples using PCA.
We conclude that this simple approach is applicable for low-cost differentiation of individual beer samples. Unlike other complex systems, typically relying on MALDI-MS, it can easily be deployed in QC, stability testing or identification for use across the field of beer production, handling and trade.

Keywords: *HPLC, ion exclusion, clustering, database*

Acknowledgement: *This project was suported by grant FV10490*

B61
ASSESSING FOOD AUTHENTICITY IN LACTOSE-FREE BRAZILIAN COMMERCIAL MILKS BY ULTRA HIGH PRESSURE LIQUID CHROMATOGRAPHY-CORONA CHARGED AEROSOL DETECTION

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Primary Lactase deficiency is genetically determined and characterized by the down regulation of lactase enzyme activity in human body. According to European Food Safety Authority (EFSA) Lactose Threshold Guideline, lactase deficiency ranges from 4% (Ireland) to 56% (Italy) of EU citizens, and lead to undesirable symptoms as bloating, flatulence, nausea, abdominal pain and diarrhea.
Regarding these costumers, many companies in dairy segment improved their dairy products processing technology, converting lactose into glucose and galactose, claiming and labeling their products as "lactose-free". Recent regulations in European Union (EFSA 2010-1777) and Brazil (RDC 136/2017) establish lactose thresholds in lactose-free products, ranging from 10mg $100mL^{-1}$ to 100mg $100mL^{-1}$, according to each country.
Many analytical strategies have been employed in lactose determination, as High Pressure Liquid Chromatography-Refraction Index Detection, High Pressure Anion Exchange Chromatography-Pulsed Amperometric Detection and Polarimetry, although the disadvantages of each one of them like poor sensitivity, dedicated system to lactose analysis and lack of selectivity. The aim of this study was to develop a robust, cheap, easy, fast, selective and sensitive method for lactose determination in a multipurpose Ultra High Pressure Liquid Chromatography-Corona Charged Aerosol Detection system (UHPLC-CAD) in order to assess lactose-free Brazilian commercial milks label authenticity. A full validation set was performed respecting Limits of Detection and Quantification, bias, recovery, linearity, matrix effect and precision following Brazilian (Inmetro DOQ 008/2016) and Eurachem (2014) validation guidelines. Eleven lactose-free milks were purchased in Brazilian market and stored at -26ºC prior to analysis. Samples were thawed at 35ºC and then 1 mL of raw samples was diluted with pure water to 100 mL in volumetric flasks. Two mL of diluted solution was filtered through a 0.22 µm syringe filter and placed in 2 mL screw cap vials. The analysis was performed in triplicate. Linear range from 10 to 300 µg mL^{-1} (R^2>0,99), LOD and LOQ were respectively, 6.5 µg mL^{-1} and 10.5 µg mL^{-1}, recovery from 91-102% and evaluated method relative standard deviation was 4.9%. Seven samples out of eleven were under LOD, 3 samples were under Brazilian thresholds: 99.41 mg 100 mL^{-1}, 83.87 mg 100 mL^{-1}, 71,40 mg 100 mL^{-1} and 1 was above Brazilian thresholds 107.93 mg 100 mL^{-1}. The validated method showed good linearity, precision, recovery and limits of detection and quantification. Overall UHPLC-CAD was a cheap, easy, fast and reliable method for lactose quantification in lactose-free milks, and a great analytical strategy for food authenticity assessment.

Keywords: *UHPLC-CAD, lactose free, food authenticity*

Acknowledgement: *This work was supported by Brazilian National Council for Scientific and Technological Development (CNPq). We thank Thermo Fisher Scientific for all analytical support and expertise coaching from their application scientists.*

B62
AUTHENTICATION OF FRUIT JUICE USING AMINO ACID PROFILES – APPLICATION IN A FOOD CONTROL LAB

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Fruit juice is known to be one of the most fraud-prone foods on the marketplace. Predominantly, orange juice and apple juice have been often reported to be associated with food fraud and numerous records are listed in the USP Food Fraud Database [1]. Motivation towards committing adulterations in fruit juices involves financial reasoning in a high demand market area. The most common fruit juice adulterations include dilution (e.g., use of water, cheaper juices), addition of juice from rotten fruit, and addition of natural ingredients (e.g., sugars, acids, flavor, dyes) [2].
Identifying adulteration of juices, both in the industry and laboratory, involves simple methods like taste testing. However, depending on the kind of adulteration and the amount of adulterant used, tasting often doesn't suffice to identify fraudulent products. Therefore, different analytical methods are applied to the authentication of fruit juices, such as profiling of sugars, organic acids, anthocyanins, carotenoids, and also amino acids. Approaches using amino acid profiles for example, have been reported in scientific literature since the 1970s and reference values have been published for a range of fruit types in the AIJN Code of Practice [3]. The employment of multivariate analysis has been successful; however, this methodology is rarely implemented in routine. Laboratories involved in authentication of juices often consider only single amino acids, e.g., proline, rather than evaluating whole amino acid profiles.
In our work, we suggest strategies for authentication of fruit juices and fruit nectars using amino acid profiles from a practical point of view. Amino acid profiles of more than 300 samples, obtained from fruit juice producers and grocery stores, have been analyzed by HPLC equipped with conventional ninhydrin derivatization. Different procedures of data analysis (univariate, multivariate) were carried out and different datasets (concentration, area; including pre-processing) were compared. Best strategies for authentication of fruit types (i.e., species) and juice types (e.g., direct juice, juice from concentrate) were set up using supervised classification procedures combined with decision trees. In our contribution we show that latest questions concerning fruit juice authentication (e.g., blood orange vs. orange, black currant vs. red currant) can be tackled using these strategies. Furthermore, relevant questions from a practical point of view (quality assurance issues, concept of data analysis) using these kinds of analysis will be highlighted.

[1] USP Pharmacopeial Convention Food Fraud Database, available via www.foodfraud.org
[2] Food Fraud and "Economically Motivated Adulteration" of Food and Food Ingredients, Congressional Research Service Report R43358, Washington D.C., United States of America.
[3] AIJN Code of Practice, AIJN - EUROPEAN FRUIT JUICE ASSOCIATION, Brussels, Belgium.

Keywords: *authentication, fruit juice, amino acids, HPLC, chemometrics*

B63
SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION TANDEM MASS SPECTROMETRY: AN INNOVATIVE ONE-RUN METHOD FOR THE COMPREHENSIVE ASSESSMENT OF CHOCOLATE QUALITY AND AUTHENTICITY

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To assess chocolate quality and authenticity comprehensively, a combination of various analytical procedures is involved, thereby making the process time-consuming and costly. Thus, we developed and validated an innovative method for the simultaneous determination of key chocolate quality features by utilizing the high potential of ultra-high performance supercritical fluid chromatography coupled to high-resolution tandem mass spectrometry (UHPSFC-HRMS/MS). By combining hexane and water extracts from sequential extraction, a single eight-minute analytical run enabled us (i) to determine cocoa butter equivalents and milk fat content according to triacylglycerols, (ii) to calculate dry non-fat cocoa solids based on theobromine and caffeine content, and (iii) to screen sugars. For 40 different chocolate samples, our results and those obtained using standard methods were in good agreement suggesting that our method is a fast, cost-effective and efficient tool for chocolate quality and authenticity control.

Keywords: *triacylglycerol analysis, cocoa butter equivalents, milk fat, cocoa solids, sugars*

Acknowledgement: *This work was supported by the Technology Agency of the Czech Republic (project No TA04010331) and "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 – No.: MSMT-43760/2015) and by specific university research (MSMT No 20-SVV/2017).*

B64
TARGETED AND UNTARGETED METABOLOMICS AS AN ADVANCED TOOL FOR THE DETECTION OF JUICE-TO-JUICE ADULTERATION

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Economically motivated adulteration (EMA) of food, also known as food fraud, is the intentional adulteration of food for financial advantage. A common form of EMA is the undeclared substitution with alternative ingredients and could also pose a health risk to consumers due to potential allergic reactions. Fruit juices have been in the top-7 foods reported from 1980 to 2010 as the most common targets for adulteration. Several fruit juices, as orange and pomegranate juice, have become popular with regard to high levels of antioxidants, presumed to be associated with positive health effects. Similarly, to other highly prized food commodities, the economic value and large-scale production of these valuable fruit juices have made them a likely target for adulteration and fraud. One of the most frequent profit-driven fraudulent procedures is extension of authentic juice with cheaper alternatives (typically juices obtained from apples, grapes, grapefruits, and others). Consequently, there is a substantial need for effective food control systems to protect consumers from adulterated food products. In the present study, the main objective was to explore the feasibility of using targeted and untargeted analysis, using ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry, UPLC-QToF-MS, to discriminate authentic and adulterated fruit juices. Data corresponding to the phenolic composition of fruit juices and their LC-HRMS metabolic fingerprint were considered as a source of potential descriptors for the classification of juices and detection of adulteration. The data set was treated using advanced chemometric techniques in order to identify possible markers. Finally, pomerganate-fruit extracts were adulterated with different amounts (5–20%) of grape and apple juice and the phenolic profile and chromatographic fingerprinting data was evaluated for authentication purposes.

Keywords: *juice, adulteration, authenticity, high resolution mass spectrometry, chemometrics*

Acknowledgement:
The Post-Doctoral Research was implemented under IKY scholarship funded by the "Supporting Post-Doctoral Researchers" Act of the Operational Programme "Human Resources Development, Education and Lifelong Learning" with Priority Axes 6, 8, 9 and co-funded by the European Social Fund (ESF) and Greek National Resources.

B65
DRIED PAPRIKA POWDER QUALITY AND AUTHENTICITY ASSESSMENT EMPLOYING SPME-GC/TOFMS

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Paprika powder, which is made by grinding of dried *Capsicum annuum* L. fruits, is one of the most cultivated and traded spices worldwide. Paprika powder, or extract thereof (oleoresin), are widely used ingredients in food and cosmetics, because of their unique properties such as flavour and colour. Hungary, Spain, USA and India are the major producers of sweet, mild and pungent varieties (their category depending on capsaicin content). However, with regards to a number of producing countries, traceability of paprika origin might be rather difficult, especially in case of developing countries. In this context, and when considering the economic value of this commodity, adulteration / mislabelling of paprika powder has become an issue of serious concern.
In this study, based on the measurement of volatile compound profiles, we focused on quality and authenticity assessment of sweet paprika spices. Altogether, 35 samples of paprika powder were bought at the Czech retail market, they differed in brand, lot number, producer, and country of origin. In addition, six samples were provided by industry partner who guaranteed their authenticity.
Profiles of paprika headspace volatiles were obtained by solid phase microextraction sampling followed by gas chromatography and mass spectrometry employing the time of flight mass analyzer (SPME-GC-TOFMS). The primary data acquired by Pegasus 4D instrument (LECO, USA) were processed after deconvolution by the Statistical Compare feature of the ChromaTof software. Following compounds aligning and response normalization, the statistical analysis was performed in SIMCA software (Umetrics, Sweden).
Large differences between the samples of paprika in volatile profiles were found. High relative abundances of 3-methylbutanal and 2-methylbutanal were typical for paprika samples from Hungary, while for those from Spain, terpenes, such as limonene and α-pinen, seemed to be markers. The chemometric processing of volatile profiles using Principal Component Analysis (PCA) showed the separation of samples according to the country of origin.

Keywords: *paprika, Capsicum annuum L., volatile compounds, SPME-GC-MS*

Acknowledgement: *This work was supported by the Technology Agency of the Czech Republic (project No TA04010331) and "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 – No.: MSMT-43760/2015) and by specific university research (MSMT No 20-SVV/2017).*

B66
ASSESMENT OF SYNTHETIC ADULTERANTS IN DIETETIC PRODUCTS ON SERBIAN MARKET

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Slimming and erectile dysfunction drugs used as illegal adulterants in dietetic products production may be harmful to human health so their content or presence of their homologues and metabolites needs to be controlled. Our studies comprised the determination of erectile dysfunction drugs (tadalafil, sildenafil, vardenafil, hydroxyhomosildenafil, sulfoaildenafil, thiosildenafil) and slimming drugs (sibutramine, orlistate and bisacodyl) and fluoxetine (Prozac) during first eight months of 2017 by liquid chromatography tandem mass spectrometry (LC-MS/MS) with electrospray ionization (ESI). The preparation was simplified and based on organic solvent extraction and dilution. Analytical characteristics evaluated were linearity, LOD and repeatability. Erectile dysfunction drugs were detected in two out of 23 samples analyzed (Sildenafil, 50 mg per dose). Slimming drugs were detected in two out of 56 samples analyzed (sibutramine in one sample, 8 mg per dose and fluoxetine, 9 mg per dose). Fluoxetine known as antidepressant was also confirmed by 1H NMR, 2D NMR and 13C NMR spectroscopy. Those positive samples are a possible warning that in Serbian market the new substances of abuse have emerged. Multilevel monitoring of food safety must be kept, aimed at the successful prevention of harmful drugs of abuse and their effects on human health.

Keywords: *synthetic adulterants, dietetic products, determination, LC-MS/MS*

B67
UNDECLARED INGREDIENTS IN FOOD SUPPLEMENTS

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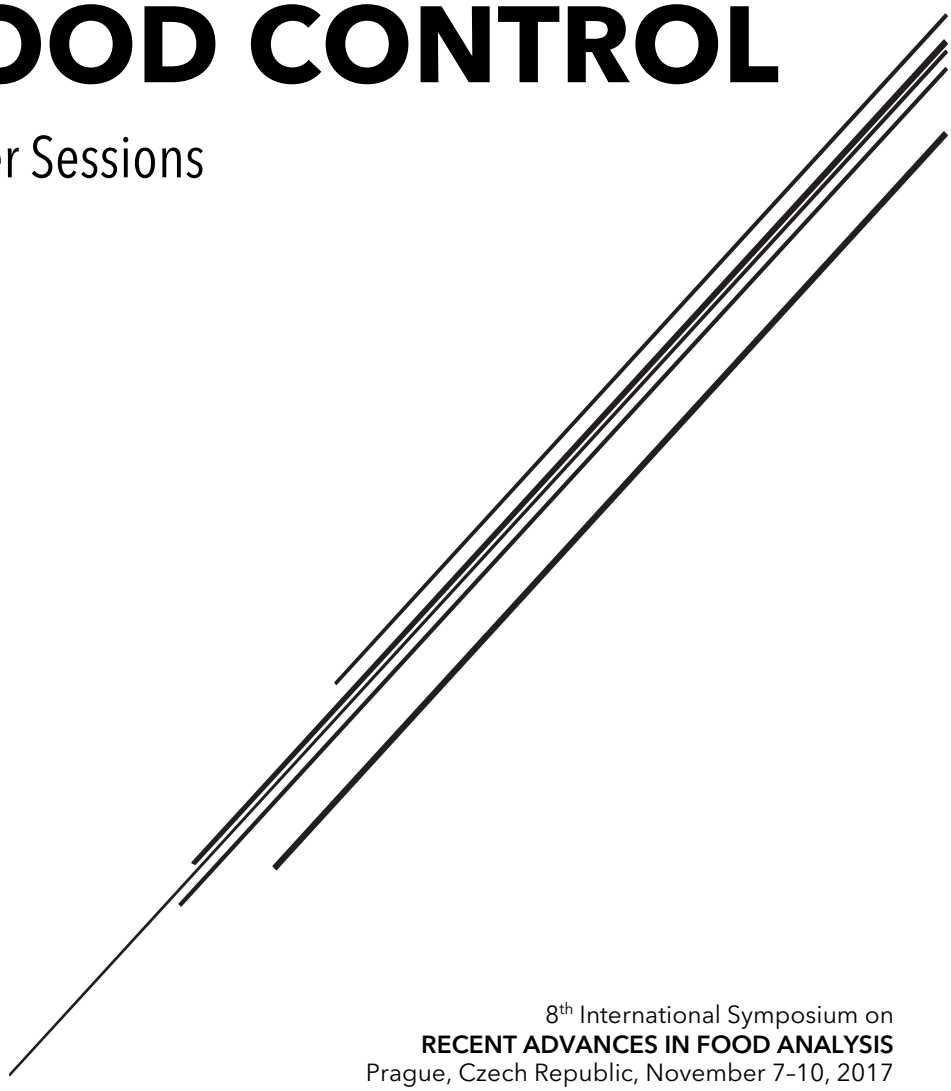
There is an increasing number of products marketed in the Community as foods containing concentrated sources of nutrients and presented for supplementing the intake of those nutrients from the normal diet. A wide range of nutrients and other ingredients might be present in food supplements including, but not limited to, vitamins, minerals, amino acids, essential fatty acids, fibre and various plants and herbal extracts.
In order to regulate them, the EU creates Directive 2002/46/EC and defines as food supplements "foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form to be taken in measured small unit quantities". For this Directive purposes nutrient means only vitamins and minerals, making it clear they are not medicines and therefore can not contain substances cataloged as active medicinal or pharmaceutical principles. Member States shall ensure food supplements marketed within the Community comply with the rules laid down in this Directive.
In recent years, in Spain, notifications have been made through the Medication Alert Network and the Food Alert Network, concerning products marketed as supplements in which the presence of undeclared active ingredients with pharmacological activity is detected, being the most frequent ones: products for the treatment of erectile dysfunction (sildenafil and derivatives), products to inhibit appetite (Sibutramine) and products for muscle development (anabolic steroids).
For this reason and in order to carry out compliance with current legislation, in the Autonomous Community of the Basque Country a Special Food Program is carried out since 2013 that includes the analytical search of such active ingredients and in 2016 the Spanish Agency of Consumption, Food Security and Nutrition (AECOSAN) elaborated the "COORDINATE PROGRAM OF VERIFICATION OF THE USE OF PROHIBITED SUBSTANCES AS INGREDIENTS NOT DECLARED IN THE COMPOSITION OF FOOD SUPPLEMENTS". In its execution, supplements that advertise invigorating physiological effects (looking for sildenafil and derivatives), slimming agents (looking for sibutramine, phenolphthalein and 2,4-dinitrophenol or DNP) and muscle development (looking for testosterone and derivatives) have been sampled at national level.
The prospective analysis of the samples corresponding to the first two types of complements was carried out at the Public Health Laboratory in Gipuzkoa of the Department of Health and Additions of the Basque Government using HPLC/MS*MS (TQD). A total of 42 samples were analyzed, and 5 (12%) have been found positive or non-conforming. The pharmaceutical principles detected were: Sildenafil, Hidroxythiohomo Sildenafil, Thiodimethyl Sildenafil, Thio Sildenafil, Tadalafil, and Yohimbine.

Keywords: *food supplements, analysis, undeclared ingredients*

BIOANALYTICAL METHODS FOR FOOD CONTROL

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
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BIOANALYTICAL METHODS FOR FOOD CONTROL

C1
SYNTHESIS OF CONJUGATE OF GOLD NANOPARTICLES WITH COVALENTLY CO-IMMOBILIZED ANTIBODY AND HRP AND ITS USE IN CHEMILUMINESCENT ELISA OF CITRININ

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Citrinin (CTN), one of fungal secondary metabolites produced by *Aspergillus* and *Pennicilium*, may be detected in some cereal grains, such as corn, wheat, barley and rice. Also, CTN is detected in red yeast rice after its fermentation in the presence of *Monascus purpureus* used as a food colorant and flavor enhancer in the Orient. Since International Agency for Research on Cancer (IARC) classified CTN as a carcinogen (group 3), some countries have legally banned the production of foods with high content of CTN. So, the maximum level of CTN in red yeast rice, allowed in Taiwan and Japan, is 5.0 and 0.2 ppm, respectively. Toxicity of CTN stimulated a development of analytical methods of its detection. In this work we developed indirect ELISA for the determination of CTN in red rice. As a revealing conjugate we used gold nanoparticles (GNPs) with co-immobilized specific antibody (sAb) and horseradish peroxidase (HRP). Varying GNPs size and a molar ratio of sAb and HRP the conditions of synthesis of the triple conjugate were optimized. The activity of HRP was measured by the chemiluminescent method using luminol and 3-(10′-phenothiazinyl)propane-1-sulfonate as a substrate and enhancer, respectively. Detection limit (IC_{10}), IC_{50} and working range (IC_{20} –IC_{80}) for the proposed assay were 5.7, 44 and 12–171 ng/mL, respectively. This assay was applied to measure CNT content in red rice samples.

Keywords: *ELISA, citrinin, gold nanoparticules, enhancement, conjugation*

Acknowledgement: *This work was financially supported by the Russian Foundation for Basic Research (Grant No. 17-54-52005\17).*

C2
DETERMINATION OF FUMONISIN B1 ON CONTAMINATED IRAQI AND INDONESIAN CORNS USING A PIONEERING ELISA BASED ON MOLECULARLY IMPRINTED POLYMER NANOPARTICLES

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Fumonisin B1 (FB1) is categorised as a human and animal carcinogen produced by Fusarium moulds which occur mainly in corn, wheat and other cereals. FB1 outbreaks have been reported and are a major concern as a public widespread causing disease where maize products are the dietary staple. For decades, enzyme immunoassay (ELISA) have been developed as the reference stablished method for FB1 determination in food and animal feed. Unfortunately, the current assays in use are inefficient due to factors such as temperature instability of the antibody (recognition element) and enzyme elements in the immunoassay, the presence of natural inhibitors in the samples tested and high levels of non-specific protein binding. Another important factors are the time consumption when results are needed rapidly and the cost of use. In order to overcome these limitations, we propose the pioneering development of an ELISA based on molecularly imprinted polymer nanoparticles (nMIPs) where the nanoparticle is a direct replacement to the primary antibody recognition element used in ELISA. The aim of this study is to determine FB1 in Iraqi and Indonesian corn using nMIPs based assay. The nMIPs for FB1 were synthesised using a computationally designed recipe where ethylene glycol methacrylate phosphate (EGMP) was chosen as the most appropriate monomer for synthesis of nMIPs validation by experimental study using solid phase extraction (SPE) for binding to FB1 (binding capacity of 95%). nMIPs using EGMP were synthesized by solid phase polymerisation in water and characterised by Dynamic Light Scattering (DLS) and UV-vis spectroscopy where the nMIPs size was found to be 362 ± 31 nm in a 0.22 mg ml^{-1} solution. Optimisation of the nMIPs based ELISA was carried out by finding the adequate concentration of conjugate (enzyme horseradish peroxidase-fumonisin B1, HRP-FB1) and nMIPs, and subsequently tested with a standard solution. The optimized protocol was stablished at 1/400 dilution of the conjugate HRP-FB1 and 0.06 mg ml^{-1} of nMIPs. Analysis of 22 Iraqi and 28 Indonesian corn samples was carried out using sample extraction with a standard protocol AOAC 2001.06-2005 using methanol 70%. A linear response (0.989) was obtained in standard solutions in a concentration range of 0.1 pM to 0.1 fM in PBS (1×10^{-13} to 1×10^{-16} M). The dynamic linear concentration range of FB1 in extract corn samples was from 0.04 to 123.10 pM, which is lower than the level of minimum residue limit required the European Commission that is 2 µM. The limit of detection and recovery of FB1 using this method are 0.02 pM and 80 % respectively. The nMIPs based assay developed could be used as a highly effective, efficient and alternative method for determination of FB1 in corn. Future research will focus on validation of this method with the view of introducing the nMIPs based ELISA as a robust, reliable and cost-effective commercial product.

Keywords: *fumonisin B1, ELISA, nMIPs, corn*

Acknowledgement: *The authors gratefully acknowledge funding by Indonesian Agency for Agriculture Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia*

C3
ENZYMATIC QUANTIFICATION OF ETHANOL IN KOMBUCHA, ALCOHOL-FREE BEER, AND JUICES USING ENZYTEC™ LIQUID ETHANOL

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In 2016, the Stakeholder Panel on Strategic Food Analytical Methods (SPSFAM) adopted standard method performance requirements (SMPR®) for the quantification of ethanol in kombucha, as laid down in SMPR 2016.001. We would like to present a robust and simple enzymatic test for quantification of ethanol in kombucha, alcohol-free beer, and juices that is fit-for-purpose according to SMPR 2016.001. The test kit consists of a ready-to-use buffer solution and a second ready-to-use solution that contains nicotinamide-adenine dinucleotide (NAD) and alcohol dehydrogenase (ADH). The enzyme converts ethanol to acetaldehyde and reduces in parallel NAD to NADH, which is measured at 340 nm within 15 min. Depending on the ethanol content of the samples, and due to the high sensitivity of the system, a simple dilution step might be necessary before measurement. The linear range is between 3 and 500 mg/l ethanol. Highest precision (CV < 2%) was shown between 30 and 300 mg/l ethanol. Other aliphatic alcohols showed side-chain activity but will not disturb the ethanol measurement, since under natural conditions these higher alcohols are present at concentration factor 1000 lower than ethanol. Analysis of certified reference materials proved that the enzymatic system shows no bias. Spiking experiments of different beverages and juices showed excellent recoveries. As expected for an uncomplicated enzymatic system, precision is also high. Overall, the system is suitable for quick and easy analysis of ethanol in beverages and juices, even by technicians with a low level of experience.

Keywords: *ethanol, enzymatic quantification, kombucha, trueness, juice*

C4
DEVELOPMENT OF EASY TO USE ANALYTICAL METHOD FOR DETERMINATION OF COMMERCIAL ß-GALACTOSIDASE ACTIVITY

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Quality assurance of every material of biological origin (enzymes, strains, proteins, DNA, etc.) used in laboratory or industry should be performed. Currently, no strict rules apply to the methods or parameters which should be used for enzyme identity or activity testing. On another hand, it is advised to follow quality/activity of the biological material in use.
Recombinant enzymes are common instruments in dairy industry. Heterologous expressed ß-galactosidases (from various hosts) are used for lactose hydrolysis in different milk processing steps. Industrial producers of ß-galactosidase define its activity in various ways (NLU·g^{-1}, LAU·g^{-1}, LAU·mL^{-1}, U·mL^{-1}, U·mg^{-1}, U), what sometimes makes it hard to repeat in the food producer's laboratory.
Enzyme activity testing usually involves the breakdown of chromogenic substrate and subsequent measurement of dye accumulation dynamics over time. These measurements are performed by spectrophoto- or fluoro- meters, devices which are not always present in dairies.
The aim of the present study was to find cost effective solution for commercial ß-galactosidase activity measurements optimal for dairies.
In the study, there was used o-NPG (2-nitrophenyl β-D-galactopyranoside) hydrolysis in 96-well plate as a standard method, as well as direct lactose hydrolysis to glucose and galactose by HPLC and several glucose strip test systems.
Different glucose strip tests are based on different enzymes, therefore crossreactivity with lactose/galactose might be an issue (this was found to be the case for glucose dehydrogenase based systems). In principle, blood glucometers can be used as a fast and simple alternative to "golden methods" for estimating commercial ß-galactosidase activity.

Keywords: *enzyme activity testing, commercial ß-galactosidase, glucometer*

Acknowledgement: *„Strengthening Research Capacity in the Latvia University of Agriculture". Contract No. 3.2.-10/2017/LLU/27*

C5
THE PROTEOLYTIC PROPERTIES OF LACTIC ACID BACTERIA FROM EWES' LUMP CHEESE

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Lactic acid bacteria (LAB), mainly *Lactobacillus* spp. and *Lactococcus* spp., play a major role in ripening of cheeses. Besides formation of lactic acid, they produce enzymes that transform various components of the matrix to aroma-active compounds. A range of key volatile aroma-active compounds, mainly C_3 -C_6 alcohols, aldehydes and ketones, are formed by tranformation of aminoacids, which are formed from milk proteins by proteolysis. Therefore, LAB-harboured genes whose products are involved in these processes, can positively influence aroma of cheeses.
Ewes´ cheeses represent a matrix with a specific composition. This is reflected also by different microorganisms that are typically involved in ripening of ewes´ cheeses, where autochthonous LAB produce the typical aroma profile of ewes´ lump cheese, barrelled ewes´ lump cheese and of bryndza cheese. Several aroma-active compounds of these Slovakian ewes´ cheeses are apparently formed by proteolysis and by subsequent transformation of aminoacids. Although LAB are weakly proteolytic, they possess a comprehensive proteinase/peptidase system. Besides the cell envelope-associated proteinase PrtP, they possess several intracellular peptidases, including a general aminopeptidase PepN and a prolyl-dipeptidyl aminopeptidase PepX. PrtP contributes to the formation of small peptides in cheese by hydrolysing larger peptides. Other enzymes important for formation of aroma-active compounds by LAB in cheeses are transaminases, which transform aminoacids to alpha-ketoacids. BcaT initiates, in this way, conversion of branched-chain aminoacids to major aroma precursors. Presence of genes encoding proteases and aminoacid-transforming enzymes is a prerequisite for their expression, subsequent growth of LAB in cheese matrix and for formation of a class of aroma-active compounds. Knowledge on the presence and sequence variability of these genes is important to for determination of their expression and for characterization of activities of microflora during ripening of cheese. To facilitate the detection of selected genes, we developed PCR-based systems to detect the genes prtP, pepN, pepX and bcaT. Then, we applied the systems to a panel of representative LAB strains isolated from ewes´ lump cheese or similar artisanal cheeses.
Positive results were confirmed by sequencing and corresponded, in lactobacilli and the lactococcus, with the predicted genes. An interesting point was that the primers oriented to pepN of lactobacilli could detect the gene also in *Leuconostoc* spp. On the other hand, primers oriented to bcaT in lactobacilli produced a false positive result, as revealed by sequencing. Negative results may mean either absence of the gene or altered sequence in the region targeted by the primer(s), which might have avoided their detection by PCR. In order to clarify this, whole genome sequences of the strains are being analysed for the presence of the genes.

Keywords: *cheese, LAB, PrtP*

Acknowledgement: *This work was supported by the Slovak Research and Development Agency under the contracts No. APVV-14-0025 and No. APVV-15-0006.*

C6
PRODUCTION AND CHARACTERIZATION OF A LIBRARY OF POLYCLONAL ANTIBODIES FOR IMMUNOAFFINITY-BASED DETECTION OF SOY PROTEINS

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Soy derivatives are considered as important food commodities, but they can contain proteins that are classified as allergens, and thus may pose a risk for allergic consumers. Among the different tools used for the detection of soy allergens, immunoaffinity assays (ELISA and immunostrips) and polymerase chain reaction (PCR) are consolidated standard choices. Nonetheless, soy food commodities can undergo a large variety of technological processes involving mechanical, thermal and chemical modifications, thus making complex the generation of antibodies able to detect both "native" and "denaturated" soy allergens. In this study, polyclonal antibodies (pAbs) were produced by eliciting the immune response of rabbits against mixtures of protein extracts from different soy commodities (i.e. soy flours). These immunogens differ for their qualitative and quantitative protein content, as revealed by gel electrophoresis (data not shown), and can be parametrized to generate descriptors. These descriptors were compared with the results emerging from the characterization of the pAbs, consisting on the determination of titers and cross-reactivities (toward common food commodities) through ELISA. This rational comparison not only allowed the selection of the most performing sera, but also the possibility of designing a second set of immunizations aimed at generating pAbs able to better recognize the different forms of soy allergens. The resulting library of pAbs was screened by ELISA for the determination of the analytical sensitivity toward soy protein standards spiked in different food matrices (i.e. biscuits, paprika, ice-cream, chocolate), or incorporated into incurred samples (i.e. rice, cookies, milk). Regarding the sensitivity, the pAbs were able to detect soy proteins in extracts (originating from different standard/commodities) with the following order: soy flour > texturized soy proteins > soy protein isolates, with LOQs in the range 0.25 – 2.5 ppm for the soy flours (around 0.125 – 1.25 ppm of proteins) and 10 - 20 ppm for textured soy proteins (3 – 7 ppm of proteins). Interestingly, all of these pAbs are able to detect the presence of soy flour (normal and toasted) at 10 ppm contamination (5 ppm of soy proteins) in some incurred samples, sometimes outperforming other commercial kits. Two pAbs are also able to detect soy proteins in extracts originating from a highly processed soya derivative (Tofu). In conclusion, this study attests the feasibility of a rational immunization design for the generation of a library of pAbs targeting soy allergens belonging to food commodities that have undergone different extent of technological processing. Thanks to the biomolecular recognition patterns of the sera and the combination with diverse chemistry strategies developed for grafting antibodies onto a variety of functional (nano)surfaces, this library of biological reagents can be used in a wide range of analytical platforms aiming at soy protein monitoring.

Keywords: *immunizations, polyclonal antibodies, soy proteins, immunoaffinity assay*
Acknowledgement: *The authors acknowledge the Walloon region for funding support (CWALITY Program).*

C7
THE BIO END USER SENSOR TREE: AN OPEN-SOURCE AND USER-FRIENDLY CLASSIFICATION SYSTEM FOR BIOSENSORS

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Biosensor classification traditionally follows useful criteria for biologists and/or engineers. The system is reviewed according to the bio-recognition elements and/or transducers used thus making sensor nomenclature informative for professionals in the aforementioned fields. This facilitates keyword searches for publications relevant to their work. However, such a classification system does not create a decision support system for the end-user who is more interested in the capabilities of the sensor instead of its biological/electrical build-up. Moreover, scientific literature regarding comparative performance studies and newly developed sensors can be lengthy and impede non-academic dissemination of new information. On the other hand, open access to summarized information regarding recent advances and discoveries in biosensor development can stimulate citizen science initiatives, allow increased participation of researchers in developing countries and, ultimately, boost biosensor development and publicity. To this end we propose a novel end-user orientated classification system that we named the "Bio End-user Sensor Tree" or BEST system. BEST is based on a classification system that uses 5 criteria in the following consecutive order 1) expert training needed 2) sensor portability 3) quantification ability 4) single or multiplex screening ability and 5) single or multi use sensor type. These criteria are used to build a decision tree that enables end-users throughout the production chain (from the agriculture sector to the consumer) to make an informed decision regarding which biosensor best fits their needs. After being directed to a certain niche of sensors a list of available possibilities is shown. These sensors can then be compared based on provided summaries of current scientific literature and consumer reviews. In this section space will also be provided for the dissemination of information regarding the development of novel sensors thus stimulating the public awareness of such projects. In order to improve and further develop BEST we have made the system open-sourced via a public folder. We invite other enthusiasts to participate in the further development of BEST to create a vibrant, fully comprehensive database providing useful information to the end user regarding a myriad of Biosensors for compound detection in food and feed.

Keywords: *biosensor classification, citizen science, open source, BEST, decision support system*

Acknowledgement: *The research was funded by the Horizon 2020 Framework program for research and innovation (Grant Agreement number: 720325).*

C8
DETECTION OF FRESHWATER CYANOTOXINS AND MEASUREMENT OF MASKED MICROCYSTINS IN TILAPIA FROM SOUTHEAST ASIAN AQUACULTURE FARMS

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Recently, there has been a rise in freshwater harmful algal blooms (HABs) globally, as well as increasing aquaculture practices. HABs can produce cyanotoxins, many of which are hepatotoxins. An ultra-performance liquid chromatography tandem mass spectrometry method was developed and validated for nine cyanotoxins across three classes including six microcystins, nodularin, cylindrospermopsin and anatoxin-a. The method was used to analyse free cyanotoxin(s) in muscle (n=34), liver (n=17) and egg (n=9) tissue samples of 34 fish sourced from aquaculture farms in Southeast Asia. Conjugated microcystin was analysed by Lemieux oxidation to ascertain the*total* amount of microcystin present in muscle. Some tilapia accumulated *free* microcystin-LR in the muscle tissue at a mean of 15.45 µg/kg dry weight (dw), with total microcystin levels detected at a mean level of 110.1 µg/kg dw, indicating that the amount of conjugated or masked microcystin present in the fish muscle accounted for 85% of the total. Higher levels of cyanotoxin were detected in the livers, with approximately 60% of those tested being positive for microcystin-LR and microcystin-LF, along with cylindrospermopsin. Two fish from one of the aquaculture farms contained cylindrospermopsin in the eggs; the first time this has been reported. The estimated daily intake for *free* and *total* microcystins in fish muscle tissue was 2 and 14 times higher, respectively, than the tolerable daily intake value. This survey presents the requirement for further monitoring of cyanotoxins, including masked microcystins, in aquaculture farming in these regions and beyond, along with the implementation of guidelines to safeguard human health.

Keywords: *UPLC-MS/MS, aquaculture, microcystin, bioaccumulation, human health*

Acknowledgement: *The authors would like to acknowledge the contributions of collaborators on this project: Katrin Kittler from the BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Straße 11, 12489 Berlin, Germany. This research was funded by the Advanced ASSET project, partly funded through Invest NI and from the European Sustainable Programme 2007–2013 under the European Regional Development Fund (ERDF).*

C9
TOTAL DNA QUANTIFICATION TO CONFIRM THE ABSENCE OF CROSS-CONTAMINATION IN VETERINARY-PRESCRIBED DIETS FOR ANIMALS WITH ADVERSE FOOD REACTIONS (AFR)

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As Humans, animals are prone to food allergies, requiring specific nutritional managements, which have been answered through two strategies in the pet food industry:
Diets based on limited ingredients, with usually one single source of novel protein.
Diets based on hydrolyzed proteins to prevent the possibility of epitope recognition and thus the potential for an adverse food reaction.
To be reliable for diagnostic and management, both types of diets must be free of ancillary proteins. As for human food, cross-contamination with unwanted ingredients in production plants is of high concern, especially in diets dedicated to the allergic population.
To insure the absence of cross-contaminated products, three key elements are required: fully characterized raw materials, fit-to-purpose industrial equipment and cleaning processes, and the analytical means to validate the absence of contamination.
By using extensively hydrolyzed proteins and DNA free ingredients, a new approach for cross contamination surveillance can be envisioned.
This led to the development of a quick and sensitive method to insure the absence of cross contamination in ANALLERGENIC Canine and Feline (Royal Canin, Aimargues, France), two veterinary-prescribed diets for pets suffering from complex food allergies.
Unlike classical diets, Anallergenic formulas are DNA free, so the monitoring of DNA content was found to be a sensitive indicator of potential cross contamination during production.
For all sources of proteins present in the plants, calibration curves between DNA and protein concentrations have been developed, allowing for the determination of protein concentrations based on species-specific DNA measurement.
In order to assess the level of potential cross-contamination, each production batch is analyzed by this 3 step-DNA testing method:
Step 1: Measurement of the total DNA content and comparison to DNA Level conformity threshold (7ng/ml) established during a multicentric preclinical efficacy trial in refractory AFR dogs. This level was clinically proven to support the resolution of the dermatological symptoms in all animals.
Step 2: In case of DNA detection at level higher than the conformity threshold, PCR analyses are conducted to identify the source of the contamination.
Step 3: Determination of the concentration of the ancillary protein level from the total DNA measurement using the appropriate species specific calibration curve.
Since the launch of the products, this method was used to validate every production (>3000 individual tests), a key element to insure the clinical efficacy. Interestingly, the first step of the method, the total DNA measurement, was found to be usually enough to qualify the production as in 99,9% of the cases DNA was below threshold. As total DNA quantifications are easily performed, this approach was found to be very efficient in industrial settings.

Keywords: *allergens, adverse food reaction, cross-contamination, petfood, total DNA quantification*

C10
LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) FOR FOODBORNE PATHOGEN DETECTION: EVALUATION OF DIFFERENT GENETIC TARGETS FOR SALMONELLA ENTERICA SEROVAR ENTERITIDIS AND TYPHIMURIUM CHARACTERIZATION

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Salmonella Enteritidis and *Salmonella* Typhimurium continue to be the most frequently identified serovars among confirmed cases of salmonellosis [1]. There is a need of rapid and reliable methods to detect, and identify the source of foodborne pathogen contamination, in this sense molecular methods, such as those based on DNA amplification, have the capacity to overcome some of the limitations of traditional culture based techniques.
Novel *in vitro* DNA amplification techniques have emerged in the last number of years with the objective of providing analytical solutions in some specific cases. Among them, isothermal DNA amplification techniques are specially interesting. Loop-Mediated Isothermal Amplification (LAMP), presents several advantages such as higher specificity due to the use of six primers instead of two, and the result of the amplification can be observed by the naked eye since positive samples become turbid [2]. Moreover, LAMP can be run using relatively impure sample materials reducing the time for sample preparation step. However, LAMP based analytical methods need to be carefully designed and evaluated in order to provide reliable results of analysis.
Different genetic targets have been proposed for the specific identification of Salmonella Enteritidis and Salmonella Typhimurium [3–5]. In the current study a comparison among several was performed in order to determine their capacity to accurately identify them by LAMP. *Salmonella* Enteritidis, was characterized based on *safA* and *Sdf I*. it was observed that both performed equally well regarding their specificity, but the former resulted more sensitive. The evaluation in spiked samples resulted in minor differences among them. Regarding the targets for Typhimurium, STM4497 and *typh*, were selected. The former demonstrated to be more specific and sensitive. This observation with pure cultures was confirmed in spiked food samples. These results highlight the importance of an adequate evaluation of the genetic targets selected, before their implementation for routine analyses.

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Keywords: *Salmonella Enteritidis, Salmonella Typhimurium, LAMP, characterization*

Acknowledgement: *This work was supported by a Marie Curie COFUND Action (Project No: 600375 NanoTRAINforGrowth - INL Fellowship programme in nanotechnologies for biomedical, environment and food applications), by the project Nanotechnology Based Functional Solutions (NORTE-01-0145-FEDER-000019), supported by Norte Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).*

C11
SCREENING FOR DRUGS OF ABUSE AND ACTIVE INGREDIENTS IN DIETARY SUPPLEMENTS

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Fat-burners are food supplements often marketed with claims for improving sport performances and facilitating weight loss. They contain ingredients, such as synephrin or green tea extracts, with proposed fat-oxidation, impaired fat absorption and thermogenic properties. Scientific evidences for the proposed mechanisms of action are however limited. Adulteration of fat burners with illegal substances was commonly observed during recent years; most common adulterants being appetite suppressors (sibutramine), stimulants and antidepressants. The objective of this study was to evaluate the occurrence of illegal substances in fat burners sold on the Swiss market, including internet. The potential benefit of a drug screening approach originally designed for the analysis of human serum or urine in forensic and clinical toxicology laboratories was also tested.

Thirty two fat burners were collected in local stores and on internet, using only websites with Swiss domain names or allowing logging in using Switzerland as country. The products were suspended in methanol, centrifuged, and the supernatant was analyzed by two LC-MS/MS approaches. First, a method targeting 124 active botanical ingredients, sibutramine and 2,4-dinitrophenol was used. Second, the Bruker Toxtyper™ screening system, based on data-dependent tandem mass spectra acquisition followed by comparison with a library containing 851 spectra of active pharmaceutical compounds, illicit drugs and metabolites thereof, was used. Identified compounds of interest were semi-quantified by LC-MS/MS using external calibration or isotopically labeled standards.

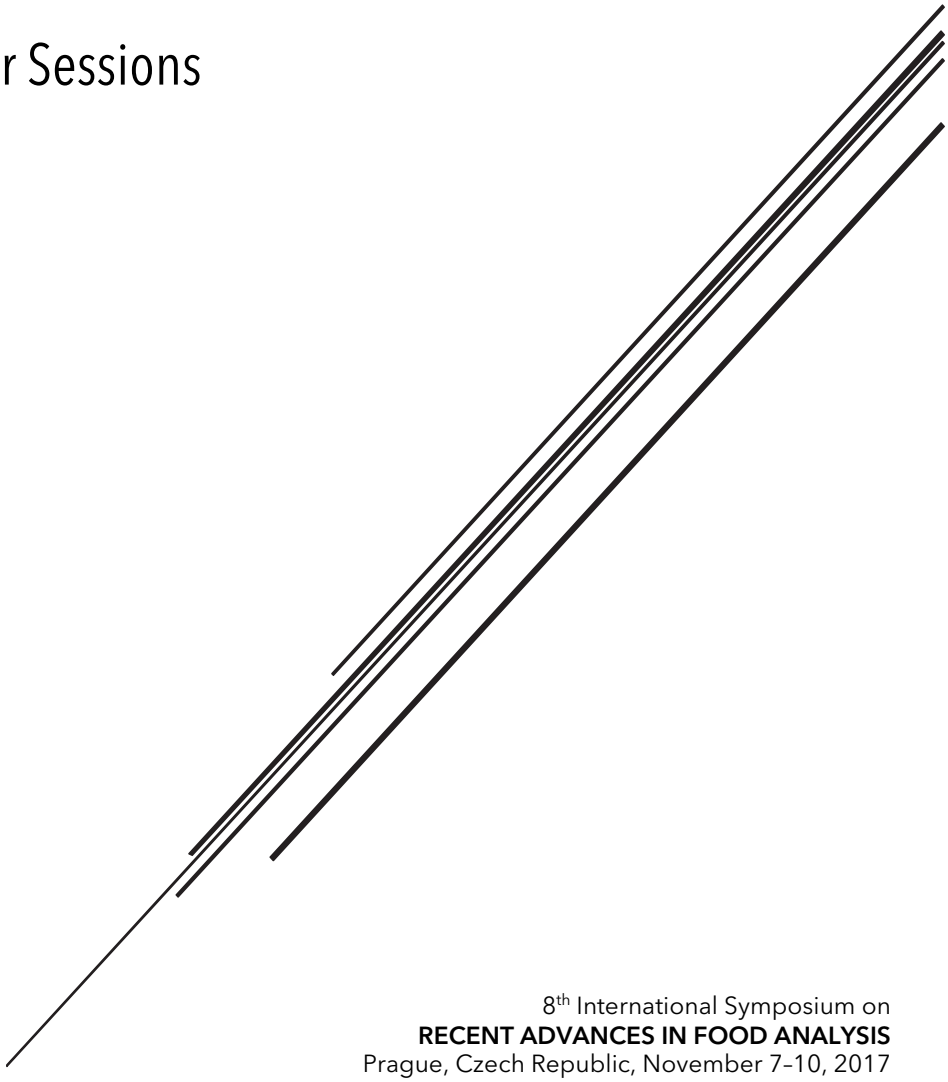
Among the 32 collected samples, 26 did not satisfy the Swiss legislation regarding their declared ingredients and/or their quantity, and other missing mandatory information. More worrying, seven of the 32 products contained either illegal or regulated pharmaceutical compounds. The targeted method enabled the identification of yohimbine, hyperforin and gingkolide A. The screening method detected amphetamine, phenethylamine and yohimbine. Among these molecules, only the presence of phenethylamine could have been expected from the declared ingredients (cacao extract). Phenethylamine was present at 150 mg/kg in this sample. Concentrations of yohimbine varied between 0.4 and 2.0 mg/kg in the three samples. One product contained 120 mg/kg hyperforine. Ginkolide A was present at 3 and 4 mg/kg in two products, and amphetamine at 24 and 770 mg/kg in two other products.

This study shows that adulteration of food supplements with illegal drugs and active compounds is still common practice, despite multiple surveys and alerts. Our results also demonstrate that the combination of different LC-MS/MS approaches, including tools developed in the biomedical and diagnostic field was effective for the screening of active compounds in foodstuff. The screening method allowed detecting amphetamine, an unsuspected substance in food supplements.

Keywords: *dietary supplements, fat burners, drugs of abuse screening*

BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

Poster Sessions



8th International Symposium on
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BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

D1
OPTIMIZATION OF EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM QUASSIA AMARA L. LEAVES USING RESPONSE SURFACE METHODOLOGY

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The food industry uses some synthetic antioxidants, such as butylated-hydroxy-anisole, butylated-hydroxy-toluene and propyl-gallate in order to prolong the storage stability of food. However, the demand for natural products by consumers has recently increased because of the toxicity and carcinogenicity of synthetic antioxidants. Thus, most of the recent study has been directed towards identification of novel antioxidants from natural sources, particularly of plant origin. The *Quassia amara* is a native plant from northern Brazil, belonging to Simaroubaceae family. The objective of this work was to determine the best extraction conditions of bioactive compounds present in the *Quassia amara* leaves by means of factorial design and response surface methodology (RSM) and to identify and isolate their active substances. The best extraction conditions of *Quassia amara* leaves bioactive compounds were methanol at 70 °C temperature and 60 min-extraction. Phenolic compounds identification performed by high-performance liquid chromatography (HPLC) revealed the presence of gallic acid (58.12 µg/g) and catechin (42.4 µg/g). By means of ^{1}H and ^{13}C NMR spectroscopic analysis, it was possible to identify the alkane octadecane, which was isolated for the first time in the *Quassia amara* leaves. The methanolic extract of *Quassia amara* showed high total phenolic content (209.39 mg GAE/g sample, GAE: Gallic acid equivalent) and antioxidant activity by the DPPH radical method (1898.02 µmol Trolox/g). In order to confirm the antioxidative effect of this promising plant, a further survey which uses other kinds of antioxidant assays is now underway. This survey includes also the application of this extract in food product. However, in view of this, the plant has potential for substitution to synthetic antioxidants widely used in the food industry.

Keywords: *factorial design, phenolic compounds, antioxidant activity, spectroscopic analysis, HPLC analysis*

Acknowledgement: *The authors thank the Brazilian National Research Council for financial support, Coordination for the Improvement of Higher Level Personnel (CAPES), Araucaria Research Foundation for the scholarships and Analysis Center at UTFPR-Pato Branco/PR.*

D2
USE OF RESPONSE SURFACE METHODOLOGY FOR THE ASSESSMENT OF THE PHENOLIC COMPOUNDS WITH BIOACTIVITY OF CROTON LECHLERI SAP CO-PRODUCT EXTRACT FROM AMAZON FOREST, BRAZIL

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The objectives of this study were to optimize the extraction of total phenolic compounds (TPC), total anthocyanin (TA) and to evaluate the antioxidant activity (AA) from *Croton lechleri* sap co-product extract. A factorial 2^3 design (FD) with three factors were conducted for optimization using response surface methodology (RSM). The temperature (35 and 70 °C), the time (30 and 90 min), and the solvent (ethanol and water) were designed for the extraction. This design was composed of eight trials performed in triplicate. The TPC was determined by Folin Ciocalteu, the TA was performed by the pH-differential method, while AA by scavenging activity of the DPPH• (2.2-diphenyl-1-picrylhydrazyl) radical. The TPC, TA and AA ranged from 192.32±0.56 to 61.44±3.65 mg GAE/g sap co-product (GAE: Gallic acid equivalent), 2.48±0.24 to 21.71±1.83 mg/100 g sap co-product and 4.03±0.31 to 16.13±0.11 µmol de Trolox/g sap co-product, respectively. In the best extraction condition recommended by FD and RSM for the total phenolic compounds content and antioxidant activity, the ethanol was used as solvent at 70°C for 60 min. However, the aqueous extract at 70°C for 30 min showed the highest TA content (21.71±1.83 mg/100 g sap co-product) and had no statistical differences between the aqueous extract at 70°C for 90 min (20.04±2.83 mg/100 g sap co-product). The statistical analysis showed that the most significant factor in the extraction of antioxidant compounds was the solvent, followed by temperature, and time. The phenolic compounds were determined by High-Performance Liquid Chromatography. High contents of gallic acid (94.88 ±2.08 mg/kg), catequin (260.11±18.23), epicatequin (22.05±2.38) and siringic acid (28.68± 2.15) were detected after treatment with ethanol at 70°C for 60 min. The extract analyzed under these conditions showed inhibitory activity against *Staphylococcus aureus*, *Salmonella bongori*, *Salmonella thyphimurium* and *Candida albicans* at the concentration of 0.005, 0.010, 0.005 and 0.150 mg/mL, respectively. In addition, these extracts showed bactericidal activity against *Salmonella thyphimurium* (0.630 mg/mL). This study illustrated that the optimal conditions for extraction of the phenolic compounds from *Croton lechleri* sap co-product extract have a high potential to produce functional ingredients. Furthermore, these results confirm the possibility to use the Croton lechleri sap co-product as alternative antioxidant and antimicrobial agent from sustainable sources in the food industry.

Keywords: *antioxidant activity, antimicrobial activity, pehnolic compounds, HPLC analysis*

Acknowledgement: *The authors thank the Brazilian National Research Council for financial support, Coordination for the Improvement of Higher Level Personnel (CAPES), Araucaria Research Foundation for the scholarships and Analysis Center at UTFPR-Pato Branco/PR.*

D3
EFFECTS OF VARIOUS LED LIGHT ILLUMINATION ON THE ANTIOXIDANT PROPERTIES OF PHYCOBILIPROTEIN EXTRACT FROM SPIRULINA PLATENSIS

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Spirulina platensis, a photosynthetic blue-green microalga (cyanobacterium), is considered as valuable source of high-worth compounds like proteins, fatty acids (γ-linolenic acid) and accessory pigments: chlorophyll a and phycobiliproteins, particularly phycocyanin, which can be applied as food additive and in biomedical researches. Natively, phycobiliproteins act as part of photosynthetic apparatus of cyanobacteria, where absorb solar energy and transfer it toward the reaction centres. Bright pigmentation and the lack of toxicity cause phycobiliproteins great natural colorants for food, cosmetics and pharmaceutics. The more that several studies confirm anti-inflammatory, anti-cancer, neuroprotective or antioxidant properties of these substances. Reactive oxygen species (ROS) are involved in pathogenesis of e.g. Parkinson or Alzheimer diseases, what opens the doors to use phycocyanin also in medical treatment. Because of mentioned properties the extracts containing this blue pigment gain importance as nutraceuticals. *Spirulina platensis* is cultivated majorly in open ponds, under sunlight conditions, because of relatively low costs of such a way of maintenance. The drawbacks of this system are most of all poor availability of light, especially in case of high biomass productivity and contaminations by other algae, bacteria or protozoa. Therefore, the use a closed photobioreactor system, which allows to control numerous culturing parameters such as: colour and intensity of light, temperature, nutrient composition, pH or oxygen concentration, might be the convenient tool for phycocyanin production. Light-emitting diode (LED) - based illumination systems are considered as a greatly promising artificial lighting for cultivation of photosynthetic microorganisms. Low energy consumption, long life cycle and narrow spectral emission (illumination in selected wavelength) are just a few of advantages of LEDs.
The objective of our study was to examine the effect of various combinations of LED light illumination on the growth of *S. platensis* and the content of photosynthetic pigments, which affect the antioxidant activity of phycobiliprotein containing extracts. The spectrophotometric methods were used to determine the chemical composition, radical scavenging activity (using 2,2-diphenyl-1-picrylhydrazyl (DPPH•)) of the crude and initially purified extracts of cyanobacterial cells, whereas the TEAC assay allowed to measure the ability of obtained extracts to quench a radical cation ($ABTS^{\bullet+}$). Received results indicated that the highest phycocyanin content was obtained after cultivation with blue LED and after combined sequence of red and blue LED light. Surprisingly the strongest antioxidant properties were the valor of partially purified extracts, what indicating that phycobiliproteins are the main factor responsible for antioxidant activity of these matrices.

Keywords: *spirulina platensis, LED illumination, phycobiliproteins, nutraceuticals, antioxidative activity*

Acknowledgement: *Reported study was supported by Ministry of Science and Higher Education of Poland, in the frame of "Young Scientist' Research Grants 2017" Faculty of Chemistry, University of Opole.*

D4
COMPARING THE PHENOLIC COMPOSITION AND ANTIOXIDANT PROPERTIES OF CAMEROONIAN AND EUROPEAN HONEYS

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Cameroon is a country in West Afrika stretching from desert in the north, mountains in the central regions and tropical rainforest in the south. The richness of forests and grassland is an ideal habitat for bees that produce dark honey with an interesting taste. In Cameroon, the people's health mainly depends on natural therapies and traditional medicine. Therefore, honey and other bee products are an important natural remedy, which presents antibacterial and antiradical activity. However, chemical composition of Cameroonian honey has not been described in the available literature. Antioxidant properties honeys are mainly attributed to the presence phenolic compounds which originates from nectar and pollen of the flowers. In addition, phenolic compounds are well acknowledged for their diverse biological activities, metal ion chelation and including free radical trapping. They have been shown to prevent inflammation disorders, cardiovascular diseases and protective effects to lower the risk of various cancers. Antibacterial and antioxidant agents from such natural sources may constitute potentially inexpensive treatment. Furthermore, with well-balanced diet, honey may be a good source of antioxidant. The aim of this study is to define the individual, characteristic phenolic compounds of Cameroonian honeys and mark their antioxidant activity, as well as to compare the parameters with the results for European honeys.

Keywords: *Cameroonian honey, chemical composition, phenolic compounds, antioxidant activity*

Acknowledgement: *Reported study was supported by The National Science Centre of Poland in the frame of Grant NO: 2014/15/B/N29/02182.*

D5
CHARACTERIZATION OF EXTRA VIRGIN OLIVE OIL ENRICHED WITH ESSENTIAL OILS BY SPECTROSCOPIC TECHNIQUES

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Innovation in the olive oil sector is increasingly demanded. The production of enriched olive oil is bound to open new markets as a new strategy to enhance its beneficial effects. One of the ways to enrich olive oil with bioactive compounds relies on the addition of essential oils obtained from plants/fruits usually used in cooking. Hence, a less quantity of oil will be used in cooking, which will result in an economic and sensory advantage. The present work evaluates the effects of the addition of ten essential oils, namely chili, thyme, oregano, rosemary, lemon, yuzu, basil, ginger, coriander and laurel. A total of 30 bottles will be tested. The extra virgin olive oil chosen was a blend of 'Galega' and 'Cobrançosa' oils characterized by a MUFA and PUFA contents of 74 and 8%, respectively, containing a low content of total phenols (150 mg GAE/kg) and an oxidative stability (EO) of 10 hours. The primary purpose of the study is to carry out the screening of the oils in order to evaluate the sensory and chemical changes along storage. The enriched olive oils will be evaluated through spectroscopic techniques namely FTIR, NIR and RAMAN in order to identify differences or similarities in the chemical composition of the different olive oil samples.

Keywords: *olive oil, bioactive compounds, infrared spectroscopy, essential oils*

Acknowledgement: *Project "CENTRO-04-3928-FEDER-000009 - Beira Baixa Terras de Excelência - Comunicação, Animação e Inovação" LEAF, Linking Landscape, Environment, Agriculture and Food (UID/AGR/04129/2013). Centro de Estudos Florestais (UID/AGR/UI00239/2013).*

D6
DIFFERENTIAL ANTIBACTERIAL ACTIVITIES OF FOUR STRUCTURAL CLOSELY RELATED FLAVONOIDS BAICALEIN, BAICALIN, CHRYSIN AND BIOCHANIN A ON CLINICAL ISOLATED STAPHYLOCOCCUS INTERMEDIUS AND STREPTOCOCCUS SUIS IN VITRO

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Bioactive compounds from natural products are potent candidates to treat pathogenic microbial infections since chemically synthetized antimicrobials are limited in usage. Flavonoids are respectable applicants as their multiple properties have been defined including antimicrobial activities. *In vitro* antibacterial activities of four structural closely related flavonoids; three flavones of baicalein, baicalin and chrysin and an isoflavone, biochanin A to clinical isolated *Staphylococcus intermedius* and *Streptococcus suis* were examined using standard disc diffusion assays. Two clinically pathogenic bacteria were obtained from Microbiological Laboratory, Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, Thailand. The flavonoid concentrations ranging from 5-40 mM were used. Antibiotic discs, Amoxicillin/clavulanic acid 30 µg and Sulfa-trimethoprim 25 µg were used as control standards. Baicalein showed intermediate antibacterial activity against *S. intermedius* since low-dose treatment of 5 mM and through particular concentrations. The activity showed dose-dependent manner by increasing zone of inhibitions (ZOI) from 7.67±0.50 mm at 5 mM to 10.78±0.44 mm at 40 mM treatments. In contrast to baicalein, baicalin, chrysin and biochanin A, showed no inhibitory activity to *S. intermedius* up to the highest concentration. No selected flavonoids exhibited any antibacterial activities against *S. suis*. The combination treatment of baicalein and biochanin A ratio 1:1 ranging from 5 to 40 mM did not elevate the antibacterial activities toward *S. intermedius*. Differential mechanism of inhibition from baicalein to *Staphylococcus intermedius* and *Streptococcus suis* were proposed. The antibacterial effects of baicalein over other flvaonoids could due to the presence of *ortho*-di hydroxyl groups at C-6 and C-7 positions in ring A. Focuses are on investigations of flavonoids structure-activity relationships, their kinetics, and mechanisms of microbial inhibitions.

Keywords: *baicalein, baicalin, Staphylococcus intermedius, Streptococcus sus, antibacterial activity*

Acknowledgement: *The authors thank Mr. Suksan Chamsing, Mr. Somyod Kankhuntod and Ms Montisha Suwanwong for technical supports.*

D7
CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF WHOLE BERRIES BUCKTHORN OIL OBTAINED BY SUBCRITICAL FLUID EXTRACTION

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Sea-buckthorn has been widely used both as food and in therapy, as such (as fresh or dried fruits) and also processed (as extracts and/or teas). Subcritical fluid extraction (SFE) is a rather novel technique which both provides good extraction yields and preserves the biological activity of the phytochemicals. This paper aims at the evaluation of the chemical composition of a SFE buckthorn oil and its antioxidant activity.
Whole berries of buckthorn were harvested at maturity from the lowland of Sacele area (Romania) and subjected to subcritical fluid extraction to obtain the oily extract.
In order to be analyzed for the fatty acids composition by GC-MS the oil, dissolved in chloroform:methanol (2:1, *v/v*), underwent a saponification process with KOH, precipitated with NaCl, re-dissolved in hexane and injected into the chromatograph column. The identification of the compounds was performed using NIST mass spectrum library.
The HPTLC analysis was performed on the oil dissolved in chloroform and on the saponificated sample as described above and had in view the sterols content. Three mobile phases were used and the best results were obtained using benzene:ethyl acetate (75:25, *v/v*).
The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was used to assess the free radical scavenging activity of the oil diluted in ethanol. The antioxidant activity of the oil was also evaluated with $ABTS^{\cdot+}$ and FOX-PCA assays, using standard curves obtained with ascorbic acid for DPPH and $ABTS^{\cdot+}$ and with cumene hydroperoxide for FOX-PCA.
The GC-MS analysis allowed the identification of palmitic and oleic acids as major components, followed by stearic acid. Among less important components, the tridecanoic and 9-octadecanoic acids were undoubtedly identified.
As for the phytosterols, stigmasterol was identified at Rf=0.59. The presence of beta-sitosterol was uncertain, as for neither of the three mobile phases it could not be separated from stigmasterol.
The scavenging effect of the oil against DPPH decreased with the increase of the oil dilution from 1:10 to $1:10^6$, maintaining a dose-dependency. The ABTS assay was proved to be a good method for the assessment of the alcohol diluted oil; the antioxidant effect is not dose dependent for this model. The FOX-PCA method revealed that the oil samples are charged with hydroperoxides, possibly due to the thermal treatment during the preparation stage.
In conclusion, the oil extracted from sea buckthorn berries by SFE preserves its main components (fatty acids and phytosterols), and also a high antioxidant effect, despite the rather great level of hydroperoxides present in the sample.

Keywords: *Hippophae rhamnoides L., subcritical fluid extraction, fatty acids, phytosterols, antioxidant activity*

Acknowledgement: *This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-2801. We hereby acknowledge the structural funds project PRO-DD (POS-CCE, O.2.2.1., ID 123, SMIS 2637, No 11/2009) for providing the infrastructure used in this work.*

D8
ANALYSIS OF EIGHT CATECHINS AND FOUR THEAFLAVINS IN TEA SAMPLES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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This paper describes the simultaneous determination of catechins and theaflavins in green, black and oolong teas, using high performance liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (HPLC–MS–MS) in multiple-reaction monitoring (MRM) mode. The tea polyphenols analyzed included (+)-catechin (C), (-)-catechin gallate (CG), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), (-)-epigallocatechin (EGC), (-)-gallocatechin (GC), (-)-gallocatechin gallate (GCG) and (-)-epigallocatechin gallate (EGCG) theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3′-gallate (TF2B) and theaflavin-3, 3′-digallate (TF3). These polyphenols separated within 26 min by HPLC. HPLC-MS/MS parameters were optimized for simultaneous analysis of all the target analytes in a single injection. The optimized HPLC-MS-MS methodology is selective and specific, and was validated for eight catechins and four theaflavins widely reported in different teas. Satisfactory linearity was achieved in linear range (0.02-5μg mL^{-1} for catechins and 0.02-20 μg mL^{-1} for theaflavins) and fine determination coefficient (r^2 > 0.9935). The recoveries ranged from 65% to 115% with the RSD ranging from 2.37% to 6.72%. The optimal analytical conditions of this method were investigated for the convenience and reliability for routine analysis of the target polyphenols concentration in three types of tea samples.

Keywords: *HPLC–MS–MS, catechins, theaflavins, tea*

Acknowledgement: *This study was supported by the National Natural Science Foundation of China No. 21525730, the Strategic Priority Research Program of the Chinese Academy of Sciences No. XDB14030401 and XDB14030402.*

D9
MACROALGAE AS A SOURCE OF BIOACTIVE PEPTIDES. IDENTIFICATION IN PROTEIN HYDROLIZATES BY HPLC-ESI-QTOF/MS

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Marine organisms exhibit a rich chemical content with unique structural secondary metabolites compared to that of terrestrial ones. Many of these compounds show important biological activities. Macroalgae are a diversified group of marine organisms that have emerged growing in highly dynamic and complex competitive environments, and generating a wide group of functional biomolecules to support survival under stress conditions such as extreme salinity and temperature, light and nutrient deficiencies. For instance, high protein contents exist in marine algae, generally 3-15% of the dry weight in brown algae (*Phaeophyta*), 9-26% of dry weight in green algae (*Chlorophyta*) and 10-47% of the dry weight in red algae (*Rhodophyta*). Recently, there is a big interest in employing marine algae proteins as a source of bioactive peptides. Marine algae peptides could be involved in several biological functions according to their amino acid sequence, such as antioxidant, anticancer, antihypertensive, antiatherosclerotic or immunomodulatory effects.
The objective of this study was to generate and identify bioactive peptides from protein hydrolyzates from three different edible macroalgae used for human consumption. An extraction method was developed to obtain aqueous and alkaline soluble proteins. The proteins obtained were submitted to enzymatic digestion previously to their analysis by reverse phase-high performance liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometry (RP-HPLC-ESI-QTOF/MS) and *de novo* sequencing was employed to characterize the active peptides. Around forty peptides were identified in the three macroalgae being six of them common in brown and red macroalgae. Any of the forty peptides identified in this study had previously been reported in macroalgae. After checking against different databases, several sequenced peptides were found within longer peptides with potential bioactivities.

Keywords: *macroalgae, bioactive peptides, extraction, liquid chromatography time-of-flight mass spectrometry, de novo sequencing*

Acknowledgement: *Authors thank financial support from the Comunidad of Madrid and European funding from FEDER program (project S2013/ABI-3028, AVANSECAL). R.P.M. thanks the University of Alcalá for her pre-doctoral contract. M.C.P. and M.P. thank the Spanish Ministry of Economy and Competitiveness (MINECO) for their "Ramón y Cajal" (RYC- 2013-12688) and "Juan de la Cierva" (IJCI-2014-22143) research contracts, respectively.*

D10
BIOACCESSIBILITY OF LEGUMES POLYPHENOLS BY AN IN VITRO DIGESTION MODEL

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Legumes are worldwide staple food representing a good source of phenolic compounds with proposed health benefits. Prior to consumption legumes are processed in a number of ways, including soaking, boiling, steaming, roasting and fermentation. These practices often improve the palatability, enhance the bioavailability of bioactive compounds but they can also affect the stability of various nutrients. Specifically, heat treatment may change the phenolic profile by leaching hydrophilic compounds, degradating and/or transforming polyphenols, and stimulating additional chemical reactions converting bound phenolic forms to the corresponding free/soluble phenolic forms. Degradation, metabolism and absorption of polyphenols can occur during gastric, small intestinal and/or colonic digestion, but according to our knowledge no previous studies on the polyphenols characterization in cooked legumes have incorporated an *in vitro* digestion model. Therefore the aim of this study was to characterize the content and the distribution of 12 polyphenols (phenolic acids, flavonoids and anthocyanins) detected in both free and bound forms in 3 legumes varieties (Beluga lentils, Black beans and Pinto beans) after cooking and in vitro digestion. An HPLC-DAD method identifying and quantifying 12 phenolics (4-hydroxybenzoic acid, chlorogenic acid, syringic acid, ferulic acid, rutin, kaempferol 3-glucoside, quercetin, cyaniding 3-5 diglucoside, delphinidin 3-glucoside, cyaniding 3 glucoside, pelargonidin 3-5-diglucoside, cyaniding chloride) has been validated through linearity, interday and intraday repeatability, limit of detection, limit of quantification and recoveries. Ferulic acid was the predominant free and bound phenolic in cooked black beans and pinto beans. Delphinidin 3-glucoside and chlorogenic acid were the prevalent free phenolics in lentils cotyledons after cooking (1.9 mg/100 g and 1.8 mg/100 g respectively). The boiling process significantly increased syringic acid content in black beans (0.9 mg/100g), possibly due to the disintegration of cell wall matrices, thus facilitating its liberation. A validated digestive model in vitro has been used in order to mimic the gastro-intestinal environment; during each step, the substrate was incubated for a specific time with simulated gastric and small intestine digestive fluids, respectively. Polyphenols percentage of release didn't show a common trend in all the samples, possibly due to the differences of the food matrices composition and/or to the interactions between different molecular environments. During the digestive phases chlorogenic acid and ferulic acid were the stablest phenolics and the highest release was ranged between 76.8-73.6% and 19.6-32.4% respectively in pinto beans. In conclusion cooking process resulted in consistent phenolic change and distribution in legumes. Along the GI tract, legumes may therefore function as a useful source of polyphenols with beneficial implications in human health.

Keywords: *polyphenols, digestion, bioaccessibility, HPLC, legumes*

D11
ANALYTICAL VALIDATION OF HPLC-DAD AND LC-ESI-MS METHODS FOR THE QUANTIFICATION OF BIOACTIVE COMPOUNDS IN GENTIANA LUTEA EXTRACTS

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Gentian root (*Gentiana lutea*) is a well-known "herbal medicine" used as bitter stomachics and sedatives listed in the European Pharmacopeia. Gentian extracts are also used in food products and in food supplements as natural flavouring or bioactive ingredients. Iridoids and secoiridoids form the largest class of naturally occurring monoterpenes, well represented in *Gentianaceae* family. The quality of Gentian root is evaluated by assessing one of its contents, gentiopicroside, a major bitter compound, but other bioactive iridoids and secoiridoids characterize the Gentian chemotypes. The biological and pharmacological activities of these compounds include cardiovascular, stomachic, choleretic and antihepatotoxic effects [1]. More recently, mounting evidence showed that bitter-tasting compounds modulate eating behaviour through bitter taste receptors in the gastrointestinal tract, leading more attention towards bitter compounds in food and botanicals [2].
Different analytical techniques were used for the separation and quantification of gentiopicroside and other bioactive compounds from Gentian (TLC, chromatographic methods, NMR), with special emphasis of hyphenated techniques involving liquid chromatography [3]. Moreover, few chromatographic methods report a complete analytical validation.
The aim of this study was to validate in parallel two chromatographic approaches (HPLC-DAD and LC-ESI-MS), leading to useful tools for the quantitative analysis of Gentian hydro-alcoholic extracts, as well as for the evaluation of the bioactive compounds in spray-dried powders. LOD, LOQ, intra-day and inter-days repeatability, accuracy (recovery) were determined in both methods; the statistical validation was obtained applying the Barlett test (homoscedasticity), the Shapiro-Wilk test (normality of residuals), Mandel and Lack-of-fit tests (linearity).
The results confirmed the usefulness of these approaches for the analysis of the Gentian samples.

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Keywords: *gentian, bioactive iridoids and secoiridoids, analytical validation, botanicals and food supplements*

Acknowledgement: *The authors sincerely thank MB med. Srl (Rivalta di Torino, Turin, Italy) for the supply of the Gentiana lutea samples.*

D12
EFFECTS OF INFRARED TREATMENT ON SAPOGENINS OF QUINOA

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In recent years, the interest about quinoa (*Chenopodium quinoa*) is highly increasing because it is a highly nutritious pseudocereal. Saponins, found in many plant genera as well as quinoa seeds, have been considered as antinutritional factors and in some cases have limited usage due to their bitter taste. Therefore, most of the earlier research on processing of saponins targeted their removal (by dehulling and/or washing of seeds) to facilitate human consumption. However, in recent years, saponins have come into renewed focus due to increasing evidence of their health benefits such as cholesterol lowering, antibacterial, antioxidant, anticancer, antidiabetic and antiobesity properties. These have resulted in the reevaluation of existing technologies for their extraction or development of new processes/processing strategies with the main purpose to optimize the yield in order to accommodate the recent need.
Nowadays, there has been an increasing trend towards the utilization of infrared (IR) in the food industry due to its advantages. It is an efficient and energy saving food processing technology due to the characteristics such as thermal efficiency, fast heating rate/ response time, wavelength and direct heat penetration into the product.
In this study, effects of infrared treatment on sapogenin content of quinoa were investigated. Infrared treatment at 735W, 1015W, 1215W, 1310W, 1425W powers was applied to quinoa. Laboratory scale infrared equipment was used in the study. The system includes a closed drying chamber fitted with two aeration channels and twelve 150W halogen lamps with a pronounced peak at approximately 1 μm. The distance between the lamp system and sample tray was adjusted to 20 cm for the study. Suitable conditions for extraction of saponin were determined in the study. Separation of sapogenins (hederagenin and oleanolic acid) was performed by injecting 10 μl of sample into HPLC equipped with a column (Supelcosil LC-18, 25 cm x 4.6 mm, 5 μm). Methanol:water (85:15) at a flow rate of 1 ml/min was used. Detection wavelength was 210 nm.
The results showed that higher sapogenin (hederagenin, oleanolic acid) can be obtained from quinoa by using suitable extraction conditions and/or infrared power. The amount of hederagenin and oleanolic acid extracted from control quinoa sample was 2.569±0.0751 mg/g dry sample ve 6.047±0.2351 mg/g dry sample, respectively. Statistically insignificant decreases and increases were observed in hederagenin content of infrared treated quinoa samples. As compared to control quinoa, increases in hederagenin and oleanolic acid content were determined in the quinoa samples infrared treated at 1310 W. The increase was significant for the oleanolic acid content of sample treated at 1310 W. Since sapogenin had health improving effects, increasing the extractability of sapogenin by using suitable extraction conditions and/or infrared power will contribute to the knowledge in literature.

Keywords: *quinoa, infrared treatment, sapogenin, extraction conditions*

Acknowledgement: *The authors wish to thank Hacettepe University, Scientific Research Projects Coordination Unit for the financial support (Project No: FHD-2016-8997).*

D13
OPTIMIZATION OF ANTHOCYANIN EXTRACTION FROM GLYCINE MAX (L.) MERR. CHEONGJA4HO USING RESPONSE SURFACE METHODOLOGY (RSM)

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This study was conducted to optimize the extraction method of total anthocyanin from black bean (*Glycine max* (L.) Merr. *Cheongja4ho*). The effect of three independent variables on total anthocyanin yield was determined by single factor experimental designs. Based on single factor analysis, response surface methodology (RSM) with central composite design (CCD) was applied with three independent variables, including the concentration of hydrochloric acid in distilled water (X_1), solid-liquid ratio (X_2), and extraction temperature (X_3). The second order polynomial model well fitted for all the response surface plots (R^2=0.97). The optimum combination was hydrochloric acid concentration of 0.39% in distilled water (X_1), solid-liquid ratio of 1:54.5 (X_2), and extraction temperature of 69.1°C (X_3). Under these conditions, 132.08 mg/100 g total anthocyanin content was observed experimentally, which was similar to the theoretical prediction of 133.18 mg/100 g. These results suggest that the estimated models were reliable and valid for anthocyanin extraction.

Keywords: *response surface methodology, Glycine max (L.) Merr., total anthocyanin*

Acknowledgement: *This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(2017R1D1A1B03028841).*

D14
TRI-P-COUMAROYLSPERMIDINE IN THE FLOWERS OF SOME SALVIA SPECIES

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Sage is a popular medicinal plant which has been well known since ancient times. Its dried leaves are applied as a spice, and extracts thereof are used for medicinal and cosmetic products. In Germany, the Bombastus Company also uses sage flower extracts (*Salvia officinalis* L.) for various applications. To distinguish the different sage extracts, it is necessary to analyze their individual chemical compositions. One characteristic compound might be established as a marker to differentiate between sage flower extracts and leaf extracts. Tri-*p*-coumaroylspermidine (Tpcs) can be detected only in the flowers of sage plants and has been described for *Salvia* species (*Salvia officinalis, S. tomentosa*, *S. nemorosa*, *S. cadmica*, *S. sclarea* and *S. lavandulifolia*) for the first time. These results confirm that spermidine conjugates are typical for these flowers [1-3]. The analytical observation of the blooming period and the flower development of *S. officinalis* showed an increasing correlation between the flowering and the Tpcs biosynthesis in the plant. Thus, Tpcs can be used as a marker to identify flowers which have been processed as alcoholic-aqueous extracts or have been included in the crushed plant material of *Salvia* species and to provide a better assessment of the optimal crop time for the flowers.

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Keywords: *salvia, flowers, tri-p-coumaroylspermidine, marker*

D15
NEW SALVICHINONE DERIVATIVES FROM THE FLOWERS OF SALVIA OFFICINALIS L.

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In Germany, the Bombastus Company uses sage flower extracts for medicinal and cosmetic applications. An ancient extraction method first described in 1778 is still in use [1]. The extraction is carried out by maceration in an ethanol-water-mixture of fresh sage flowers in big stoneware pots. After the distillation of the ethanol, the extract splits into an aqueous extract and an insoluble residue which can be obtained by decantation and subsequent drying. This residue is called "sage flower resin" and contains mainly nonpolar compounds including natural compounds and also unknown artefacts for sage in high concentrations. Compounds such as abietane diterpenes showed some interesting reactions in alcoholic-aqueous sage extracts. Similar transformations have already been described in the literature and have their origin from their contact with oxygen [2,3]. Furthermore, in this study, the oxidation cascade of abietane diterpenes could be expanded and some new compounds be isolated and elucidated, thus having been described here for the first time.
Alcoholic sage flower extracts stored under air contact result in a decreasing amount of carnosol and an increase in rosmanol, galdosol, and the newly identified compounds salvichinone A and B. Hereby, salvichinone A is able to describe the advanced oxidized state of sage extracts, including abietane diterpenes such as carnosol, thus making it suitable for use as a marker for quality proof.

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Keywords: *Salvia officinalis L., flowers, sage resin, salvichinone derivatives*

D16
EFFECT OF STORAGE ON FLAVONOID CONTENTS OF SORGHUM GENOTYPES

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Sorghum [*Sorhgum bicolor* (L.) Moench] is an important cereal in the world, which can grow under adverse environmental conditions, such as very dryness, saline and hot areas, where the production of other cereals is uneconomical. The cereal is used for food in Africa and Asia and for animal feed in most other countries. However, there is an increased interest in using sorghum for human consumption because it is a gluten-free cereal and has high levels of phenolic compounds, such as the flavonoids flavones and flavanones, which contain health benefit properties. Flavones (luteolin and apigenin) have being related to antioxidant, anti-tumor, anti-microbial and anti-inflammatory activities and flavanones (naringenin and eriodictyol) have being associated with antioxidant properties. However, these compounds are sensitive to the physico-chemical environment, thus, the storage period and conditions may lead to their degradation with antioxidant properties alteration. Despite this, information has not been found regarding the stability of the flavones and flavanones during storage. Thus, this work aims to evaluate the flavone and flavanone contents in the sorghum genotypes SC319 (flour and grain) and TX430 (flour and bran) stored for 180 days at 4, 25 and 40°C. Analyzes were performed using a high-performance liquid chromatograph equipped with a diode array detector. There was an effect of storage time which favored the flavone and flavanone reduction in the two evaluated genotypes. The storage temperature influenced the flavones more than the flavanone contents. Although there was a storage effect on flavone and flavonone levels, it were observed retentions ranged from 70.27 to 88.72% for luteolin, 69.04 to 99.15% for apigenin, 56.92 to 88.51% for naringenin and from 77.10 to 93.42% for eriodictyol at the three temperatures at the end of 180 days. The total flavones and flavanones were better preserved at 4°C (about 88% retention). At room temperature (25°C), the retention of total flavones was at least 77% and 85% for total flavanones. The apigenin of the flour and grain of the genotype SC319 was the most preserved compound, with retentions from 92 to 99%. Naringenin presented the lowest retention (from 56.92 to 71.25%) in the same materials.

Keywords: *Sorghum bicolor (L.) Moench, storage temperature, dietary flavonoids, bioactive compounds*

Acknowledgement: *The authors acknowledge FAPEMIG for the financial support and Dr Joseph Awika, from Texas A&M University (TX, USA) for the donation of the genotype TX430 samples.*

D17
OPTIMIZATION OF ANALYTICAL STRATEGY FOR DETERMINATION OF ORGANIC SELENIUM SPECIES IN SELENIUM-ENRICHED MICROSCOPIC ALGAE

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Microscopic algae, which are exposed to selenium in the form of selenite, are able to incorporate this element to its cells thus organo-selenium compounds with higher biological availability are formed. The most common compounds are amino acids selenocysteine and selenomethionine, which are occurring in a free form or incorporated into peptides. In order to understand the biological effect of selenium and its metabolism, thorough identification of its individual chemical forms is needed. Atomic absorption spectroscopy (AAS), fluorescent spectroscopy (AFS) and induction captured plasma-mass spectrometry (ICP-MS), mainly hyphenated to liquid chromatography (LC), belong among the basic techniques for determination of selenium. Another alternative is represented by LC coupled to high resolution mass spectrometry (HRMS) which provide valuable information on the molecular structure of unknown selenium compounds.

The aim of the presented study was to develop a methodology for determination of organo-selenium compounds in microscopic algae and evaluate the potential of various strains of microscopic algae to produce these compounds. Within the first part of the study, enzymatic hydrolysis optimization using *Streptomyces griseus* protease representing a critical step ensuring cell walls disintegration, was performed. Three methods of physical cell disintegration were tested on *Vischeria helvetica* algae utilizing (i) balotina (glass beads), (ii) shock freezing and (iii) ultrasonic needle of which the third option provided the best results. Additionally, to assess the bioavailability of selenium from microscopic algae, digestion under conditions simulating human gastrointestinal tract conditions, was tested.

For the analysis of targeted organo-selenium compounds (selenomethionine, selenocysteine and Se-(Methyl)selenocysteine), two instrument types differing in ionization and mass analyser, (i) high resolution tandem mass spectrometer with hybrid quadrupole-orbital ion trap arrangement (Q-Exactive Plus, Thermo Scientific) and (ii) ICP-MS (Elan DRC-e, Perkin-Elmer), were tested. Detection parameters were optimized for both instruments.

Keywords: *microscopic algae, organoselenium compounds, mass spectrometry, cell disintegration, hydrolysis*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by the Technology Agency of the Czech Republic, project No TE01020080. This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

D18
FAST GAS CHROMATOGRAPHY/MASS SPECTROMETRY (FAST GC/MS) ANALYSIS FOR MULTIPLE DETERMINATION OF CANNABINOIDS IN INDUSTRIAL HEMP INFLORESCENCES AND HEMPSEED OIL

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Recently, in relation with the therapeutic use, allowed in many countries, and the resumption of hemp cultivation for fiber and food there is an exponential increase of labs analyzing cannabinoids in *Cannabis sativa L*. However existing procedures are often inefficient, require a high analytical expertise and use large volumes of environmentally hazardous solvents. Moreover, the solvent composition, mass-to-solvent ratio, extraction technique, derivatization reactions and time vary considerably between methods. The objective of this work was to develop and validate an accurate and quick method and to propose and comment an easy Standard Operating Procedure (SOP) for analyzing cannabinoids in cannabis raw materials and finished products. A fast gas chromatography coupled to mass spectrometry (fast GC/MS) method for multiple cannabinoids analysis was developed, using a capillary GC column coated with 5% diphenyl-polysiloxane. The main cannabinoids tetrahydrocannabivarin (THCV), cannabidiol (CBD), cannabichromen (CBC), Δ8-tetrahydrocannabinol (Δ8-THC), Δ9-tetrahydrocannabinol (Δ9-THC), cannabigerol (CBG), cannabinol (CBN), cannabidiolic acid (CBDA), tetrahydrocannabinolic acid (THCA) and cannabigerolic acid (CBGA) were well separated in 3.30 min, with a good peak resolution (R>1.5), repeatability (interday <9.90%; intraday <7.22%) and sensitivity (LOD 0.2 µg/mL; LOQ 0.7 µg/mL). The Fast GC/MS method for the determination of cannabinoids was tested on industrial hemp inflorescences and hempseed oil and can be conveniently used for the analysis of numerous samples per day.

Keywords: *cannabinoids, fast GC/MS, industrial hemp, hemp inflorescences*

D19
SCREENING OF MACROALGAE TOWARDS THE PROMOTION OF A HEALTHY DIET

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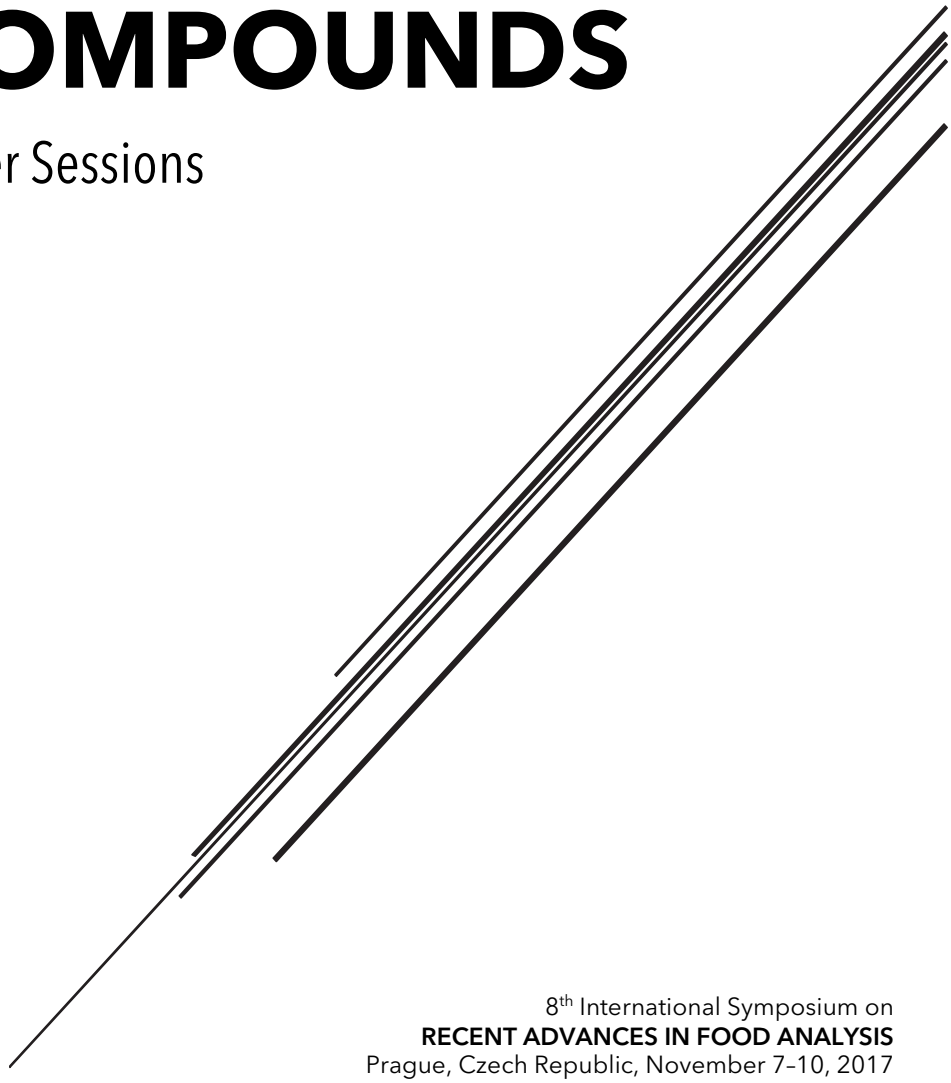
The increased life expectancy represents a major concern for public health, since aging is associated with several chronic-degenerative diseases, such as obesity, diabetes, hypertension and cancer. Hence, there is currently a great pressure for development of healthier practices, including the promotion of dietary patterns.
Macroalgae are currently included in the list of superfoods. They are a rich and balanced source of nutrients and bioactive phytochemicals and their consumption is correlated to the prevention of several diseases. Although the direct consumption of macroalgae as foods is still very insipient in Europe comparing to the Asiatic countries, food and nutraceutical industries have shown a growing interest to introduce macroalgae as ingredients of functional foods, boosting the macroalgae dietary habits in the European countries. In this context, the present work intended to evaluate the feasibility of four economically-relevant European macroalge, namely *Ulva rigida*, *Gracilaria* sp, *Fucus vesiculosus* and *Sacharina latissima*, to be used as food ingredients in functional foods or as supplements, either in the direct form or as an extract. Hence, the nutritional composition and the content of phytochemical antioxidants of the macroalgae were measured. Moreover, the extracts´ richness in nutrients, as well as their bioactive potential, including antioxidant activities and ability to inhibit key enzymes with impact in obesity and diabetes (α-amilase, α-glucosidase and pancreatic lipase) or in heart tension (angiotensin converting enzyme), were evaluated.
Overall, protein, ash and fiber contents of the focused macroalgae ranged from 9-24 % dw, 20-32 %dw, 37-45 %dw, respectively. *Gracilaria* sp revealed the highest protein content and a high ash content with a Na/K ratio of about 0.2, which is very favorable when considering cardiovascular health. On the other hand, the brown macroalgae *F. vesiculosus* stood out for its superior fiber content and levels of phenolic compounds. This last fact was reflected by the high antioxidant activity of extracts obtained from this algae (either with water or with hydro organic mixtures), in comparison to those from the other species, and probably partially associated to the ability of F. vesiculosus extracts to inhibit α-glucosidase activity.
In addition to these beneficial properties, *F. vesiculosus* aqueous extracts were shown to be a good source of minerals (ashes 28-30 % dw). Indeed, from the nutritional point of view, all the macroalgae extracts were in general very rich in minerals. This was particular relevant for *Gracilaria* sp extracts, where ash levels accounted for about 56% dw, but also for *U. rigida* (ash content of about 34-37% dw). In addition, the extracts from the last algae seemed to be very interesting in terms of soluble fiber content (34-37% dw), particularly if we have in mind that its major polysaccharides are ulvans, which are generally accepted to exert distinct bioactivities.

Keywords: *seaweeds, diabetes, hypertension, nutritional value, bioactive compounds*

Acknowledgement: *This work was financed by the Project Seaweed for Healthier Traditional Products (SHARP), Research & Development Co-promotion No. 3419, supported by European structural and investment funds (FEEI) under the Program "Portugal 2020". Thanks to FCT the European Union, QREN, FEDER, COMPETE, for funding the Organic Chemistry Research Unit (QOPNA) (project PEst-C/QUI/UI0062/2013; FCOMP-01-0124-FEDER-037296).*

FLAVOUR SIGNIFICANT COMPOUNDS

Poster Sessions



8th International Symposium on
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FLAVOUR SIGNIFICANT COMPOUNDS

E1
UTILIZATION OF FREEZEFRAME® TECHNOLOGY TO CAPTURE KEY FRESHNESS AROMA COMPOUNDS IN SAVORY FOODSTUFF

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In order to capture key freshness imparting molecules Givaudan developed FreezeFrame® technology. This technology uses liquid nitrogen to freeze the sample and preserve molecules that would normally be lost rapidly through evaporation, oxidation or enzymatic degradation, thus maintaining the integrity of the fragile freshness metabolome of the sample. While the sample defrosts under a protective nitrogen atmosphere the volatile constituents are continuously collected using refined trapping techniques and analysed via GC-MS and GC-Olfactometry. In the past this method was applied to a range of fruits enabling both fragrance and flavour creation to capture the freshest aspects of the fruits.
Current savory flavour creation tools often use aromatic essential oils to create herb or spice profiles; however, due to the production process these oils lack the volatile molecules that provide freshness. In order to create savoury flavours imparting 'freshly-chopped' freshness, the FreezeFrame® approach was applied to a range of savory foods such as basil and onion. Comparing the analysis of the basil and onion essential oils with the analysis of the headspace samples obtained using the FreezeFrame® approach and with GC-O information key differences in the aroma profile can be highlighted.
The basil FreezeFrame® headspace sample collected during the first 20 min contains many fresh, volatile monoterpenes such as myrcene, α- or β-pinene, eluting before 35 min in the GC analysis, the basil FreezeFrame® headspace sample subsequently collected between 20 and 40 min shows already a shift towards less volatile and fresh compounds and the basil oil sample is dominated by compounds eluting after 35 min such as γ-cadinene, T-cadinol (heavy woody) or cubenol (spicy herbal) and contains little of the typical green, fresh, citrus, herbaceous notes which characterize the fresh profile of basil.
Comparing the onion FreezeFrame® headspace sample with the essential oil highlights the presence of green, fresh, alliaceous notes in the headspace sample resulting from compounds such as propyl mercaptan, propyl thioacetate or methyl propyl disulfide. The oil sample shows mainly compounds eluting after 35 min missing therefore the freshness imparting compounds and is dominated by sulfurous, cooked alliaceous notes evoked by compounds such as methyl propyl trisulfide.
The better in-depth understanding of the freshness metabolome of savory foods enables flavour creation to develop fresher and more authentic profiles. Quantitative Flavour Profiling was conducted with a trained sensory panel to compare tomato soup flavoured with either a basil flavour based on essential oils or a new freshness flavour based on FreezeFrame®. This revealed clear differences in the sensory profiles. The soup containing the FreezeFrame® flavour showed significantly more 'basil fresh' and significantly less 'cooked tomato' base characteristics.

Keywords: *FreezeFrame®, freshness, basil, onion*

E2
VOLATILE FINGERPRINT BY SPME-GC-FID TO DISCRIMINATE OLIVE TREE VARIETIES INFECTED BY XYLELLA FASTIDIOSA

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Xylella fastidiosa is a Gram-negative, plant-pathogenic bacterium that causes diseases in different plant species [1]. This phytopathogen can be vectored in the foregut of sharpshooter leafhoppers, which feed on the sap of the plant xylem. This plague is causing severe damages in Italian economy whose olive/oil industry is a primary asset, and in the European Union, which is facing the first confirmed record in its territory of this pathogen [2,3].
It is noted that the VOCs emitted by plants are a very complex mixture of several hundreds of components that play an important role in trophic relations in diverse ecosystems and provide important cues for insects in their search for hosts. Moreover, these compounds may work out as direct and indirect plant defense and to attract insects for pollination [4]. For this reason, a headspace solid-phase microextraction/gas chromatography-spectrometry (HS-SPME-GC-MS) technique was proposed to highlight VOC composition differences between twigs coming from healthy and *Xf* infected olive trees.
Four different fibers (polydimethylsiloxane, Carboxen/polydimethylsiloxane divinylbenzene/Carboxen/polydimethylsiloxane, and polydimethylsiloxane/ divinylbenzene StableFlex) were tested and GC-MS conditions were evaluated in order to optimize the number of VOC detected by the proposed method. Finally, differences between samples healthy and *Xf* infected were analyzed by means of a chemometric analysis (PCA, T-test and F-test).
A total of 152 compounds were identified in the analyzed samples: 3 acids, 9 esters, 17 alcohols, 46 methyl esters, 11 other esters, 14 aldehydes, 24 hydrocarbons, 26 terpene derivates, 2 amides, 4 aromatics, 3 furanes and 5 ketones. The results obtained showed in the infected twigs the formation of new methyl esters, a lower content of carbonylic compounds (ketones and aldehydes) and a higher content of hydrocarbons, indicating new metabolites produced by the interaction host/plant that can be involved in the defensive mechanism paths of the olive tree and/or in the infective action of *Xf*. Furthermore, the statistical analysis, which highlights differences among healthy and *Xf* infected trees, has been used to set-up a quick, easy and solvent-free screening method to evaluate the presence of *Xf* in olive trees.

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Keywords: *Xylella fastidiosa, volatiles, olive tree, SPME-GC-MS, chemometric analysis*
Acknowledgement: *This work was financially supported by the Apulia Region, Research Programme "Sperimentazione Finalizzata alla Prevenzione e al Contenimento del Complesso del Disseccamento Rapido dell'olivo (CODIRO)" – Project EZIOCONTROL.*

E3
COMPARISON OF AROMA COMPOUNDS IN WHISKY BY SPME-GC×GC–TOF MS

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Over 1000 compounds from a wide range of chemical classes are known to contribute to the aroma of whisky, and these include alcohols, phenolics, fatty acids, esters, lactones, aldehydes and nitrogen-containing compounds. It is important to be able to confidently identify these volatiles, for quality control and authentication purposes, as well as in the engineering of new aromas.
Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is ideal for the analysis of such complex samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute.
This study investigates the application of flow-modulated GC×GC–TOF MS using a reverse-fill/flush device, which allows separation of volatiles ranging from C1 to C40 (and above), the flexibility to change the loop volume in method optimisation, splitting for simultaneous detection and efficient modulation of whisky volatiles that have boiling points similar to, or lower than, pentane.

Keywords: *aroma, GCxGC, whisky, flow modulation*

E4
BARRELLED EWES´ CHEESE – A PRINCIPAL INTERMEDIATE IN WINTER BRYNDZA CHEESE PRODUCTION: GAS CHROMATOGRAPHY-OLFACTOMETRY STUDY

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Bryndza cheese is a type of PGI Slovakian soft spreadable traditional cheese, produced from unpasteurized ewes´ milk in mountain regions. Diversity of microbial consortia and comprehensive qualitative and quantitative profiles of volatiles - in focus on the revelation of key odour-active compounds and quantification of their odour-activity - were studied in barrelled ewes´ cheese, which is a long-ripened intermediate product in the production of winter bryndza cheese. Eight samples of barrelled ewes´ cheese were obtained from the bryndza-producing dairy in Middle Slovakia region. Each cheese was produced from ewes´ lump cheeses from 1 to 5 mountain farms and ripened for 2-3 months in wooden barrels. Microbial cultures were studied by both culture-based and culture–independent approaches. The data obtained demonstrate that it is a product with specific microflora, dominated by lactococci, lactobacilli and *Geotrichum* spp. Although it is produced from raw ewes´ milk, the levels of potential pathogens are acceptable. A headspace solid-phase microextraction (HS-SPME) was used to isolate the volatile fractions of barrelled cheeses which were subsequently analysed by gas chromatography procedure. Odour–active compounds as principal constituents of the overall aroma were investigated applying gas chromatography-olfactometry (GC/FID-O) technique, supported by gas chromatography – mass spectrometry (GC/MS). GC-O methodology couples instrumental GC analysis with sensory detection and evaluation by olfactometry, in order to recognize odour-active compounds in complex mixtures, as the human nose is a much more sensitive detector than the conventional instrumental ones (FID or MS) for many volatiles, that are often present in products at concentrations lower than the instrumental detection limit. At this regard, the GC/FID-O revealed almost forty olfactory perceptions in samples of barrelled ewes´ cheese. Experimental data obtained highlighted that barrelled cheese is characteristic by a specific and much richer profile of odorants, different from shorter-ripened ewes´ curd cheese, potentially May bryndza cheese. We found 30 specific odour-active compounds which are characteristic exclusively for barrelled ewes´ cheese. Odorants butanoic acid, ethyl butanoate, isovaleric acid, hexanoic acid, octanoic acid, decanoic acid, methyl octanoate, ethyl hexanoate, ethyl octanoate, *p*-cresol, and δ-decalactone were principal for its overall aroma, being detected at significant intensities in all samples. On the basis of results of our study is obvious that the overall GC/MS profiles of volatiles of barrelled ewes´ cheeses were, to a certain extent, similar to the GC/MS profile of volatiles of raw ewes´ milk-based Pecorino Crotonese cheese ripened for 60-120 days. It would be interesting to compare profiles of odorants with other long-ripened ewes´ cheeses, however from methodological point of view, GC/FID-O data are necessary to facilitate comprehensive comparison.

Keywords: *cheese, microbial consortia, volatiles, odorants, gas chromatography-olfactometry*

Acknowledgement: *This work was supported by Slovak Research and Development Agency under the contracts No. APVV-14-0025 and No. APVV-15-0006.*

E5
COMPARISON OF SIMULTANEOUS DISTILLATION EXTRACTION, SOLVENT ASSISTED FLAVOR EVAPORATION AND SOLID-PHASE MICROEXTRACTION FOR THE DETERMINATION OF VOLATILE FLAVOR COMPONENTS IN BROCCOLI

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The brassica vegetables are characterized by specific aroma and taste properties, which are not always accepted by consumers. The sulfur aroma and bitterness are usually the most common reason for cruciferous rejection. Although, they are desirable in daily diet, due to the wide proven health-related values resulting from their intake. Those values are mostly correlated with the presence of isothiocyanates, products of glucosinolates hydrolysis. Isothiocyanates are presented in volatile fraction of Brassica vegetables, whereas glucosinolates are their non-volatile precursors. Broccoli is one of the most popular Brassica representative, presented in our daily diet.
The goal of this study was to compare the usefulness of three extraction methods: SPME (solid-phase microextraction), SAFE (solvent-assisted flavor evaporation) and SDE (simultaneous distillation extraction) for isolation of aroma components. Subsequent analysis was performed by gas chromatography – olfactometry (GC-O) to identify odor fractions. To identify odoriferous compounds GC-MS and GCxGC-ToFMS were used.
SAFE extract was a source of 30 potent odorant compounds, SDE extract contained 27 potent odorant compounds and SPME 21. It was difficult to point out the most suitable extraction technique. SAFE gave the most abundant number of aroma active components and SPME allowed for identification of early eluting compounds. The combination of SAFE and SPME gave full profile of aroma active compounds after GC-O analysis. However, SDE technique despite many disadvantages, allowed for identification of pyrazines. The aromas of pyrazines: green peas, earthy etc. were presented in all extracts. However, the pyrazines were not present on GC-MS or even GCxGC chromatograms of SPME and SAFE extracts. In SDE, high temperature applied during the process caused an increment of pyrazines concentration what enable to identify those components. High temperature applied in SDE also caused an increment of some aromas intensity and degradation of few aroma components. That is why it was not possible to apply SDE instead of SAFE. It seems, that all three techniques are useful in aroma-active compounds identification. In the percentage proportion, the most abundant group in all extracts were alcohols and sulfur compounds. The profiles of volatile composition were relatively similar. However, in SDE extract contained some components occurring in high temperature conditions, like higher pyrazines or dimethyl pentasulfide concentration. Presented research indicated that all applied techniques were useful for volatile isolation and identification of aroma active components in broccoli, however undoubtedly SAFE and SPME would be methods of choice for further research on key aroma components, because of their reliability and simplicity. Those techniques allowed to focus on components present in fresh broccoli, without high temperature application, which caused significant changes in volatiles composition.

Keywords: *broccoli, SPME, SDE, SAFE, GC-O, GCxGC, volatiles, aroma-active compounds*

Acknowledgement: *This work was supported by the National Science Centre (Poland) under Grant 548 2015/18/M/NZ9/00372.*

E6
EXTENDING QUANTITATIVE HEADSPACE EXTRACTION TO INCLUDE LESS VOLATILE ORGANIC COMPOUNDS IN DIFFICULT MATRICES: MEASUREMENT OF VOCS TO SVOCS IN DAIRY PRODUCTS USING VACUUM ASSISTED SORBENT EXTRACTION (VASE) AND GCMS ANALYSIS

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The headspace of common dairy products, including cheese and milk, were analyzed utilizing a new solvent-free technique called Vacuum Assisted Sorbent Extraction (VASE). Dairy products are typically challenging to analyze using headspace techniques due to the low volatility of many compounds of interest, and the high fat content, which creates a high affinity for most organic compounds to the sample matrix. Most headspace techniques do not yield much information from fatty matrices, leaving solvent extraction as the only effective technique which recovers low level flavor and odor compounds, as well as contaminants such as pesticides. VASE enables reproducible headspace extractions of VOCs to SVOCs, including low volatility compounds with minimal matrix effects, therefore increasing the number of applications compatible with headspace analysis. Heavy volatile compounds with low vapor pressures having little to no response by SPME are extracted 10-50x more efficiently with reproducible recoveries. A tube containing a solid adsorbent, called a Sorbent Pen, is placed directly into the headspace of a vial holding a liquid or solid sample. A seal is formed between the side of the Sorbent Pen and the top of the vial to create a closed system for extraction. The top of the Sorbent Pen outside of the vial contains a seal which allows the vial to be evacuated through the adsorbent immediately after insertion. Once under vacuum, the VOCs and SVOCs can diffuse into the headspace and onto the adsorbent faster than when extraction is performed at atmospheric pressure. VASE allows the sample and headspace to come to an equilibrium in a closed system, causing analytes to diffuse onto and collect at the very front of the adsorbent bed. Therefore, VASE achieves a much better recovery of heavier compounds while eliminating the common carryover issues. Heating of the sample while under vacuum further increases the extraction rates, while alternating between heating and cooling can optimize SVOC extraction efficiency as the sample is cycled through boiling and cooling repeatedly under vacuum. Heating of the sample while under vacuum further increases the extraction rates, while alternating between heating and cooling can optimize SVOC extraction efficiency as the sample is cycled through boiling and cooling repeatedly under vacuum. Running multiple extractions offline in parallel allows for high sample throughput despite the longer extraction times. Data includes chromatograms featuring the full range of VOC to SVOC aroma profiles of dairy products, and recoveries and calibration curves of organochlorine pesticides extracted from milk samples. The results show the extensive range of compounds extracted using VASE and demonstrate its potential as a routine method for solvent-free analysis of multiple-target compounds and contaminants in the difficult fat containing matrices of various dairy products.

Keywords: *food and flavor analysis, headspace analysis, thermal desorption, GCMS, vacuum assisted sorbent extraction*

Acknowledgement: *I would like to thank each of the engineers and research scientists at Entech Instruments whose innovative efforts provided milestones towards the successful development of this powerful new extraction and desorption technique.*

E7
CHEMICAL AND SENSORY PROFILES OF BRAZILIAN MERLOT WINES

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The aroma is probably the most important characteristic that, among many factors, contributes to the quality of the wine. The volatile composition of wine is the result of a technological and biochemical sequence, influenced by the varietal components, as well as by the compounds produced during fermentation and aging. Many compounds are released in the winemaking process, either by chemical and enzymatic hydrolysis or by the action of microorganisms such as yeast and lactic acid bacteria, resulting in a complex chemical composition, both qualitatively and quantitatively. This study presents data on the sensory profile and volatile composition of Brazilian Merlot wines. A method of MHS-SPME-GC-MS was used to identify and quantify 103 volatile compounds in 19 samples of commercial Merlot wines. The quantitative descriptive analysis was applied to all samples, and the sensory attributes were correlated with the quantitative chemical data to explore relationships between the chemical composition and the sensory profile of wines. The esters were the class of volatiles compounds found in the highest proportion in the analyzed samples, with emphasis on ethyl octanoate and ethyl decanoate, as the majority esters with aroma perceptible by humans. The fruity and floral character of aroma was evidenced by the descriptive analysis for all wines. Differences between samples from different regions of origin were observed, highlighting the role of the volatile varietal compounds for the classification of wines according to the origin, especially α-terpineol and β-damascenone. The fermentation process was also significant for the character of the wines, with emphasis on the maceration technique and the yeast strains used in the fermentation, for their contribution to the differences in the content of 1-hexanol, fatty acids, and esters. Substantial correlations between chemical composition and sensory attributes were also reported, with emphasis on the herbaceous character provided by 1-hexanol, isobutyric acid correlated to animal and greasy aromas and β-damascenone related to fruity and floral flavors.

Keywords: *volatile compounds, quantitative descriptive analysis, MHS-SPME-GC-MS, principal component analysis, red wine*

Acknowledgement: *The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing Prodoutoral scholarship (S. G. Arcari).*

E8
THE USE OF VAC-HSSPME-GCXGC-TOFMS FOR PHENOLS' ANALYSIS IN BEERS

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Solid-phase microextraction (SPME) is a widely used solventless extraction technique in the volatile/flavor compunds´ analysis. It doesn´t required the use of solvent, offers certain selectivity based on the fiber coating choice and high preconcentration degree for analytes, therefore is nowadays a proedominant extraction method in food volatiles analysis.Vacuum-assisted headspace solid-phase microextraction (Vac-HSSPME) is a relatively novel approach which, thanks to the extraction under vacuum conditions allow to isolate volatile compounds using shorter time and lower temperatures than with the use of standard SPME. Removing air from the sample before the fiber injection speeds up the extraction kinetics. Moreover, Vac-HSSPME is easy to perform because the only requirement is to maintain vacuum in the sample before and during extraction.
In many alcoholic beverages volatile phenols play important role in flavor formation, especially in peated whiskeys and craft beers produced from smoked malt. In this study the optimization process for the extraction and analysis of volatile pehnols in beer is shown using SPME-GCXGC-ToF with (I) DB-5 and (II) Supelcowax-10 columns combinaction. Classic approach using ambient pressure HSSPME was compared to Vac-HSSPME. Method was elaborated for 2-methyl phenol, 3-methyl phenol, 4-methyl phenol, 4-ethyl phenol, 2,4-dimethyl phenol, 4-ethyl-2-methoxy phenol, 2-methoxy-4-methyl phenol, 2-methoxy-4-propyl, 2-methoxy-4-(2-propenyl) phenol 2,3,5-trimethyl phenol and 2,4,6-trimethyl phenol.
CAR/PDMS fiber was selected for analysis performed for 30 min extraction at 60 °C, using 10ml of beer with 2g of NaCl. The calibration curbes were performed in the range of 0.01-0.5 ppm. The extraction with the use of Vac-HSSPME resulted in significantly higher peak areas than with the use of ambient pressure HSSPME in similar extraction times. The most abundant volatile phenols in smoked beers were trimethyl phenols, 4-methylguaiacol and 4-propyl guaiacol.

Keywords: *phenols, beer, aroma, vacuum-assisted headspace solid-phase microextraction, GCxGC-ToFMS*

E9
RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY FOR HIGH THROUGHPUT SCREENING IN FOOD ANALYSIS: THE CASE OF BOAR TAINT

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Increasing awareness on animal welfare has led to a European Treaty announcing a voluntary ban on the surgical castration of piglets by 2018. One viable alternative for the surgical castration is the raise of entire males. However, the main setback of the latter is the possible occurrence of boar taint, an off-odour caused by the release of androstenone (AEON), skatole (SK) and indole (IND) when meat of boars is heated. In order to prevent adverse consumer reactions, there is an urgent need for rapid methods allowing the detection of boar taint at the slaughter line.
In this study, rapid evaporative ionization mass spectrometry (REIMS) was used as a new emerging technique to develop a predictive model for accurate high-throughput identification of boar taint in pig adipose tissue. A first of its kind step towards achieving at-line classification of boar carcasses. Pig adipose tissue was sampled using the iKnife handheld sampling device, which was connected directly to a Xevo G2-XS Q-TOF mass spectrometer equipped with REIMS source. Untargeted mass spectrometric profiling in both negative and positive ionisation mode of pig neck fat samples enabled the construction of a predictive model for the classification of boar carcasses in boar taint positive or negative groups. The predictive model showed high accuracy (93%) and a very low false positive and false negative rate (alpha and beta-error ≤ 5%), demonstrating the potential and applicability of this model on pig carcasses. Moreover, as the REIMS technique delivers a result within 10 seconds from sampling to result, this technique guarantees point-of-control analysis, which represents a major step forward in high-throughput screening of aberrant pig carcasses.

Keywords: *boar taint, rapid evaporative mass spectrometry, meat quality, chemometrics, flavour /odour profile*

E10
PHENOLIC AND VOLATILE COMPOSITION OF PORTUGUESE EXTRA VIRGIN OLIVE OILS

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Nowadays, for olive oil technologists improving sensory properties of extra virgin olive oil are the main goal, as this can be one of the choice drivers from the consumer point of view. To study the sensory characteristics, it is important to know the compounds that have impact on flavour. Volatile and phenolic compounds are the main compounds that affect the aroma and taste of olive oil. Apart sensorial characteristics, the presence of different bioactive compounds in virgin olive oil influences its nutritional and oxidative stability. The recent authorization of the European Food Safety Authority (EFSA) for health claims on olive oil labelling can be a future important issue both from the consumer and industrial points of view. The aim of this study was to evaluate volatile and phenolic compounds of olive oils produced from the most important cultivars for olive oil in Portugal, 'Cobrançosa' and 'Galega Vulgar', harvested in early ripening stages. Volatile compounds were identified by GC×GC-ToF-MS and phenolic compounds by HPLC-DAD. Pattern recognition techniques (Principal Component Analysis, PCA, Cluster Analysis, CA, and Discriminant Analysis, DA) were used for multivariate data analysis. Olive oils characterized by their fatty acid composition were grouped by cultivar. Under optimized conditions, 22 volatile compounds were quantified in all samples. *(E)*-2-hexenal was the most abundant of the quantified compounds. Both (E)-2-hexenal and hexanal, are the main compounds responsible for the green attributes of 'Galega Vulgar' and 'Cobrançosa' olive oils. Secoridoid derivatives that have significant biological activities, like oleacein (3,4-DHPEA-EDA) represent the highest percentage of phenol compounds in both olive oils. 'Cobrançosa' olive oil had higher contents of flavonoids. The ripening stage of fruits showed to be a key factor on the amount and profile of bioactive and volatile compounds of olive oil.

Keywords: *olive oil, odorants, secoridoid derivatives*

Acknowledgement: *The authors thank to the Portuguese national funding of FCT - Fundação para a Ciência e a Tecnologia, to the research unit of LEAF (UID/AGR/04129/2013).*

E11
FLAVOUR PROFILING OF MILK USING HIGH-CAPACITY SORPTIVE EXTRACTION AND TD–GC×GC–TOF MS

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Milk, as a liquid product produced on a vast scale, is highly susceptible to contamination – from chemicals used in agriculture, from animal feed, or from the transport, processing and packaging processes. Reliable analysis of the volatile components of milk is therefore valuable to ensure high quality.
Traditionally, solid phase micro-extraction (SPME) has been used for flavour profiling. It is a fast and simple technique, but can be limited in terms of sample capacity, reproducibility and sensitivity.
Here we utilise high-capacity sorptive extraction for immersive sampling of milk. The high-capacity PDMS phase results in higher sample loadings than SPME methods, and combined with pre-concentration by thermal desorption (TD) offers greater sensitivity across a wider analyte range.
Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) is ideal for the analysis of such complex aroma samples, because the enhanced separation capacity allows analysts to screen the entire composition in a single analysis, with confident identification of compounds that would ordinarily co-elute.
This study investigates the application of flow-modulated GC×GC–TOF MS (using reverse-fill/flush dynamics) to ensure that even the most volatile components could be reported from a range of milk samples.

Keywords: *GCMS, SPME, milk, flavour*

E12
MONITORING THE EFFECT OF POST-HARVEST STORAGE ON FRUIT QUALITY BY TD-GC×GC-TOF MS

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The fruit quality (FRUITY) project aims to provide a better understanding of post-harvest storage conditions of fruit, to allow improved sensorial and internal quality of fruit throughout the supply chain.
The project uses a multi-trait approach - including sensory profiling, monitoring of the volatile organic compounds (VOCs) produced by the fruit and investigation of biochemical reactions - with the overall goal of providing a suite of simple diagnostic checks to monitor fruit quality.
In this presentation, we will focus on the VOC bouquets from peach cultivars in an attempt to identify molecular markers for objective quality assessment.
Thermal desorption (TD) enables rapid and robust in-situ sampling of VOCs, on to sorbent tubes that can be subsequently capped for safe transport to the laboratory for analysis. Here, we use comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC–TOF MS) to provide enhanced separation of these complex samples. The pre-concentration effect of TD, combined with improved separation and highly-sensitive detection by GC×GC-TOF MS provides a comprehensive chemical fingerprint in a single analytical run. The VOC profiles at the time of harvest and after storage at low temperature will be compared and correlated with results from sensory evaluation.

Keywords: *fruit quality, GCxGC, thermal desorption, flow modulation, volatiles*

E13
FLAVORS, ODORS, AND CONTAMINANTS IN ALCOHOLIC BEVERAGES USING VACUUM ASSISTED SORBENT EXTRACTION (VASE) AND GC/MS ANALYSIS

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Analysis of flavor compounds in alcoholic beverages presents challenges for extraction, separation, and quantitation. This is not only due to the high amount of ethanol, but also the low odor thresholds and very low concentrations of odor causing compounds in final products. Matrix affinities for volatile compounds are high, and matrix interferences may saturate adsorbents. Additionally, aromas may represent multiple compounds of different volatility, polarity, and chemical properties, which often dictate the extraction technique. Most current extraction procedures have several disadvantages including poor quantitation due to low recoveries, matrix inferences, or carryover. A new technique for analyzing the full range of volatile to semi-volatile organic compounds in the headspace of alcoholic beverages using Vacuum Assisted Sorbent Extraction (VASE) paired with GCMS is presented. VASE greatly improves upon headspace analysis of samples by both enhancing extraction efficiency and reducing matrix interferences. Application of VASE to alcoholic beverages allows reliable extraction of aromas, off-flavor compounds, and contaminants with minimal ethanol-induced matrix effects due to the high phase ratio and surface area of the adsorbent. VASE is a powerful extraction technique which places a sample vial under vacuum in the presence of a 70mg adsorbent cartridge (Sorbent Pen) to effect near exhaustive extractions of GC compatible compounds. Sample preparation for GCMS injection is performed by placing samples into 20-125 mL vials with liners creating a seal with the Sorbent Pen. A vacuum is created through a micro seal at the top of the Sorbent Pen. After the vacuum source is removed, the sample vial remains under vacuum, causing VOCs to transfer to the adsorbent faster than at atmospheric or higher pressures. The vacuum extraction process may continue for 30 min-4 8hrs at 4-100°C. Sample injection is performed with a thermal desorption unit fitted into an open GC injection port, which brings the Sorbent Pen within an inch of the analytical column, eliminating transfer losses. Absence of dynamic purging keeps the extracted compounds near the front of the adsorbent for rapid injection onto the column resulting in sharp peaks without refocusing. Results from several high-alcohol containing beverages will be presented. Wine has been analyzed for aromas, and for trihaloanisole quantitation to part per quadrillion levels using single quad GCMS systems. Cognac and whiskey have been studied to provide a quantitative method to monitor phthalate levels for regulatory compliance. Variations in aroma profiles are shown for several light, dark, and aged rums. Data reveals both the reproducibility and lack of carryover which results from thermal desorption of the extracted sample compounds onto the front of the adsorbent bed under static vacuum conditions where headspace chemicals are not driven deeper into the adsorbent as seen with dynamic headspace sampling.

Keywords: *vacuum assisted sorbent extraction, headspace analysis, thermal desorption, GCMS, food and flavor analysis*

Acknowledgement: *I would like to thank each of the engineers and research scientists at Entech Instruments whose innovative efforts provided milestones towards the successful development of this powerful new extraction and desorption technique.*

E14
CHEMICAL PROFILE OF VOLATILE COMPOUNDS FROM CAMEROONIAN HONEYS

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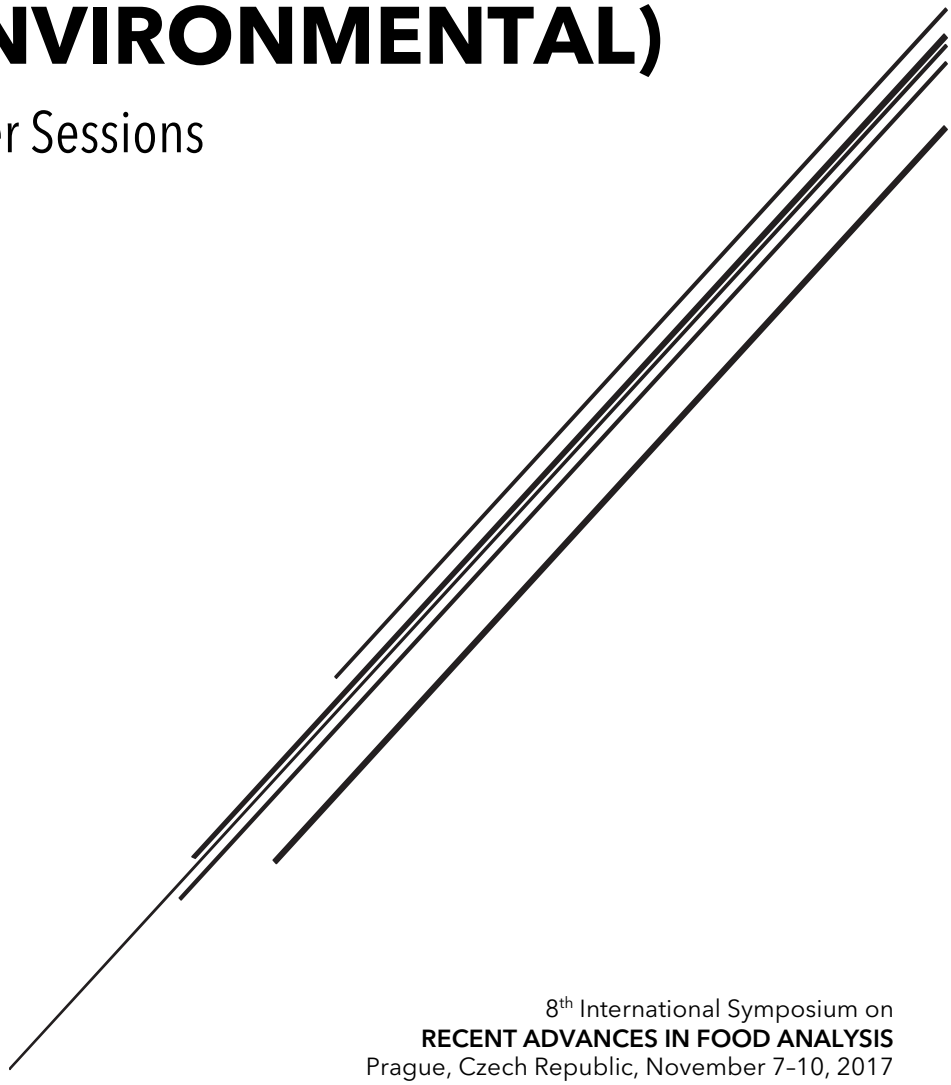
Honey is natural food product made by honeybee (*Apis melifera*) from plant, honeydew or both. It is used not only in food industry but also is popular product in the cosmetics as well as medical industry. Furthermore, consumer interest in this product is constantly increasing. In the market is a wide range of diverse types of honeys with different botanical and geographical origin. Each of them is characterized by unique flavor, nutritional values and therapeutic effects. The chemical composition of honey is variable and strongly depend on huge number of varied factors, such as bee species, climate, soil characteristic, botanical and geographical origin, age of honey, storage methods and honey processing (harvest technology and condition). There is also observed increasing interest of unusual honeys from uncommon geographical origin on international market. Among them are also honeys from Cameroon or other African countries.
The purpose of presented study was evaluation of volatiles chemical content of Cameroonian honeys from different regions of Cameroon. Because of huge ecological diversity of this country chemical composition, honey colour, taste and consistency are strongly dependent from the harvesting region e.g. in the centre there is humid topical lowland rainforest while on the south and east there is savannah. For extraction of volatile compounds three different methods of extraction were applied. Each extract was analysed by GC-MS and for each honey chemical profile of volatile compounds was create. Moreover, HPTLC method was applied for constructing something like code-bars, which were useful for differentiate honeys from different region of Cameroon. Obtained results show that combination of GC-MS and HPTLC analysis create a specific fingerprint of honeys with different botanical origin and that those methods are powerful techniques for differentiation origin of honeys.

Keywords: *volatile compounds, chemical profiles, HPTLC, Cameroonian honeys*

Acknowledgement: *This study was carried out with financial support from the National Science Centre in Poland project No. 2014/15/B/NZ9/02182 "Chemical markers of unifloral honeys".*

FOOD CONTAMINANTS (ENVIRONMENTAL)

Poster Sessions



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FOOD CONTAMINANTS (ENVIRONMENTAL)

F1
OPTIMIZATION OF A METHOD FOR SHORT-CHAIN CHLORINATED PARAFFINS (SCCPS) DETERMINATION IN BIVALVE MOLLUSK SAMPLES

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Short-chain chlorinated paraffins (SCCPs) are a complex mixture of halogenated contaminants carbon chain lengths from 10 to 13. Technical mixtures are used as extreme pressure additives in lubricants, flame retardants in plastics and paints, plasticizers in coatings, sealants and adhesives and in leather fat liquors.
Several studies have shown the extensive presence of SCCPs in several marine compartments however there is little information regarding bivalve mollusks limited to coastal waters in the Chinese Bohai Sea [1]. Bivalve mollusk accumulate POPs very efficiently, hence they are used as bioindicator in marine environment.
In this work, a method for the determination of short-chain chlorinated paraffins (SCCPs) in bivalve mollusc is optimized. Because of the high number of variables that potentially affect paraffins determination, a Plackett-Burman 2^7 factorial design (Minitab 16 statistical package) was applied. Seven experimental extraction and purification variables were optimized (sample amount, extractant volume, boiling and rinsing times, basic alumina and acidic silica gel amounts and purification solvent volume).
Freeze-dried mussels spiked with a mixture of SPCCPs were extracted in a Soxtec™ 2055 System (FOSS Analytical AB, Höganäs, Sweden) with *n*-hexane:dichloromethane (1:1). Soxtec extracts were purified in a multilayered column that was packed with hydrated basic alumina (6%), activated silica, acidic silica and anhydrous sodium sulphate. The target analytes were further recovered with *n*-hexane:dichloromethane (1:1). Extracts have been analyzed by gas chromatography coupled to tandem mass spectrometry GC-QqQ-MS/MS (EI).
The factorial design results indicate that sample amount, extractant solvent, rinsing time and acidic silica amount had a statistical significance on extraction efficiency of SCCPs. The most significant factor was the sample amount (p=0.006) followed by the rinsing time (p=0.015) and extractant volume (p=0.023), these one with positive sign. The acidic silica gel amount (p=0.043) was the least significant and with negative sign. Further experiments were conducted to fine tune variables and shifted in the direction imposed by factorial design. The optimal conditions were, sample amount 4 g, extractant solvent 50 mL, boiling time 2 h, rinsing time 1.5 h, basic alumina amount 8 g, acidic silica gel amount 4 g (15%) and purification volume 50 mL. Validation of method was performed in terms of quantification limit, recovery and reproducibility. The optimized method was applied to analysis of real bivalve mollusk samples collected in Galicia.

[1] Yuan, B., Wang, Th., Zhu, N.L., Zhang, K.G., Zeng, L.X., Fu, J.J., Wang, Y.W., Jiang, G.B. 2012. Sea. Environ. Sci. Technol. 46. 6489-6491.

Keywords: *SCCPs, GC-QqQ-MS/MS, bivalve, mollusk*

Acknowledgement: *This work was financially support by Plan Estatal de Investigación Científica y Técnica y de Innovación Project (CTM2014-56628-C3-2-R).*

F2
DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS WITH LC-LC-GC-MS/MS

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The polycyclic aromatic hydrocarbons (PAHs) are ubiquitously present. This substance group contains up to 250 different compounds. The US environmental agency EPA (Enviromental Protection Agency) distinguishes "light PAH" and "heavy PAH" (with five or more rings). There are different PAH priority lists: the US EPA list (16 PAH) complaints primarily the occurrence in the environment. The EFSA list of the EU is aimed to PAHs in food only taking into account their carcinogenic or genotoxic effects. All manufacturing and treatment processes, in which foodstuffs are strongly heated or come into contact with combustion gases or fumes, can lead to higher PAH contents of the products. These include drying oilseeds or open-fired cereals, baking bread, roasting coffee, frying and smoking and grilling meat over an open fire. High levels of PAH (in particular benzo(a)pyrene) were occasionally detected in black or hot smoked meat. The acute toxicity of the PAH is low. The potential hazard is primarily due to the carcinogenic properties of some representatives of this substance group. Some of these substances are classified as likely to be cancerous for humans. Benzo(a)pyrene, the most known and best studied substance, is used as a guiding substance for the health assessment. Among PAH 4, benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene are summed together. These are very suitable as indicators, since they can be detected in almost all foods. Regulation (EC) No 835/2011 lays down maximum amounts for benzo(a)pyrene and for PAK4 for the particular foodstuffs.
The analysis of PAH in complex foods often indicate a complicated and also work-intensive sample preparation. Chromatographic interferences are often the case. The determination by LC-LC-GC-MS requires only a simple and fast sample preparation. The preparation consists merely of an extraction and saponification. The further cleanup is automated by coupling two HPLC columns. The sample extracts are purified by HPLC (removing triglycerides and biogenic interfering substances), fractionated and the fraction with the PAH is transferred into the gas chromatograph. Subsequent gas chromatographic separation is carried out with a selection PAH column, detection using a mass-selective detector (MS/MS) [1]. The Chronos software also optimizes sample preparation by interlacing time. The LC cleanup does not start after the analysis of a previous sample has been completed, it starts the new sample while the analytic at the GC is still running. This significantly reduces waiting times for analytical systems. This automatization reaches correct and accurate results and also minimized cross-contamination due to an intensive sample cleanup.

[1] Nestola, M., Friedrich R., Bluhme P., *Anal. Chem.*, 2015, 87 (12), pp 6195–6203, "Universal Route to Polycyclic Aromatic Hydrocarbon Analysis in Foodstuff: Two-Dimensional Heart-Cut Liquid Chromatography-Gas-Chromatography-Mass Spectrometry"

Keywords: *food contaminants, polycyclic aromatic hydrocarbons, PAH4, environmental contamination, LC-LC-GC-MS*

F3
ANALYSIS OF THE BIOACCUMULATION AND BIOTRANSFORMATION OF ORGANOPHOSPHORUS ESTERS IN WHEAT (TRITICUM AESTIVUM L.)

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Organophosphate esters (OPEs) are widely used as flame retardants and plasticizers in a variety of industrial applications and household products, particularly after the implementation of regulations phasing out the production and usage of polybrominated diphenyl ethers in many countries in recent years. Due to the fact that OPEs are mainly used as non-reactive additives in these products and are liable to leach out to the environment, their behaviors in the environment have attracted increasing attention from both the public and researchers.
Plant uptake and accumulation may provide the potential for OPEs to transfer into the food chain and cause a threat to human health. However, to date, the study of plant uptake of OPEs is scarce. Biotransformation is an important process for organic contaminants in the environment, which may bring additional adverse influences to bear on the environment and human health due to the fact that the biological effects of metabolites may be different compared with precursor compounds. Therefore, the uptake, translocation and biotransformation of organophosphate esters (OPEs) by wheat (*Triticum aestivum* L.) were investigated by a hydroponic experiment. The results demonstrated that OPEs with higher hydrophobicity were more easily taken up by roots, and OPEs with lower hydrophobicity were more liable to be translocated acropetally. A total of 43 metabolites including dealkylated, oxidatively dechlorinated, hydroxylated, methoxylated, and glutathione- and glucuronide- conjugated products were detected derived from eight OPEs, with di-esters formed by direct dealkylation from the parent tri-esters as the major products, followed with hydroxylated tri-esters. Molecular interactions of OPEs with plant biomacromolecules were further characterized by homology modeling combined with molecular docking. OPEs with higher hydrophobicity were more liable to bind with *Ta*LTP1.1, the most important wheat non-specific lipid transfer protein, consistent with the experimental observation that OPEs with higher hydrophobicity were more easily taken up by wheat roots. Characterization of molecular interactions between OPEs and wheat enzymes suggested that OPEs were selectively bound to *Ta*GST4-4 and CYP71C6v1 with different binding affinities, which determined their abilities to be metabolized and metabolite products in wheat. This study for the first time provides both experimental and theoretical evidence for the uptake, accumulation and biotransformation of OPEs in plants.

Acknowledgement: *This work was funded by the National Natural Science Foundation of China (Projects 21537005 and 21621064) and the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant XDB14020202).*

F4
META-ANALYSIS OF COMBINED TOXICITY DATA FOR HUMAN AND ECOLOGICAL RISK ASSESSMENT OF MULTIPLE CHEMICALS: CASE STUDIES TO SUPPORT METHODOLOGICAL DEVELOPMENT

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Human and ecological risk assessment of combined exposure to multiple chemicals "chemical mixtures" is a challenging topic for scientists, risk assessors and risk managers. A key aspect of this challenge relates to the potential substantial number of chemicals involved and the toxicological profiles and exposure patterns of these chemicals in humans and species present in the environment. This research effort aims to compute and analyse scientific evidence dealing with combined toxicity of multiple chemicals to support the development of harmonised methodologies for human and ecological risk assessment. Extensive literature searches were performed to compute relevant toxicity data for species of human (e.g. rat, mouse, dog and rabbit) and ecological interest and ecological (fish, rainbow trout, zebrafish) into excel databases. Combined toxicity data for binary mixtures were analysed for each study to quantify magnitudes of interaction, as the mean effect ratio between single compounds and binary mixtures following acute, subacute, sub-chronic or chronic exposure. These meta-analyses allow, 1. the transparent reporting of measured toxicological endpoints using a weight of evidence approach, 2. testing the dose dependency of toxicological interactions and 3. illustrate how meta-analysis can support hazard characterisation of combined toxicity of multiple chemicals. Following the preliminary analysis of the case studies available, further work is proposed including the development of predictive models using the combined toxicity databases, dose-response modelling and toxicokinetic data for species of human and ecological relevance.

Keywords: *risk assessment, human health, ecological health, combined exposure, multiple chemicals*

F5
DETERMINATION OF PFAS IN FISH BY HPLC-QTOF

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Perfluorinated compounds (PFC) are a large family of industrial chemicals (over 3000) which contain fluorine atoms in carbon chains. Due to their high stability and useful properties PFC are widely used in paints and coatings, commercial cleaning materials, as compounds of degreasing and polishing mixtures, also in water repelling agents, fire-fighting foam and fluids, etc. PFC are persistent contaminants able to bioaccumulate and, therefore, they are detected in human serum, tissues, breast milk. At present, the world production of PFC is reduced, due to information about their potential health risks, including risks of cancer promotion. Selected PFC groups are included in the Annex B of Stockholm Convention on Persistent Organic Pollutants (POPs).
For the determination of 19 compounds from PFOA and PFOS groups in fish samples, the following approach was implemented in our laboratory: the analytes are extracted from tissue samples with methanol on vortex type of extractor for 30 minutes. The extract is sonicated for 30 minutes, centrifuged at 4750 rpm, and filtered through 0.2 µm syringe filter. After 5-fold dilution, the extract is purified by SPE on WAX cartridge. The cartridge was conditioned with water and methanol, washed by 1% formic acid; the elution step was done by 0.5 % aqueous ammonium hydroxide.
HPLC separation was carried out on ACQUITY BEH column (1x 100, 1.7 µm) in gradient mode. Detection was performed on Xevo G2 QTOF mass-spectrometer in negative ionization mode. The retention times for all the compounds were between 5.71 and 9.25 min. The following compounds were analyzed using this method: PFBS, PFPeS, PFHxS, PFOS, PFNS, PFDS, PFDoS, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDa, PFHxDA, PFODA. The described method has a LOD of 0.2 µg/kg.

Keywords: *PFOA, PFOS, fish sample preparation*

F6
THE ANALYSIS OF ENVIRONMENTAL SAMPLES USING HIGH RESOLUTION MASS SPECTROMETRY TO IDENTIFY NOVEL PFAS COMPOUNDS

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Introduction
There are a very wide number of PFAS compounds that are actually present in environmental samples that are not part of the current EPA methods for PFAS analysis. The goal of this work was to use a QTOF mass spectrometer with Non Target Screening workflow to locate and identify unique PFAS compounds that were not known to be in the sample.
Methods
Water samples were analyzed using a Sciex X500R QTOF system set up to acquire data in a Non Target Screening workflow. The instrument first performed a TOF scan from 100-1500. This scan was follwed by a data dependent scan where ions above a set threshold were selected for fragmentation and acquisition of a full scan MS/MS spectrum. The resulting MS/MS spectra were searched against a high resolution library to identify potential PFAS of compoundscern present in the samples.
Preliminary Data
Perfluoroalkyl substances (PFASs) encompass a range of fully fluorinated alkyl compounds and are prevalent in Aqueous Film Fire Foam (AFFF). In addition, PFASs are ubiquitous as they are used in many household goods and have been found in various environmental and biological samples. Here, we demonstrate the use of QTOF technology to exploit the power of high resolution mass spectrometry and the use of product ion spectra to identify novel compounds in AFFF contaminated samples. The use of library searching to identify unique and unexpected compounds in these samples will be presented.
Novel Aspect
Novel PFAS compounds are identified using high resolution mass spectrometry.

Keywords: *PFAS, LCMS, environment*

F7
THE ANALYSIS OF HALOGENATED PERSISTENT ORGANIC POLLUTANTS USING GC-Q-ORBITRAP: A STEP FORWARD

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HRMS hybrid instruments, such as quadrupole-Orbitrap, are being introduced as analyzing systems in food safety control laboratories. This instrumentation is a very valuable tool for complex analysis, but also, as a second stage, to confirm doubtful results obtained with low resolution MS/MS.
Since 2011, the Laboratori de l'Agència de Salut Pública de Barcelona has been using HRMS in more than 15 internal procedures for the analysis of veterinary drugs, pesticides, marine biotoxins and organic pollutants, which involve the analysis of around 3000 samples / year, using LC-HRMS.
In December 2015, with the introduction of the GC-Q-Orbitrap, we have focused on the analysis of halogenated persistent organic pollutants at extremely low levels, as well as on the effort to resolve matrix interferences in complex samples with the help of this new system.
In the present work, some examples showing how the GC-Q-Orbitrap can help analyzing halogenated persistent organic pollutants will be discussed:
Non-dioxin-like-PCBs: a new recent Regulation requires lowering the limits for the official control of the levels of non-dioxin-like PCBs in certain foodstuffs. New methodologies should be optimized to fulfill these requirements.
Polybrominated diphenyl ethers: very low limits (0.01 ng/g) should be achieved as described in the European Recommendation (2014/118/EU), to report monitoring data to EFSA in order to monitor trends in exposure.
Polychlorinated naphthalenes: there is scarce data reported on polychlorinated naphthalenes congeners, although they are in the Stockholm Convention list. There has been an increasing interest of these compounds, due to the toxicological studies and they are likely to be regulated.
Outstanding results have been obtained, but some critical issues, during the method development had to be addressed: different acquisition modes and instrumental parameters have been assessed, in order to establish an internal standard procedure for the analysis of these compounds in different food commodities.

Keywords: *HRMS, GC orbitrap, food contaminants*

F8
CHARACTERISATION OF CHLORINATED PARAFFIN PROFILES BY LC-ESI(-)-HRMS AND SEMI-AUTOMATIC POST-ACQUISITION DATA TREATMENT

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Chlorinated paraffins (CPs) are a family of high production volume chemicals, with applications as additives in cutting oil and lubricants as well as plasticizers and flame retardants. Technical mixtures consist in a range of homologue groups exhibiting different chemical formulas ($C_nH_{2n+2-x}Cl_x$), each one comprising an undefined number of isomers. It is usual to distinguish short-chain (SCCPs, C_{10}–C_{13}) from medium-chain (MCCPs, C_{14}–C_{17}) and long-chain (LCCPs, $C_{\geq 18}$) CPs.
From both environmental and human health perspectives, CPs are of growing concern. Particularly, SCCPs are currently candidate substances to be listed in the Stockholm Convention. For efficient risk assessment, data regarding their environmental occurrence, fate, toxicity and presence in human food chain are urgently needed.
From an analytical point of view, CP determination represents a challenge due to the complexity of their mixtures. So far, no technique succeeded in resolving isomers. Generally, gas chromatography coupled to low resolution mass spectrometry with electron capture negative ionization has been applied to estimate mean chlorination degree of mixtures, with important limitations in terms of interferences and response factors. Recently, high resolution mass spectrometry (HRMS) was combined with atmospheric pressure chemical ionization under chlorine-enhanced conditions in the direct introduction mode to address the issue of interferences between homologue groups. Some limitations related to the resolution (R=10,000) were however reported. Postacquisition procedures were also introduced in order to (i) counterbalance the absence of chromatographic separation based on a pattern deconvolution and (ii) elucidate the closest linear combination of standard mixtures to CP patterns in environmental sample.
In the present work, we propose an alternative approach combining (i) liquid chromatography (LC) separation, (ii) electrospray ionization (ESI) with the detection of acetate adduct ions and (iii) HRMS on an Orbitrap technology mass analyser set at a mass resolution of 140,000 (at *m/z* 200) for data acquisition. Regarding post-acquisition data treatment, we describe the fast automatic integration of signals from 338 homologue groups in the open source R environment and apply the procedure developed by Bogdal *et al.* (*Anal. Chem. 2015, 87, 2852-2860*) to elucidate the closest linear combination of standard mixtures. The method was applied to environmental samples as a proof of concept with expected perspectives for an application to food samples.

Keywords: *chlorinated paraffin, LC-HRMS, suspect screening, R environment*

F9
EFFECTS OF PAHS EXPOSURE ON LIPID METABOLISM OF MICE

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The fine particulate matter (PM2.5) with the peculiarities of low-dose, persistent, and multi-path has become an increasing concern due to its harmful for health. Polycyclic aromatic hydrocarbons (PAHs) are among the PM2.5 constituents of greatest concern, which can enter deep into the respiratory system, and eventually cross the air-blood barrier, leading to the dysrugulation of lipid metabolism, thus linked with heart or lung diseases. Herein, we investigated the lipid changes of mice liver tissue exposed to benzo[a]pyrene (B[a]P) using a non-targeted lipidomics platform of RPLC-Qtrap-MS. Male and female C57BL/6 mice in exposure group mice were exposed to B[a]P of in corn oil by intratracheal instillation for 1d, 3d, 7d, and 21d. The equivalent volume of corn oil was instilled intratracheally in mice of control groups. The lipids of liver tissue were extracted with the traditional MTBE method and analyzed by ultra-performance liquid chromatography coupled with a Q-Exactive Orbitrap mass spectrometer. Raw data was performed using the Progenesis QI software. Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed on the normalized data sets for all detected lipids. Different days of exposure treatments resulted in more or less different alterations of lipids profiles in liver samples from mice. As a result, there are 3 and 21 differential lipids detected in HESI positive & negative ion mode respectively. The volcano plot showed that almost all the differential lipids displaying a rising trend in three B[a]P exposure groups compared with the control, nevertheless when exposed for 21 days, the lipids were back to the original state. These inherent biological variations in lipids could at least partially underlie the self-regulation of mice after intervention by B[a]P exposure.

Keywords: *lipid metabolism, exposomics, particulate matter, benzo[a]pyrene, intratracheal instillation*

Acknowledgement: *National Natural Science Foundation of China (No. 91543105)*

F10
LEVELS OF PCDD/F AND PCB IN SWEDISH MARKET BASKET STUDY

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Since 1999 the National Food Agency in Sweden has performed several market basket surveys analyzing sampled food for the content of nutrients and toxic compounds. The content of the baskets was based on per-capita-consumption data.
This latest market basket study from 2015 contained five different food baskets collected from five different grocery chains in Uppsala, each basket contained about 250 food items.
The foodstuff in the baskets was divided into 12 categories based mainly on the Swedish Food circle and in some cases divided further into subgroups. Dairy was for example divided into two subgroups, fluids and solids due to prior problems with homogenization.
The persistent organic pollutants polychlorinated biphenyls (PCBs), dibenso-p-dioxins (PCDDs) and dibensofurans (PCDFs) were analyzed in food groups of animal origin and/or a high fat content. In total 32 samples was analyzed for PCBs and PCDD/PCDFs in eggs, fats/oils, fish/fish products (fresh and cooked), meat/meat products and dairy products (liquid and solid).
For all samples except the liquid dairy sample, pressurized liquid extraction (PLE) was preformed using a system from Fluid Management Systems (MA, USA). The liquid dairy sample was extracted by liquid-liquid extraction. The fat content was determined gravimetrically. Removal of the fat and fractionation of the samples was performed on a PowerPrep™-system from Fluid Management Systems (MA, USA). Finally, the samples were analyzed on a GC-HRMS (Agilent Technologies 7980 GC and an AutoSpec Premier, Waters) with isotopic dilution technique. All samples were fortified with ^{13}C-labled standards before analysis.
Generally low levels of the compounds were found. The highest concentrations of PCDD/F and PCBs were found in fish. High concentration of dioxinlike PCBs was also found in some of the egg samples.
Earlier studies have shown that the intake of the PCB congener CB153 is correlated with the total PCB intake and this congener was therefore used as a marker for total PCB in this study. Fish is the foodstuff that contributes most to the total intake. Fish constituted more than 60% (CB153) or 40% (PCDD/F/PCB TEQ) of the total intake. Eggs contributed almost nothing to the CB 153 intake but the contribution was more than 15% for the intake of PCDD/F/PCBs.
The median total intake for PCBs was estimated to 3.7 ng/kg body weight/day and for PCDD/Fs 0.47 pg TEQ/kg body weight/day. The results from the market basket surveys show that the intake of PCB and PCDD/F has decreased since 1999.

Keywords: *PCBs, PCDD/Fs, dioxins*

F11
THE ASSESSMENT OF HUMAN DIETARY EXPOSURE TO ORGANIC CONTAMINANTS BASED ON THE TOTAL DAILY DIET STUDY

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Humans are continuously exposed (via inhalation, ingestion and dermal contact) to effects of environmental pollutants where the intake of contaminated food is one of the most important sources of compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), perfluoroalkylated substances (PFASs) and brominated flame retardants (BFRs). Many of them may accumulate in the body what can lead to negative effects on organisms especially during long-term exposure. This study is focused on the determination of all above mentioned groups of contaminants (n=100) in the total daily diet. Within the summer 2017, duplicates of the total diets (n=20) were obtained from colleagues from our laboratories.
The aim of the study was to analyze 20 samples of total daily diets for the occurrence of PCBs (n=8), OCPs (n=13), PAHs (n=24) and BFRs (n=22). For the isolation of nonpolar compounds such as PCBs, OCPs, PAHs and BFRs, the extraction method using shaking with ethyl acetate, water and salts ($MgSO_4$ and NaCl) was applied. As a purification step, solid-phase extraction using a silica column was used. The isolation of more polar compounds represented primarily by PFASs and some BFRs was performed by the modified QuEChERS method using acetonitrile. Within the purification of an organic extract, dispersive solid phase extraction using different sorbents such as Z-sep, ENVI-Carb, C_{18} and a mixture of all mentioned sorbents was tested. The Z-sep sorbent was finally selected as the best option due to the higher efficiency of the crude extract purification. The identification/quantification of nonpolar analytes was realized by gas chromatography coupled to (tandem) mass spectrometry with electron ionization for PCBs, OCPs and PAHs and negative chemical ionization in case of BFRs. PFASs and some BFRs were analysed by ultra-high performance chromatography coupled with tandem mass spectrometry.
The total concentrations of PCBs, OCPs, BFRs and PAHs in all samples ranged from <0.003 to 0.65 ng/g; <0.003 to 1.47 ng/g; <0.003 to 0.573 ng/g and <0.003 to 63.2 ng/g. The most abundant contaminants from the group of PAHs; OCPs; PCBs and BFRs were FA, PY, PHE, BbFA and IP; *p,p´*-DDE and HCB; CB 153, 180 and 138; BDE 154, 99, 66 and 100. Within the assessment of human exposure, the average daily intake of Σ8PCBs; Σ16PBDEs; ΣDDTs and 16ΣPAHs were 2,95; 2.06; 3.39 and 265 ng/kg bw/day, respectively. Our results are comparable with the other studies from different countries (Spain, Italy, Belgium) [1, 2, 3].

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Keywords: *daily diets, dietary exposure, environmental pollutants, GC-MS, LC-MS*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and the specific university research (MSMT No 20-SVV/2017). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

F12
SIMPLE, FAST, INNOVATIVE AND AUTOMATED DETERMINATION OF 27 POLYCYCLIC AROMATIC HYDROCARBONS IN OILS AND FATS BY LC-LC-GC-MS

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Polycyclic aromatic hydrocarbons (PAHs) are due to their mutagenicity, carcinogenicity and toxicity important food contaminants. In routine, the extraction of PAHs in oils and fats is either performed by saponification or by caffeine-complexation in combination with a solid-phase extraction clean-up and analysis by GC-MS [1]. This method is complex and time-intensive. The aim was to simplify the sample preparation and to decrease chromatographic interferences. Therefore, a fast and robust method by LC-LC-GC-MS was tested [2]. Prior to analysis by LC-LC-GC-MS no further extraction or clean-up is needed. The sample preparation consists of weighing, addition of internal standard and shaking for 10 seconds. The first LC dimension (silica gel) is used for the separation of matrix interferences from the fraction containing PAHs/polyunsaturated compounds. Using the second LC dimension (tetrachlorophthalimidopropyl-modified silica gel), the separation of PAHs and polyunsaturated compounds is achieved.
More than 200 oils and fats including sunflower oil, coconut oil, olive oil, sesame oil, rapeseed oil, walnut oil, soy oil, cacao butter and fish oil were analyzed by the routine method and using the LC-LC-GC-MS method. Matrix interferences were enormously reduced by LC-LC-GC-MS with results comparable to the routine method. Furthermore, verification data including intra-day precision, linearity, accuracy and robustness were sufficient and in compliance with the European Regulation.
Overall, the analysis by LC-LC-GC-MS combines a fast and easy sample preparation with an automated sample clean-up and analysis without obvious matrix interferences. Therefore, using this method the sample throughput can be enhanced by decreasing the consumables and solvent consumption and increasing the chromatographic quality.

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Keywords: *PAH, LC-LC-GC-MS*

F13
DETERMINATION OF MINERAL OIL AROMATIC HYDROCARBONS (MOAH) IN FOOD BY LC GC FID – COMPARISON OF CONVENTIONAL VS. AUTOMATED EPOXIDATION

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The determination of mineral oil hydrocarbons (MOH) in foodstuffs gained importance over the last years. Especially, the mineral oil aromatic hydrocarbons (MOAH) due to the potential carcinogenicity of certain MOAH. Several sources could be responsible for the MOH contamination of foodstuff, like lubricants used during food processing, recycled paperboard, jute bags and environmental pollution.
MOAH analysis of foodstuffs by LC-GC-FID without clean-up is complicated by the presence of various interfering substances, such as squalene and its isomerization products, sterenes and carotenoids. These substance classes co-elute with the MOAH fraction and overload the GC-column which renders the detection of small mineral oil contaminations almost impossible. Removal of these interferences by HPLC based on polarity differences is not possible. Epoxidation of interferences to increase their polarity seems to be a good possibility. Therefore, epoxidation by *meta*-chloroperoxybenzoic acid (*m*-CPBA) provided the best removal of interfering substances. But conventional epoxidation is complex and did not give suitable results for all matrices respectively interferences. A new automated epoxidation delivered good and comparable results for almost all matrices and could be a suitable alternative to the conventional epoxidation.
By the conventional epoxidation, dichloromethane as solvent and sub-ambient cooling for improved selectivity is used; the reaction is stopped by sodium bicarbonate [1]. This method is widespread and established in routine analysis for MOAH. But collaborative trials showed often high variances in the obtained results for MOAH. A new automatable method with ethanol as solvent, without sub-ambient cooling and sodium thiosulfate was recently proposed [2]. This method showed good precision and recovery for MOAH. Furthermore, the adaption of the reaction conditions and time-controlled automation increased the recovery of MOAH. Matrix interferences were enormously reduced by this epoxidation method and the results were comparable to the routine method.

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Keywords: *MOAH, LC-GC-FID, epoxidation, automation*

F14
DIRECT IMMERSION SPME IN COMPLEX FOOD MATRICES BY MEANS OF A NEW MATRIX-COMPATIBLE COATING

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The complexity and diversity of various food matrices and the need for clean extracts to be injected into analytical instrumentation may imply the use of tedious and time-consuming sample preparation strategies that often are a source of a significant amount of laboratory waste. The development of a new matrix-compatible solidphase microextraction (SPME) coating, namely PDMS/DVB/PDMS, bearing enhanced antifouling properties, enabled the analysis of complex food matrices by direct immersion SPME and helped to overcome issues related to extensive sample pre-treatment and instrumental contamination. We herein present recent advances made toward the analysis of contaminants in complex food samples by using the new matrix-compatible SPME coating. A protocol to avoid fouling from a high sugar content food matrix, as pure grape juice, was developed by implementing the typical SPME routine with two further steps of pre-desorption rinsing and post-desorption washing. This approach showed its efficiency toward analysis of various vegetable commodities. In addition, further modifications of this procedure were applied to enable for the first time the direct immersion of an SPME coating in avocado pulp (high fat content matrix) and soy milk (high protein content matrix), maintaining undermined the coating efficiency for a long series of consecutive extractions (up to 100) and avoiding instrumental contaminations due to accumulation of non-volatile fractions of the matrix into the gas-chromatograph injection port. The matrix-compatible coating showed superior performance compared to other SPME coatings under the same extraction conditions, in terms of coating lifetime and reproducibility of the analyses. Moreover, the use of the new matrix compatible coating enabled the development of a simple and efficient direct immersion-solid phase microextraction-gas chromatography-mass spectrometry (DI-SPME-GC-MS) method, for simultaneous determination of PAHs, PCBs, and pesticides in edible seaweeds. The complex nature of dry seaweed samples required a careful optimization of parameters affecting the extraction process and the rinsing/washing procedure of the coating. The method was optimized by use of multivariate approaches such as Plackett–Burman and Central Composite Design (CCD) and was validated according to FDA directives. Rewarding results were obtained, with limits of detection (LOD) in the sub/low part-per-billion level, good accuracy, and inter/intraday reproducibility. The results presented pave the way to the development of greener, fast and effective methods for analysis of food matrices by SPME.

Keywords: *matrix-compatible SPME, direct immersion SPME, quantitative analysis, food contaminants*

Acknowledgement: *The authors would like to thank the Natural Sciences and Engineering Research Council (NSERC) of Canada, Millipore-Sigma (Merk) and GERSTEL Inc. for financial support provided for this study. We would like to express our gratitude to Dr. Erica Souza-Silva, Dr. Selenia DeGrazia, Dr. Xiujuan Li, Dominika Gruszecka, Li Zhang and Viniciuos Aquaro Jr. for their contribution to this work.*

F15
CADMIUM LEVEL IN PHEASANTS' TISSUES FROM SERBIA AS BIOINDICATOR OF ENVIRONMENTAL POLLUTION DURING 2011-2016

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Cadmium (Cd) is one of the most toxic heavy metals and environmental pollutant that possess a significant health risk to the humans. It occurs on agricultural land as a contaminant of phosphorous fertilizer and in sewage sludge entering the human food chain. It migrates easily between soil layers and is taken up into the food chain plants uptake. Consequently, it is found in meat, especially in liver and kidney that accumulate higher levels. Cd in game birds as a representative of wildlife is considered to be suitable bioindicator of environmental pollution. Heavy metals are especially dangerous to birds whose metabolism is more rapid compared to other species. Serbia has a long hunting tradition with a large number of well-kept hunting grounds, where pheasants are among the most popular game species. The aim of the present study was to establish distribution of Cd in leg muscle and liver of pheasants, as suitable bioindicator of environmental pollution from Serbia.
Leg muscle (n=273) and liver (n=322) of pheasants from Serbia were collected within the Serbian National residue monitoring program during 2011-2016. Analysis of the ^{111}Cd isotope was performed by inductively coupled plasma mass spectrometry (ICP-MS). Majority of investigated samples contained Cd in detectable quantities. The mean Cd levels in leg muscle were near detection limit of 0.001 mg/kg. None of analysed leg muscle exceeded maximum residue level (MRL), 0.05 mg/kg for Cd in game muscle. Levels of Cd in leg muscle from 2014 were statistically different compared with levels from 2013 and 2016. The mean Cd levels in liver were in the range 0.022-0.277 mg/kg. During residue monitoring program, the increase in Cd levels were observed as well as the number of non-compliant samples (2011-0; 2012-4; 2013-2; 2014-5; 2015-7; 2016-11). In 2011 only, none of liver samples had exceeded MRL in liver of game and statistical analysis showed significant differences between measured Cd levels from 2011 compared to those from other years.
According to the results from this study and considering the fact that pheasants are suitable bioindicators of environmental pollution, it could be concluded that environmental pollution by Cd is gradually increasing, and that future monitoring of Cd levels is necessary.

Acknowledgement: *This work was supported by grants from the Ministry of Education, Science and Technological Development of the Republic of Serbia (project no. III 46009).*

F16
POLYCYCLIC AROMATIC HYDROCARBON (PAH) ANALYSIS IN COMPLEX FOOD MATRICES USING GAS CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY (GC-MS/MS)

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Trace contaminants of polycyclic aromatic hydrocarbons (PAHs) are closely monitored in food samples as PAH exposure is potentially toxic. In food, PAHs can be generated during preparation such as high-temperature grilling and smoking of fatty matrices. An extraction procedure was optimized to investigate several PAHs that are regulated in fatty and complex matrices. The challenge of analyzing PAHs are due to their chemical properties. PAHs are resistant to chemical reactions and have the tendency to accumulate rather than degrade. In addition, PAHs are inclined to desublime and are difficult to vaporize. Several PAHs were evaluated using a modified Gas Chromatograph Electron Ionization Triple Quadrupole Mass spectrometry (GC-MS/MS). A self-cleaning ion source allowed the source to be kept clean from PAH and sample deposition. Column backflushing (BF) enhanced the lifetime of the column by removing heavy matrix at the end of each analysis.

Keywords: *GC-MS/MS, PAH*

FOOD CONTAMINANTS (ENVIRONMENTAL)

F17
THE POTENTIAL OF GC-Q-EXACTIVE FOR SIMULTANEOUS ANALYSIS OF PBDES AND NOVEL BROMINATED FLAME RETARDANTS (NBFR) IN FOOD

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Polybrominated Diphenyl Ethers (PBDEs) and novel brominated flame retardants (NBFRs) are important industrial contaminants which are found in food and feed. The European Food Safety Authority (EFSA) published several opinions on these substances. Following these opinions, and due to the lack of data, the European Commission (EC) published a recommendation for monitoring of PBDEs, polybrominated biphenyls (PBBs), hexabromocyclododecanes, bromophenols and emerging BFRs in food. The diversity of these compound classes and the low levels (pg/g to low ng/g range) makes analytical chemistry challenging; there's a need for sensitive, selective and accurate analytical techniques. Many analytical methods focus on BFR subgroups being either PBDEs, PBBs, HBCDDs or the NBFRs. Current approaches such as GC-ECNI-MS show excellent sensitivity, but lack selectivity by monitoring the bromine trace (m/z 79 and 81), making them susceptible for inaccuracies due to co-eluting brominated compounds. GC-Q-Exactive potentially solves these issues providing sensitivity, selectivity and the possibility for full-scan acquisition. The aim of this study is to explore the potential of GC-Q-Exactive for the simultaneous analysis of PBDEs and NBFRs, combined with a simple sample clean-up for food matrices like fish, animal fats and oils.
To achieve that goal, the GC parameters and Q-Exactive settings were optimised. The BDEs included in this study are BDE 17-28-47-49-66-71-75-77-85-99-100-119-138-153-154-183-190 and 209. The NBFRs included are: α/β-DBE-DBCH, TBX, α/β-TBCO, PBT, PBEB, HBB, HBCDD, HCTBPH, BTBPE, OBTMPI, DBDPE. A TRACE 1310 GC, equipped with a PTV injector and a RTX-CLpesticides column (15m, 0.25mm, 0.25µm) was connected to a Q-Exactive operated in EI mode. With the Q-Exactive operated in full-scan excellent sensitivity and selectivity was observed with standard injections. In sample extracts of animal fat and vegetable oils, obtained with a Quechers type clean-up approach, matrix components were observed in full scan acquisition that coeluted with target analytes BDEs 85, 99, 100, 119, 153, 154, and DBHCTD, and which reduced the sensitivity of the target analytes that co-eluted with matrix, probably because the c-trap is filled with matrix. A more thorough clean-up involving oxidation (e.g. by sulphuric acid) reduced matrix interference, but at the cost of losing some labile NBFRs (e.g. PBB-Acr, EH-TBB, TDBPP, BEH-TEBP, TDBP-TAZTO). SIM can be employed to overcome the matrix load in the C-trap, allowing sensitive analysis of the target analytes and can be employed for routine monitoring. For detection of unknown BFRs, the GC-Q-Exactive is a powerful full-scan instrument. Selective non-destructive clean-up approaches are needed in order to reduce the matrix load in the Q-Exactive and employ the full potential of the instrument.

Keywords: *brominated flame retardants, PBDE, GC-Q-Exactive*

F18
MAXIMIZED PRODUCTIVITY FOR DIOXIN AND PCB ANALYSIS IN FOOD USING DUALDATA MODE WITH MAGNETIC SECTOR GC-HRMS

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Introduction:
Magnetic Sector High Resolution GC/MS is the golden standard for high sensitivity analysis of Dioxins and other POPs. Already for decades it has been proving its proficiency in this field of analysis and thus became the established analysis technique available nowadays in leading Dioxin laboratories throughout the world. For all gas chromatographic analyses a certain amount of 'dead' time is an intrinsic part of the measurement. The dead time is the time before the first relevant peak is detected and after the last relevant peak elutes. Accordingly this dead time does not contain relevant analytical information and thus can be seen as wasted time.
Dioxin analyses are typically conducted using 60 m columns that result in run times of 50-60 minutes. The dead time for such analyses can be 20-30 minutes per sample. Over a sample sequence this dead time equates to several hours per day that the average mass spectrometer is effectively idle.
The chromatographic dead time can be almost eliminated by performing alternate staggered injections using two GCs coupled to a single mass spectrometer. Depending on the ratio between dead time and acquisition time sample throughput can theoretically be doubled. This approach can be used for any type of GCMS application including combinations of different applications like e.g. Dioxins with PBDEs etc.
To realize a staggered injection sequence a hardware modification inside each GC needs to be implemented. This modification needs to ensure that only the flow of one analytical column at a time is guided into the ion source of the mass spectrometer. Therefore a time controlled dynamic flow switching system was developed using a proprietary microfluidic channel device (MCD) to switch flow between vacuum purge and MS. All restrictions and connections inside the GC oven are implemented on a miniaturized MCD.
Results and discussion:
The concept was proven by numerous experiments and again validated in full production dioxin analysis using thousands of samples at leading contract laboratories. The system as described here was tested to be able to cut out even high concentrated standards and demonstrated to work in routine with large injection volumes of 10 µL and higher. Even with large injection volumes, no solvent was found to reach the mass spectrometer, proving the 100% performance of the MCD device to switch column flow to vacuum. This gives absolute assurance that peaks from only one GC at any given point of time can reach the MS for detection. Chromatograms with and without this wafer are practically undistinguishable from one another in terms of peak shape or sensitivity. The Thermo Scientific DualData XL acquisition option can be used for different POPs analyses applications such as Dioxins, PCBs or PBDEs. Also a combination of different applications per GC is possible. The DualData Option provides a solution for maximum productivity in terms of number of samples analyzed per time.

Keywords: *dioxin, magnetic sector, high resolution MS, PCB, productivity*

F19
HPLC-APPI-MS/MS ANALYSIS OF PAH IN FOOD – ADVANCING FROM ACADEMIA TO COMMERCIAL ROUTINE

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Polycyclic aromatic hydrocarbons (PAH), typical organic pollutants in environmental and food matrices, originate from fossil fuels and incomplete combustion processes. Numerous substances from this class are well known to be carcinogenic or show other adverse health effects. They can affect human health through uptake with food or water but also via prolonged skin exposure or inhalation. Consequently, various efforts were made to define maximum permissible values in consumer products, environment or food products. On national and international level, different compounds have been selected as priority compounds for determination of PAH pollution from diverse matrices and purposes, along with varying threshold values.
To meet the analytical requirements and criteria set by (EC) 835/2011 and (EC) 836/2011, several analytical methods were developed in the past. Those methods are characterized by the effort to separate, identify and quantify isomers with very high physicochemical similarities but distinct toxicological impact. Typically, PAH are analyzed by GC-MS or LC-FID. Both methods exhibit particular restrictions in selectivity, specificity, robustness or efficiency. GC-MS may benefit from isotope dilution approach for quantification but suffers from certain separation restrictions as well as reduced column life and matrix effects. LC-FID on the other hand lacks the specificity of mass chromatography and quantification by isotope dilution.
Here we present a HPLC-APPI-MS/MS method for analysis of EU Priority PAH in fatty food and feed samples. In comparison to the standard GC-MS method, the application of HPLC-APPI-MS/MS allows for lower LOQs and higher robustness towards sample matrices. Consequently, simplified cleanup protocols can be applied, thus reducing one source of blank values from extensive solvent and simultaneously enabling higher sample throughput. The method was developed based on previously published approaches but required comprehensive adaption and optimization to develop from a purely academic approach towards a robust, economic and versatile instrument for large scale food analysis. During the optimization, innovative HPLC-column materials and combinations of columns have been tested in order to overcome the common restrictions in PAH isomer separation. A specific focus of our work was to separate PAH of interest from their common isomers as EU regulation demands but few established methods provide. Additionally, recent methods of sample preparation like SPE and Quechers were tested and optimized in combination with this method.
Overall, we present the innovative application of a state of the art approach on PAK analysis and review our results in the context of various published and established methods. To further explore the potential use of this method for routine analysis of regulated PAH in food samples, we provide extensive validation data and analytical PAH findings from various fat and oil matrices.

Keywords: *PAH, HPLC, APPI, analytical method*

Acknowledgement: *We kindly thank Agilent Technologies for their support and collaboration.*

F20
PHTHALATE AND DINCH METABOLITES IN URINE SAMPLES COLLECTED FROM CZECH MOTHERS AND NEWBORNS

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Esters of phthalic acid, also known as phthalates, belong to a group of additive plasticizers that are not bound to the material by a strong chemical bond. Therefore, these compounds are easily released into the environment as contaminants. Phthalates are known for the adverse effects on human health. They have the ability to disrupt effects of steroid enzymes, thyroid hormones and act as androgens, estrogens or antiestrogens. Furthermore, they may affect regulation of many biological processes such as proliferation of adipocytes or glucose homeostasis, which may be associated with a higher incidence of obesity and diabetes. The aim of this study was to extend and validate an analytical method for the analysis of 8 phthalate metabolites in human urine by other 4 metabolites of the compound di-iso-nonyl cyclohexane-1,2-dicarboxylate (DINCH). DINCH is widely used since 2002 as an alternative plasticizer to substitute use of phthalates. The analytical method requires only 300 µl of a urine sample. After the enzymatic hydrolysis (90 min, 37 °C and pH 5) the prepared urine sample is diluted with an organic solvent (methanol). Ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) is used to identify and quantify the target analytes. The method validation for phthalate metabolites was carried out on the NIST material SRM 3673 (urine of a non-smoker) in 6 repetitions. Limits of quantification (LOQs) ranged from 0.15–0.4 ng/mL urine with recoveries 60–127% and repeatability to 8%. The method for the analysis of DINCH metabolites, namely mono-*iso*-nonyl cyclohexane-1,2-dicarboxylate (MINCH), hydroxy-*iso*-nonyl cyclohexane-1,2-dicarboxylate (OH-MINCH), oxo-*iso*-nonyl cyclohexane-1,2-dicarboxylate (oxo-MINCH) and carboxy-*iso*-nonyl cyclohexane-1,2-dicarboxylate (cx-MINCH), was validated using a blank urine sample with an artificial contamination technique at two concentration levels (level 1 – 0.5 ng/mL urine; level 2 – 5 ng/mL urine). LOQs were 0.15 ng/mL urine with recoveries 60 – 104% and repeatability to 6%. The new validated method was subsequently used for the analysis of both above mentioned groups of target compounds in 250 urine samples collected from mothers and their newborn children living in two localities of the Czech Republic (Ceske Budejovice and Karvina) to assess the contamination of this part of Czech population. Detected were all 8 phthalate metabolites up to the concentration levels of Σ phthalate metabolites 139 µg/g creatinine.

Keywords: *UHPLC-MS/MS, urine, phthalate metabolites, DINCH metabolites, mothers and newborns*

Acknowledgement: *This study has received funding from the Czech Science Foundation Project No. 13-13458S. The authors deeply appreciate the cooperation with MUDr. Radim J. Sram from the Institute of Experimental Medicine AS CR in Prague. The "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015), the European Union's Horizon 2020 research and innovation programme (grant agreement No 692195), as well as the financial support from a specific university research (MSMT No 20-SVV/2017) are also gratefully acknowledged.*

FOOD CONTAMINANTS (ENVIRONMENTAL)

F21
HUMAN BIOMONITORING FOR THE ASSESSMENT OF DIETARY EXPOSURE TO CONTAMINANTS AND MICRONUTRIENT INTAKE AND STATUS: CURRENT ACTIVITIES AT THE ITALIAN NATIONAL INSTITUTE OF HEALTH

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Human biomonitoring (HBM) enables to assess the nutritional status of a population and the association with health and wellbeing outcomes with a view to pursuing health promotion and disease prevention. For example hypertension, a major risk factor for cardiovascular diseases in industrialized countries, is associated to excess sodium intake. Public health initiatives aimed at sodium reduction should be accompanied by monitoring of population sodium intake. Measuring urinary sodium is a convenient means for this purpose and with the same urine sample potassium intake, which plays a role in decreasing the risk of hypertension, can be assessed. HBM is a main tool for evaluating the impact of measures to improve the intake of nutrients as well as for assessing specific risks of inadequate status of key micronutrients in vulnerable groups, e.g. iodine for pregnant women and infants, iron for infants, children and premenopausal women.
For dietary contaminants, HBM gives an indication of the aggregated (i.e. dietary plus non-dietary) exposure of a population. If estimates of dietary exposure are available (e.g. by means of total diet studies), HBM may enable to assess the relative magnitude of the different exposure pathways. In many cases, typically for trace elements, the diet is by far the major source of exposure and HBM can be used to directly assess the intake through food and water.
The identification, validation and application of appropriate biomarkers (BMKs) in food safety studies are developing fields where much remains to be done. At the Italian National Institute of Health (ISS), a growing number of research activities rely on the use of BMKs of micronutrient intake and status, on the one hand, and BMKs of exposure to contaminants and other xenobiotics, on the other hand. Examples include the use of urinary iodine to assess the iodine status at the population level, of plasma selenium and selenoprotein P as BMKs of selenium intake and of the functional selenium body pool, respectively, of hair mercury as BMK of methylmercury exposure. Recently, a combination of BMKs has been used in studies aimed at assessing inorganic arsenic (iAs) exposure and individual capacity to metabolize and detoxify the ingested iAs. Toenail arsenic is used as long-term BMK, reflecting chronic exposure to iAs over several months. The sum of urinary iAs and methylated metabolites is used as BMK of recent exposure whereas the MMA/iAs and DMA/MMA ratios are determined to assess the methylation efficiency. Finally, pilot HBM studies have been performed to assess the internal levels of bioaccumulative endocrine disrupters (perfluorinated substances, polybrominated flame retardants) in Italian adults and children.
The ISS activities and results indicate that HBM, integrated with a variety of dietary-based approaches (total diet/basket-market/duplicate diet studies) and mechanism-driven toxicological data, can yield robust and novel evidence for benefit/risk assessment.

Keywords: *human biomonitoring, intake assessment, micronutrients, dietary contaminants, public health*

F22
ANALYSIS OF OXYGENATED AND NITRATED DERIVATIVES OF POLYCYCLIC AROMATIC HYDROCARBONS IN AIR USING GAS CHROMATOGRAPHY HYPHENATED WITH MASS SPECTROMETRY OPERATED IN NEGATIVE ION CHEMICAL IONIZATION (GC-MS-NICI)

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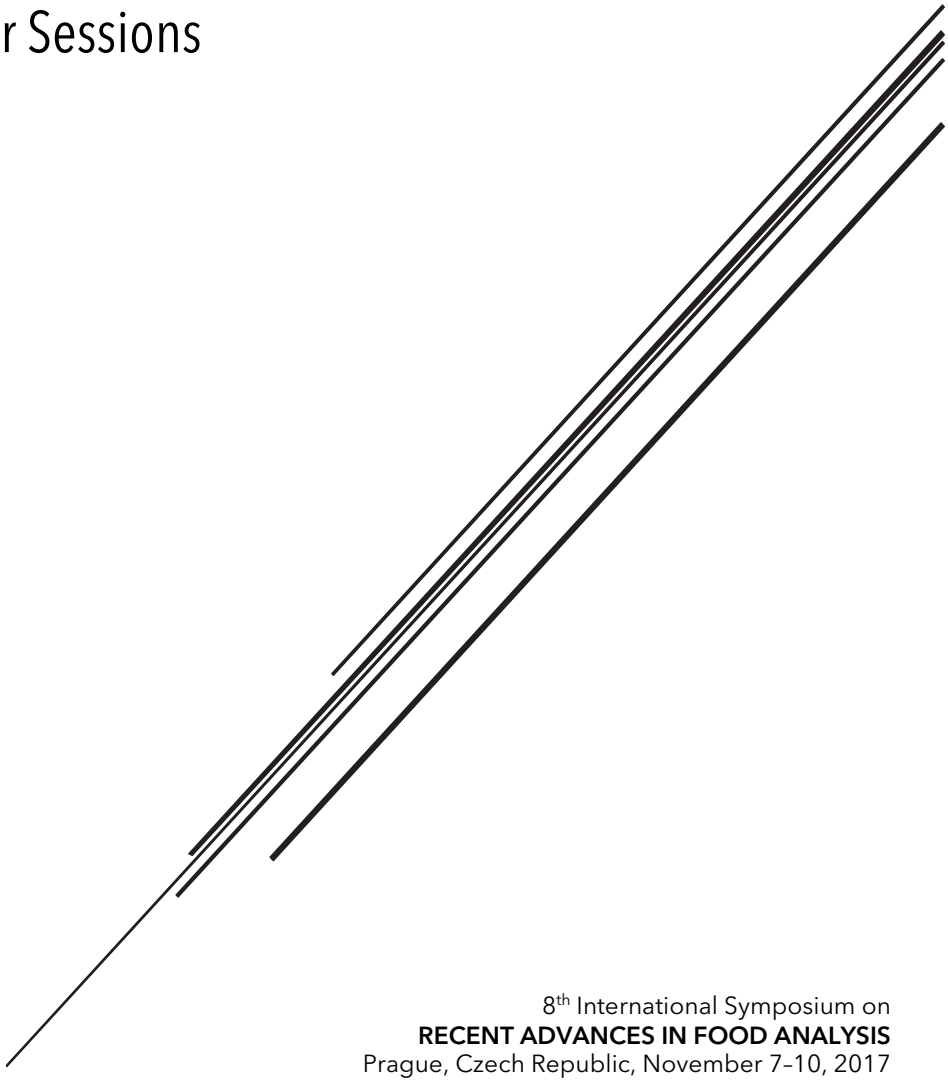
Polycyclic aromatic hydrocarbons (PAHs) belong to a wide group of well-known contaminants with carcinogenic and/or mutagenic effects which are formed during incomplete combustion processes. Nitrated and oxygenated derivatives of PAHs (NPAHs, OPAHs) are synthetized during oxidative reactions of PAHs in the atmosphere or could be formed together with their parental compounds during combustion. NPAHs could probably be 100,000 times more mutagenic and 10 times more carcinogenic compared to the classic PAHs, OPAHs are suspected oxidative stress inductors. Concentrations of NPAHs in outdoor air are two orders of magnitude lower than PAHs (in the range of pg/m^3 of air), OPAHs in outdoor air are presented at similar concentration as parental PAHs (in the range of ng/m^3 of air).
The presented study is focused on the development of a new analytical procedure for determination of 18 NPAHs and 6 OPAHs in outdoor air with great emphasis on employing gas chromatography interfaced with mass spectrometry operated in negative ion chemical ionization (GC-MS-NICI) technique. The extraction procedure is based on Soxhlet extraction into a mixture of hexane:dichloromethane (1:1, *v/v*) and subsequent clean-up using silica column. The new method was successfully developed and validated, and the limits of quantification (LOQs) for all targeted compounds were within range of 0.06–1.2 pg/m^3 of air. The method will be used for analysis of 180 samples of outdoor air in filters sampled using high-volume air samplers. The analysed samples were from two different seasons: "Summer" (sampling during July till October of 2016) and "Winter" (sampling during January till June of 2017) and from two different cities in the Czech Republic: Ceske Budejovice, which represents "background" pollution and Litvinov, which represents highly contaminated area due to high industrial pollution.

Keywords: *PAHs, outdoor air, nitrated PAHs, oxygenated PAHs, GC-MS-NICI*

Acknowledgement: *This work was supported from specific university research (MSMT No 20-SVV/2017) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015).*

GENERAL FOOD ANALYSIS

Poster Sessions



8th International Symposium on
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GENERAL FOOD ANALYSIS

G1
NEW FINDINGS ABOUT THE TOXINES OF BITTER-TASTING ZUCCHINI

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In recent years, more and more cases of acute poisoning after the consumption of bitter-tasting zucchini have been reported in the newspapers; one of them, in August 2015, was fatal. Mostly elderly individuals were affected by the intoxication, perhaps because the ability of the taste sensation "bitter" decreases with increasing age. Nevertheless, in general, the bitter taste of zucchinis can be covered by way of the preparation in the kitchen, for example, by cooking together with potatoes, with strong herbs or sauces, and so on.
Responsible for the intoxication are the naturally occurring cucurbitacins. The cucurbitacins are highly oxidized tetracyclic triterpenes nearly exclusively present in plants of the family of the *Cucurbitaceae,* namely squash plants [1,2]. Dependent on the dose, they can cause severe bloody diarrhea, strong colic, nephritis, tachycardia, and circulatory collapse even up to the death.
In 2016 and 2017, the authors had the opportunity to obtain one zucchini plant each which had previously produced bitter-tasting fruits. Therefore, not only the fruits at different degrees of maturity but also the other parts of the plants, i.e. leaves, stems, roots, and blossoms could be analyzed with regard to their cucurbitacin composition using HPLC- and UHPLC-DAD-MS/MS.
Only few of the about 80 cucurbitacins known are commercially available. Since they differ only slightly in their structure, sometimes only in the stereochemistry, this does not actually make MS-detection helpful in all cases. Therefore, we used the medically applied Cucurbitacin-rich colocynth, the medical plant of the year 2012, as a source for isolating a number of individual cucurbitacins as standard substances [3,4] indispensable for the correct assignment of the peaks in the LC-chromatograms.
The contents of the individual cucurbitacins and their distribution in regard to the glycosides and the aglyca will be presented. Noticeable is the major occurrence of the cucurbitacin-B-glucoside in comparison to its aglycon and the different contents in the fruits of the same plant. Furthermore, the influence of cooking-technological processes was studied.

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Keywords: *cucurbitacin, zucchini, cucurbitaceae, UHPLC-MS/MS*

G2
CHEMICAL, NUTRITIONAL AND FUNCTIONAL ANALYSIS OF CHILOE'S GIANT GARLIC (ALLIUM AMPELOPRASUM L.) BY HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY-AUTOGRAPHY COUPLED TO MASS SPECTROMETRY

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Garlic is a food and medicinal herb used worldwide since ancient times due to its healthy properties. It contents several kinds of bioactive molecules, mostly organosulfur compounds, to which some beneficial activities against cardiovascular, neurological, hepatic, arthritis and immunological diseases have been described. Chiloe's giant garlic (*Allium ampeloprasum var. ampeloprasum*), is a garlic specie botanically related to leek. This subspecies is mainly micro-cultivated in Chiloe island (southern Chile), declared by Food and Agriculture Organization of the United Nations as a Global Importance Agricultural Heritage System. The present work reports for the first time a detailed chemical, nutritional and functional evaluation of Chiloe's giant garlic applying traditional food analysis methods and High-Performance Thin-Layer Chromatography (HPTLC)-autography coupled to Mass Spectrometry (MS). For proximate chemical composition peeled garlic samples were analyzed using the AOAC official methods of analysis. Samples showed a moisture level of 62.34±0.35%, crude lipid 0.09±0.00%, fiber 13.10±0.05%, proteins (Nx6.25) 2.80±0.10%, minerals 0.74±0.02%, and carbohydrates 20.93±0.19%. For carbohydrates, organosulfur and antioxidant profiles, 2 grams of blanching (10 minutes) garlic samples were homogenized and extracted with 40 mL of methanol: water (8:2 v/v) solution. After filtration, carbohydrates profile was determined by HPTLC/Vis with post-chromatographic derivation with aniline-diphenylamine reagent. The most relevant carbohydrates found were sucrose (5.92±0.20 mg/g), glucose (0.11±0.00 mg/g) and fructose (0.46±0.00 mg/g). The presence of other saccharides such as mannose, maltose, xylose, arabinose, raffinose and galactose was discarded. Organosulfur profile was also determined by HPTLC/Vis with post-chromatographic derivation with ninhydrin derivatization reagent. The main organosulfur compounds found were alliin, metiin and isoalliin. The identity of each compounds was achieved coupling an underivatized plate to the mass spectrometer via TLC/MS interface, observing the following *m/z* 178.15, 152.20 and 178.15, which correspond to alliin, metiin and isoalliin, respectively. Antioxidant capacity was evaluated applying HPTLC/autography/MS for 2,2-diphenyl-1-picrylhydrazyl (DPPH*) assay. The molecules with the highest antioxidant capacity were identified as γ-glutamyl-S-allyl-L-cysteine (GSAC) and γ-glutamyl-S-(trans-1-propenyl)-L-cysteine (GSPC). This antioxidant activity has not been previously described. Thus, the detailed chemical, nutritional and functional composition of Chiloe's giant garlic is reported for the first time finding very interesting functional and nutritional molecules, similar to common garlic (*Alllium sativum*) but in quite different concentrations, particularly GSAC and GSPC, which concentrations are highly superior in Chiloe's giant garlic.

Keywords: *chiloe's giant garlic, HPTLC/autography/MS, organosulfur compounds*

Acknowledgement: *Authors The authors want to thank to the National Commission of Scientific and Technological Research (CONICYT) of the Chilean Government for the doctoral scholarship granted N° 21150629. This work was financially supported by National Fund for Scientific & Technological Development (FONDECYT), project N°1171857, FONDEQUIP 130209 and University of Concepcion.*

G3
SUBSTANCE-BASED RECYCLING OF PRESS WATER FROM BREWER'S SPENT GRAINS

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The joint project intends to develop an alternative recycling concept for brewer's spent grains as the largest waste stream during the brewing process. Thereby, the focus will be a selective membrane-supported separation/extraction of nutritionally relevant ingredients, resulting in new perspectives for use e. g. in food industry (amino acids, vitamins…).
In the initial phase, the dehydration process of wet spent grains needs to be optimized in order to achieve a maximum liquid yield. The resulting press water must then be characterized due to its so far unknown chemical composition by applying different analytical methods, for example (U)HPLC-PDA-MS/MS and HPLC-FLD/ELSD, in order to determine the valuable constituents for the subsequent membrane screening.
In a second step, several membrane experiments such as MF, UF, NF, and RO will be applied in a multi-step separation-process with the objective of a successive isolation of desired and undesired product fractions. The separation takes place in reference to chemical properties such as molecular weight as well as to other physical properties such as solubility. Biofouling/Scaling of the membranes will be considered as well. Finally, defined brewery process steps will be investigated in regard to their influencing chemical composition and stability.
The approach for the realization of the project goals will be presented.

Keywords: *brewer's spent grains, recycling, membrane technology*

Acknowledgement: *This measure was cofinanced with tax funds on the basis of the household decided by the legislators of the Saxon State Parliament. Sincere thanks go to Prof. Dr. Eßlinger (Freiberger Brauhaus, Freiberg) for providing the samples.*

G4
IDENTIFICATION STUDY BY USING LC-ESI-HRAMS

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During the last decade, the evolution of high resolution accurate mass spectrometry (HRAMS) has provided very good mass accuracy (better than 3-5 ppm), high resolving power (more than 30,000) apart from good sensitivity. Those facts allow the use of this kind of technique for trace analysis and achieve the strict maximum residue levels stablished in EU. Different workflows are available combining full scan MS and MS/MS in the same run, that have to meet the identification criteria.
SWATH® acquisition is a data independent non-target acquisition mode that divides the entire analyzed mass range into smaller segments. It was evaluated for pesticide multiresidue analysis in fruits and vegetables by using LC-QTOF-MS. The compounds were quantified in full scan MS mode and the MS/MS mode was used for identification purposes. QTOF-MS allows working using high resolution accurate full scan mass spectrometry and to obtain better selectivity as well as permit the retrospective analysis of the data, feature that cannot be afforded by QqQ-MS. Also, SWATH® workflow reduces the interferences in MS/MS spectrum because a lower number of ions (those within the range selected) are fragmented at the same time. It offered more possibilities for applying screening in the field of pesticide residue analysis.
The objective of the present work was to investigate the potential of SWATH® acquisition on a LC-QTOF-MS for identification of pesticide residue analysis. In the first step, various experiments with different mass windows (form 1 to 20 windows) were carried out. The use of ten mass isolation windows at the acquisition of the data was selected as a compromise between the required points per chromatographic peak and the reduction of interferences achieved.
Afterwards, the identification evaluation was performed through the analysis of 141 compounds in 20 vegetable matrices. A study of false positives and false negatives was accomplished following the identification criteria set in Document No SANTE/11945/2015 (retention time, two ions, mass error and ion ratio abundance). Furthermore, special attention was paid to some issues that can difficult the identification of the studied analytes working combining MS and MS/MS mode: low fragment abundance due to the use of a generic collision energy, influence of the matrix at the collision cell, effect of the concentration level, width of the mass isolation window and the presence of protomers. Finally, to verify the efficiency of the proposed method, a PT sample in lemon (EUPT-FV19) was analysed, obtaining acceptable results in terms of z score, proving that it also allows a proper quantification.

Keywords: *HRAMS, identification, SWATH, false positive evaluation, false negative evaluation*

Acknowledgement: *The authors acknowledge funding support from the European Commission, DG SANTE (Grant decision SI2.726352).*

G5
DEVELOPMENT OF DETECTION TECHNOLOGIES FOR FOODBORNE TOXINS

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Toxins are the some of the major pathogenicity factors for bacterial pathogens and plants. In an era of concern over foodborne contamination through bioterrorism, the sensitive and accurate detection of toxins is clearly essential for both food safety and biodefense. Our research unit designs and develops methods and reagents such as high-affinity monoclonal antibodies against high-consequence toxins including shiga toxins, botulinum neurotoxins, and abrin and staphylococcus enterotoxins. We combine the use of antibody-based techniques with activity assays such as cell-based and mouse bioassays that can detect enzymatic activities and provide sensitive and rapid validation for toxin detection. These detection assays are further optimized for evaluation in food matrices and evaluated for possible field-deployable use. Developing new toxin detection technologies will facilitate advancements in preventing, diagnosing, and treating foodborne intoxications. New methodologies and their further development with commercial partners will provide food processors and regulatory agencies with tools for enhanced food safety and biosecurity.

Keywords: *botulinum neurotoxins, shiga toxins, abrin, staphylococcus enterotoxins, immunoassays*

Acknowledgement: *U.S. Department of Agriculture- Agricultural Research Service, National Program 108 CRIS project 2030-42000-049-00D*

G6
STUDY ON TRIACYLGLYCERIDES IN EDIBLE VEGETABLE OILS BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM QUADRUPOLE-TIME OF FLIGHT MASS SPECTROMETRY

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Introduction
Edible vegetable oils constitute an important class of food products, which are widely used throughout the world. From a food chemist point of view, the determination of the triglyceride composition of edible oils provides information that complements that of the fatty acid composition. Knowledge of the distribution of the fatty acids within the glyceride molecules plays an important factor in considering nutritional values of edible vegetable oils. If the purpose of the analysis is to gain a better knowledge of the fatty acid composition of each triglyceride, liquid chromatography coupled with MS detection is the recommended approach. In this study, UHPLC-HRMS with ESI+ ionization mode was used to facilitate the analysis and identification of triacylglycerides in edible vegetable oils.
Methods
The analysis was performed using an Agilent 1290 Infinity II LC System coupled to an Agilent 6545 Accurate-Mass Q-TOF LC/MS system, which was equipped with the Agilent Jet Stream ion source operated in ESI+. The LC/MS method was a 23 min long gradient method with solvent A as acetonitrile containing 10% 10mM ammonium formate and 0.2% formic acid and solvent B as isopropanol containing 2% water and 0.2% formic acid. An Agilent Poroshell 120 EC-C18 2.1x100 mm 2.7 µm column was used at a flow rate of 0.30 mL/min. Column temperature was maintained at 40°C and injection volume was 1 µL. The mass spectrometer was operated in full scan MS mode at 2 spectra/second and in targeted MS/MS at 3 spectra/second.
Preliminary Data
28 triglycerides in vegetable oil were analyzed and identified by using an Agilent 1290 Infinity II LC System coupled to the Agilent 6545 Accurate-Mass Q-TOF LC/MS system. The acetonitrile and isopropanol containing water, used as solvent B, was modified with a small amount of ammonium salt and formic acid to improve the ionization efficiency of triglycerides in electrospray ionization mode (ESI+). With the optimization of the chromatographic conditions and the gradient profile, the analysis could be completed within 23 minutes, greatly improving on the efficiency and throughput of the analysis. The observed molecular ions of the triglycerides corresponded to the ammonium $[M+NH_4]^+$ and sodium [M+Na]+ adducts, and the measured mass deviation was less than 1 ppm. The fatty acid composition of the triglycerides was confirmed using MS/MS spectral information: the identified fatty acids were mainly C16:0, C18:0, C18:1, C18:2 in vegetable oil, but small amounts of C20:0, C20:1, C22:0, C22:1, C24:0, C24: were also present. Taking into account the existence and possible presence of isomeric content of the fatty acids in the studied triacylglyceride samples, two-dimensional LC (2D-LC) could be used for the additional in-depth analysis and characterization of the percentage distribution of the geometrical and positional isomers.

Keywords: *triacylglycerides, edible vegetable oil, fatty acids composition, quadrupole-time of flight mass spectrometry, two-dimensional liquid chromatography*

G7
CLASSIFICATION OF APIS MELLIFERA HONEYS FROM BRAZIL ACCORDING TO THE SEASON AND BOTANICAL ORIGIN USING DISCRIMINANT ANALYSIS (LDA)

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Introduction: Although best known as a sweetener, hThe aims of this study is were to evaluate physicochemical and antioxidant properties and total phenolic content of phenolic compounds of *A. mellifera* honey samples from Southern Brazil and to classify these samples according to to season and botanical origin classification byusing multivariate analysislinear discriminant analysis. Methods: 49 honey samples were collected in different seasons e were tested for their physicochemical and antioxidant properties and their content of phenolic compounds. Moisture, ash, electrical conductivity, pH, free acidity, color, diastase activity, sugars and HMF HMF levels were determined. Phenolic compounds were analysedanalyzed by HPLC. Levels of total phenolics and total flavonoids were measured andand the ORAC, FRAP and DPPH 2,2-diphenyl-1-picrylhydrazyl free radical assays performed to determine total antioxidant capacit. The linear discriminant analysis (LDA) was carried out to distinguish honey samples in terms of the season when they were collected and all data set (49 samples x 21 responses). Results: LDA also showed that 96% of honey samples were correctly classified into each botanic origin: all multifloral (n=6), *Pluchea sagitalis* (n=2) and *Hovenia dulcis* (n=6) honeys were correctly classified, while 80% (n=4) of *Schinus terebinthiofolius* and 96% (n=27) of eucalyptus honeys were also adroitly classified. The main discriminant responses were pH (Wilk's lambda=0.021, p=0.0004), flavonoids (Wilk's lambda=0.014, p=0.029), electrical conductivity (Wilk's lambda=0.013, p=0.037), and quercetin levels (Wilk's lambda=0.013, p=0.044). A great similarity of honey samples collected in the same season, and theThe main discriminant responses were *p*-coumaric acid levels (Wilk's lambda=0.125, p=0.018), titratable acidity (Wilk's lambda=0.123, p=0.022), diastase activity (Wilk's lambda=0.120, p=0.029), and total flavonoids content (Wilk's lambda=0.116, p=0.044). Although the number of samples was limited, one can infer a great similarity of honey samples collected in the same season. LDA also showed that 96% of honey samples were correctly classified into each botanic origin; all multifloral (n=6), *Pluchea sagitalis* (n=2) and *Hovenia dulcis* (n=6) honeys were correctly classified, while 80% (n=4) of *Schinus terebinthiofolius* and 96% (n=27) of eucalyptus honeys were also adroitly classified. The main discriminant responses were pH (Wilk's lambda=0.021, p=0.0004), TFC (Wilk's lambda=0.014, p=0.029), electrical conductivity (Wilk's lambda=0.013, p=0.037), and quercetin levels (Wilk's lambda=0.013, p=0.044). Conclusion: A great similarity in physicochemical composition, antioxidant activity and phenolic composition of honey samples collected in the same season and our results suggest that multivariate analyses (LDA) are useful tools to authenticate honeys.

Keywords: *honey, season, botanical origin, multivariate analyses., food analysis*

Acknowledgement: *CNPq, FAPESP*

G8
ANTIOXIDANT COMPOUNDS IN EDIBLE FLOWERS OF TAGETES PATULA L (ASTERACEAE) PROCESSED BY RADIATION

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Tagetes patula L. (Asteraceae), are French marigold native from Mexico and Central America, which are also popularly known and disseminated throughout the world. Furthermore, the French marigold flowers are used in culinary preparations, being also acknowledged for their phytochemical and medicinal properties. Edible flowers have been increasingly used in culinary preparations, which require new approaches to improve their conservation and safety. Nowadays, food irradiation as a Phytosanitary application is an economically and viable technology to extend shelf life of many vegetables. The purpose of this study was to evaluate the antioxidant properties of *T. patula* flowers submitted to electron beam and gamma irradiation doses of 0.5, 0.8 and 1.0 kGy, as also non-irradiated samples (control). The antioxidant activity was evaluated through Oxygen Radical Absorbance Capacity assay (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and Ferric Reducing Ability of Plasma (FRAP). The results show that the process by ionizing radiation in the flowers *T. patula* preservation can be feasible alternative ensured the properties of edible flowers.

Keywords: *tagetes patula, edible flowers, ionizing radiation, biocompounds*

Acknowledgement: *The authors are grateful to IPEN-CNEN/SP, CNEN, FCF/USP, CNPq. for the financial support.*

G9
INTERACTIONS BETWEEN FOOD COMPOUNDS: THE CASE OF ASCORBIC ACID AND GREEN COFFEE OR GREEN TEA EXTRACTS

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Plant extracts and ascorbic acid (AA) are constituents of functional foods, dietary supplements, and cosmetics. They exert antioxidant and/or antimicrobial activity thus they are used as natural additives, which are usually perceived by the consumers as safer than synthetic ones. Knowledge on the possible interactions between antioxidants may facilitate designing of food or cosmetic products with better antioxidant properties.
The aim of the present study was to determine the type of interactions between AA and green tea (GTE) or green coffee (GCE) extracts influencing their antioxidant activity. The ratio of extract to AA was 2:1 and 1:2. Interactions were measured using the TEAC (Trolox Equivalent Antioxidant Capacity) assay in aqueous solutions and in oil-in-water (40/60%) emulsions based on cranberry oil using RapidOxy oxidation stability tester. For the TEAC values, interaction indexes were calculated to describe the type of observed interactions. For emulsions, the results were expressed as the time needed to consume 5% of the initial oxygen present in the measuring chamber by emulsion sample. The protection factor W_o was calculated as the ratio of time for emulsion containing AA or/and extracts to the time for the control sample. W_o higher than 1 indicated antioxidant activity, and lower than 1 - pro-antioxidant activity. The types of interaction were determined using the summation of individual effects.
Antioxidant activity of tested antioxidants, expressed as the TEAC values, was the highest for AA, then for GTE and GCE. The TEAC values of extract-AA mixtures increased with increasing content of AA in mixture. It was found that there is no interaction between AA and GTE in mixtures of both weight ratios (additive effect), but for GCE the effect was antagonistic.
In emulsion, AA and both extracts showed antioxidant activity (W_o higher than 1.0). Mixture of GCE and AA revealed very strong protective effect against emulsion oxidation (higher than single compounds and mixture of GTE-AA in both ratios). GCE interacted with AA synergistically in both ratios, whereas GTE interacted with AA antagonistically (in the ratio of extract-AA of 1:2) or synergistically (ratio of 2:1). Differences in the type of interaction between tested extracts and AA are the result of their different polyphenol composition. GTE is rich in catechins, whereas GCE – in chlorogenic acid and its derivatives.
The results of the present study indicate that interactions between AA and plant extracts affect the antioxidant properties of their mixtures. The antioxidant properties of AA and plant extracts depend on the type of extract (polyphenol composition), the weight ratio of the ingredients present in the mixture and the nature of reaction medium.

G10
DETERMINATION OF SELECTED PARAMETERS OF PAPRIKA POWDER USING FT-NIR SPECTROSCOPY AND CHEMOMETRICS

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Recently, there is the need in industry for a cost-effective and non-destructive quality-control analysis systems. These requirements are fulfilled by the combination of near-infrared spectroscopy (NIR) and chemometrics. It allows to determine some physical or chemical parameters of products and eliminate time consuming analyses.
Paprika is a ground spice produced from red or chilli pepper (*Capsicum* L.). It is appreciated by consumers due to its flavour, aroma, and colour. The red colour of paprika derives from carotenoids, mainly capsanthin and capsorubin.
The aim of the study was to evaluate selected parameters of different types of commercial red paprika using NIR spectroscopy and chemometric analysis. The research material comprised of 18 commercial ground paprika (7 samples of sweet, 7 samples of hot, and 4 samples of cayenne). Total carotenoid and water contents were determined in tested products. Moreover, colour parameters (CIE L*a*b*) and FT-NIR spectra were measured.
Principal Component Analysis (PCA) of FT-NIR spectra was used to compare paprika samples. It was found that PCA is not applicable to differentiate paprika according to its type (sweet, hot, and cayenne) but the Partial Least Squares (PLS1) method revealed the relationship between FT-NIR spectra and water or total carotenoid contents in paprika. Based on the obtained calibration models, which were characterized by high values of R^2 and relatively low values of root mean square error of validation (RMSEV), it was shown that it is possible to predict water and carotenoid contents in paprika powder from their FT-NIR spectra. Moreover, it was found that a* value (contribution of red colour) was positively correlated with total carotenoid content.
The results of the present study demonstrated that FT-NIR spectroscopy together with chemometric analysis can be a good tool for evaluation of selected parameters of paprika powder.

G11
CARBOHYDRATES IN PRESS WATER FROM BREWER'S SPENT GRAINS BY HPLC-ELSD

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Wet spent grains are the main waste in brewing in Germany with 2 million tons each year [1]. Its recycling as animal feed requires a dehydration step for stability. Although the remaining liquid phase accounts for about 50% of the wet spent grains mass, its composition so far is unknown. Therefore, the characterization of the press water was one aim of a joint cooperation project between the TU Dresden and the TU Bergakademie Freiberg.
A TLC screening proved the presence of different mono- and oligosaccharides as expected. A quick and easy analytical tool for the quantitative determination is HPLC combined with an evaporative light scattering detector (ELSD) due to its benefit over the RI- and the PDA-detector. Consequently, a HPLC-ELSD method for press water was optimized and validated. As the applied ELSD 100 differs from its previous model, especially in regard to its changed tube drift shape, optimization parameters from earlier publications could not be adopted. Moreover the "dynamic gain" setting opens up new perspectives.
The developed method allows for a quantification of carbohydrates in press water from brewer's spent grains.
The results of the study will be presented.

[1] FEI-Projekt (AIF17170), Verwertung von Biertrebern durch hydrothermale Spaltung mit dem Ziel der Entwicklung genussfähiger Getränkegrundstoffe, Kurzbericht.

Keywords: *HPLC-ELSD, carbohydrates, dynamic gain, brewer's spent grains, recycling*

Acknowledgement: *This measure was cofinanced by tax funds of the household decided by the legislators of the Saxon State Parliament. Sincere thanks to Prof. Dr. Eßlinger (Freiberger Brauhaus, Freiberg) for providing the samples.*

G12
EVALUATION OF A NEW ULTRA INERT WAX GC COLUMN FOR THE ANALYSIS OF FATTY ACIDS, FAEES AND FAMES

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GC Columns with Polyethylene Glycol (PEG) stationary phases are commonly used for analyzing compounds with polar functional groups, and are well suited for food, flavor and fragrances applications. A current challenge with traditional PEG phases, however, is the need to incorporate functional groups, such as nitroterephthalic acid, to separate challenging analytes like acidic organic compounds. These modifications, nevertheless, typically reduces column lifetime, maximum operating temperatures and are prone to react with some active analytes.
Continuing with our recent advances in Ultra Inert (UI) technology, we are introducing a new WAX UI phase, specifically designed for the analysis of fatty acids in free and ester forms. This new WAX phase (DB-FATWAX UI), delivers superior inertness, better long-term thermal stability and greater sensitivity than any other traditional WAX column. The excellent peak shapes obtained for acidic compounds, eliminate the need to use two separate GC columns for the analysis of free fatty acids (FFAs) and their esters in the same sample.
In this work, we present a variety of applications on the analysis of free fatty acids, fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs); including determination of volatile FFAs from dairy products, analysis of Omega 3 and Omega 6 per AOCS Ce 1b-89, and FFAs and FAMEs in complex mixtures with other organic acids. The use of DB-FATWAX UI for the analysis of FFA and naturally occurring FAEEs in distilled alcoholic beverages without sample pretreatment, is also discussed.

Keywords: *FAME, free fatty acids, FAEE, inertness*

G13
A METHOD FOR THE QUANTIFICATION OF UNDERIVATIZED AMINO ACIDS IN SUGAR SNAPS BY LC-MS/MS

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Proteins are considered as the "building blocks" of living organisms. Although, being huge molecules all proteins are composed of only 20 naturally occurring L-amino acids. Out of these 20, 11 are essential and must be obtained through dietary supplements. In a wide range of food amino acids are ubiquitously present as free amino acids and can e.g. enhance flavor or taste or increase nutritional value. Due to their pivotal role for animals and humans there is a need for techniques that ensure an efficient and reliable determination of amino acids in food (supplements) and feeds.
We have developed an LC-MS/MS method for the analysis of free amino acids present in food. The analysis was performed on a Thermo Scientific™ Ultimate 3000™ RSLC system coupled to a Thermo Scientific™ TSQ Endura™ mass spectrometer. The chromatographic separation was carried out on a Discovery HS F5-3 column (Phenomenex, USA). Processing of the data was done using the Thermo Scientific TraceFinder 4.1 software.
The LOQs in neat solvent were in the low µg/L range and all correlation coefficients were above 0.995. Sugar snaps was used as example food matrix. Through standard addition it was possible to quantify 19 out of 20 amino acids from an amount corresponding to 2 µg or less of matrix. The repeatability was very good with %RSD values lying below 15 for 18 out of 20 target analytes. The method presented here offers a quick and simple way for the analysis of 20 L-amino acids without need for any derivatization.

Keywords: *amino acids, underivatized, LC–MS/MS*

G14
DEVELOPMENT AND VALIDATION OF CONVENTIONAL EXTRACTION OF BLACK BEAN FOR MAXIMAL POLYPHENOLS YIELD AND ANTIOXIDANT ACTIVITY

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Black bean contains phenolic compounds that contribute to the antioxidant activity. This study aimed at establishing the optimal extraction condition for the phenolic compounds from black bean using response surface methodology (RSM) with central composite design (CCD). Black bean was extracted using three variables, which include hydrochloric acid concentration (X_1, 0.3-0.5%), solid-liquid ratio (X_2, 1:30-1:50), and extraction temperature (X_3, 30-50°C). The complete design consisted of 19 experiments including eight factorial experiments, six axial experiments, and five replicates at the center point. The proposed quadratic model for the black bean extract was close to the fitted line (R^2=0.96). Optimal combination was hydrochloric acid in distilled water of 0.45% (X_1), solid-liquid ratio of 1:89.41 (X_2), and extract temperature of 20.1°C (X_3), giving maximal phenolic compounds yield of 1711.62 mg gallic acid equivalent/100 g. In verification experiment, no significant difference was found between predicted and experimental values for each response. The ABTS radical scavenging activity was found from 95 to 99%. These results indicate that optimization of the extraction conditions is important for accurate quantification of phenolics and antioxidant activity in black bean.

Keywords: *black bean, RSM, phenolic compound, antioxidant activity*

Acknowledgement: *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03028841).*

G15
DETERMINATION OF FUNCTIONAL COMPONENTS IN RED WINES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MS

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A method for determination of 13 functional ingredients (including resveratrol, gallocatechin, epigallocatechin, epigallocatechin gallate, epicatechin gallate, catechin gallate, taxifolin hydrate, etc) in red wine by ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry was developed. The red wine samples were diluted directly and separated on a C18 column by a liner gradient elution with acetonitrile-0.1% formic acid (*v/v*) a queous solution. Electrospray ionization was applied and the qualitative and quantitative analyses were operated under multiple reaction monitoring mode (MRM) by high resolution mass spectrometry. The calibration curves showed good linearity (R^2>0.99) in their detection range (10–1000 μg/L). The limits of detection were less than 1.0 μg/L except for epigallocatechin (1.0 μg/L), gallocatechin (1.0 μg/L) catechin gallate (3.0 μg/L) and taxifolin hydrate (3.0 μg/L). The average recoveries ranged from 80.9% to 112.3%. The method is quick, accurate, sensitive and suitable for the quantification of functional components in red wines. When the method was applied to commercial red wine samples, it was found that all selected wine samples contained catechin, epicatechin, epigallocatechin, gallocatechin, catechin gallate/epicatechin gallate and taxifolin hydrate. In addition, the concentrations of functional components are significantly different among the red wines which were made from different species of grape.

Keywords: *functional components, red wines, high performance liquid chromatography, mass spectrometry*

Acknowledgement: *This study was supported by the National Science and Technology Major Project of "Study on the Quality Detection Technology for Import and Export Medicine and Food Dual Purposes Products "(Project number:2017YFF0211000)).*

G16
COMPARISON OF ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT IN PETIOLES OF SIX SWEET POTATO VARIETIES

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Sweet potato consists of root, stem, petiole, and leave. The petiole is consumed after peeled off and blanched in Korea. The antioxidant activity and phenolic content in the petioles of six different sweet potato varieties [Hogammi (SP-1), Hayanmi (SP-2), Yeonjami (SP-3), Poongwonmi (SP-4), Jinyulmi (SP-5), and Danjami (SP-6)] developed in Korea were investigated. The petiole color varied from green to purple. The moisture content was found from 90.8% for SP-5 to 94.1% for SP-2. The phenolic content ranged from 888.35 to 1690.97 mg/g fresh weight (FW) chlorogenic acid equivalent with the highest value in SP-5. Antioxidant activities by the DPPH radical scavenging activity was highest for SP-3, with an EC_{50} value of 1.54 mg/mL. Effectiveness (EC_{50}) in reducing powers was found from 5.08 to 5.40 mg/mL and in a descending order of SP-6 > SP-5 > SP-4 > SP-2 > SP-1 > SP-3. No significant correlation was found between phenolic content, antioxidant activity, and reducing power. Individual phenolic compounds were analyzed by HPLC equipped with a diode array detector. Four phenolic compounds including chlorogenic acid, 4,5-dicaffeolyquinic acid, 3,5-dicaffeolyquinic acid, and 3,4-dicaffeolyquinic acid in all varieties. Among these compounds, the content of 3,5-dicaffeolyquinic acid was highest, followed by chlorogenic acid.

Keywords: *sweet potato petiole, antioxidant activity, phenolic compound, reducing power*

G17
APPLYING HIGH SPEED GAS CHROMATOGRAPHY FOR THE SPECIATION OF FATS IN FOODS AND EDIBLE OILS

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Determination of total fat content and its speciation as saturated, unsaturated, polyunsaturated, and trans fat for nutritional labeling is primarily accomplished by derivatization of the hydrolyzed free fatty acids as methyl esters (FAMEs) followed by high resolution GC-FID analysis. In particular, AOAC method 996.06 describes sample preparation procedures and a GC-FID method for speciation of fats in a variety of food products. Due to the high complexity and very small structural difference between analytes in a typical FAME sample the preferred GC columns are 100m or longer and use a highly polar cyanopropyl stationary phase. As a result the associated GC methods are quite slow; AOAC 996.06 requires over an hour of GC run time per sample not including oven cool down.
The analysis of FAMES has become increasingly important to food science in recent years. Unsaturated and polyunsaturated fat content is perceived favorably by consumers, who as a whole, are rapidly becoming more conscious of nutritional value. At the same time, the US FDA has issued a final determination that partially hydrogenated oils (those that commonly contain trans fats) are not GRAS and thus cannot be used in food products without specific approval. Fatty acid profiles are also useful in determining origin, authenticity, and sensory attributes of edible oils by chemical fingerprinting.
This work explores using shortened narrow bore columns, high carrier gas flows, and fast oven temperature programming as routes to reduce GC run times for FAMES analysis. The trade off' between separation performance and analysis time is explored with the conclusion that relatively complex mixtures of FAMES can be separated with greatly reduced analysis time.

Keywords: *FAME, AOAC 996.06*

G18
NITROGEN/PROTEIN AND SULFUR DETERMINATION BY COMBUSTION METHOD OF FOOD AND ANIMAL FEED

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The nutritional composition of food plays a very important role in food industries for research and quality control purposes. New regulations regarding all processed food and most raw foods include a series of tests aimed at determining food contents and their contribution to a healthy diet. One of the tests used in the production process is the determination of protein content of food and animal feed. The exact determination of the amount of protein, through the determination of the nitrogen content is fundamental for the nutritional quality of animal feed and for the safety of final food products intended for human consumption. Official regulations establish the protein content and labeling requirements, which enable consumers to define price and quality comparisons based on % protein declarations. Also sulfur is an essential component of living matter. Sulfur deficiency has a negative influence in the quality of proteins through the synthesis of amino acids such as cysteine, cystine, methionine and the synthesis of vitamins. For the cow, sulfur is a valuable element in determining the Cation-Anion Balance of Ration which is a very important parameter to ensure a good regeneration of the breast in later lactation. The importance of sulfur testing in foods and feeds has grown in the last years and many of the classical methods are now no longer suitable for routine analysis. The use of a simple and automated technique allowing fast analysis with excellent reproducibility, and that can avoid the risk of handling toxic chemicals is required. Regarding protein content, the alternative to the classical Kjeldahl method, is the Dumas (combustion) method which is approved by different associations (AOAC, AACC, AOCS, ASBC, IDF, ISO and IFFO). The Thermo Scientific Flash*Smart* Elemental Analyzer, based on the dynamic combustion method (modifed Dumas method), provides rapid and automated nitrogen and sulfur determination without use of hazardous chemicals and offers advantages in precision over traditional methods. The Flash*Smart* Elemental Analyzer allows runs at both high and low levels with no need to change configurations and without matrix effects. Trace sulfur content can be accurately determined by using the analyzer coupled with a Flame Photometric Detector (FPD). The method combines the advantages of an elemental analyzer with the sensitivity, selectivity and robustness of a FPD. The coupling is simple and it allows the determination of total sulfur up to 5 – 10 ppm in the same instrument without matrix effect. Sample protein content is calculated automatically using a conversion factor in the Thermo Scientific Eager*Smart* Data Handling Software.This paper presents N/Protein and Sulfur data of several food and animal feed in a large range of nature and concentration to show the repeatability, accuracy and precision obtained.

Keywords: *protein, sulfur, combustion, food*

G19
NITROGEN/PROTEIN DETERMINATION IN FOOD BY DUMAS METHOD USING ARGON AS CARRIER GAS

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One of the most important nutrient in food nutrition is protein. The precise and accurate determination of its amount, through the determination of Nitrogen, is fundamental to achieve the nutritional quality of finished products. For these reasons, the capabilities of the Dumas method (combustion method) for the determination of Nitrogen have been greatly improved to make faster, safer and more reliable than the traditional Kjeldahl method. Combustion Dumas method has been approved and adopted by different associations (AOAC, AACC, AOCS, ASBC, IDF, ISO and IFFO). In this light, the Flash*Smart* Elemental Analyzer, based on the dynamic flash combustion of the sample, cope with a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. However as in the last years there was large cost increasing for Helium, it is necessary to test as alternative gas, Argon which is readily available. This paper presents data on Nitrogen/Protein determination of food products with different Nitrogen concentration to show the performance of the system using Argon gas and the reproducibility of the results obtained in comparison with the values obtained using helium as carrier gas.

Keywords: *protein, food, combustion, argon, carrier gas*

G20
DEVELOPMENT OF A METHOD FOR DETERMINATION MACROLIDE ANTIBIOTICS IN HONEY USING HILIC-MS/MS

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A hydrophilic liquid chromatography-tandem mass spectrometry method was developed and validated for determination of four macrolide antibiotics including erythromycin, spiramycin, tilmicosin and tylosin in honey samples in the mass fraction range from 0.50 to 50.0 μg kg^{-1}. Parameters affecting extraction efficiency were examined during sample preparation, including the pH of extraction solvent as well as composition and volume of elution solvent. Furthermore, various chromatographic separation parameters (chromatographic column type, column temperature, mobile phase composition, flow rate) and mass spectrometric parameters (spray voltage, sheath gas pressure, capillary temperature), were optimized. Finally, macrolides were extracted from honey with PBS buffer 0.1 M, pH 8 and sample extracts were further cleaned up and analyte concentrated using solid-phase extraction on OASIS HLB columns. Separation was performed on a XBridge Amide column 3.5μm protected by a guard column using acetonitrile:water as mobile phase, (90:10, *v/v*) in the first segment and (70:30, *v/v*) in the second segment of run, at a flow rate of 0.3 mL min^{-1}. Detection was achieved by triple quadrupole mass spectrometry using heated electrospray ionization interface. Depending on macrolide chemical structures, protonated molecules form single and/or double charged precursor ions in the positive ionization mode. The analysis under MS/MS was performed using multiple reactions monitoring of two characteristic ion transitions for each analyte and their ratios were key criteria of macrolides presence confirmation in incurred samples. Quantification was performed using matrix-matched standards with the use of roxithromycin as an internal standard. This approach provided efficiently correction of losses during sample preparation as well as the matrix effects. The developed and optimized method was validated by determination of following validation parameters: selectivity, linearity, precision, accuracy, recovery, limits of detection and quantification and stability of macrolide antibiotics in honey extract. The recoveries of macrolide antibiotics from honey spiked at 0.50, 5.0 and 50.0 μg kg^{-1} were within the range of 78.0 to 110.3%, intra-day precisions were ≤ 2.41%, inter-day precisions were ≤ 3.36% and accuracy was below 10%. The limit of detection and quantification was 0.20 μg kg^{-1} and 0.50 μg kg^{-1} respectively for all studied macrolides. Through the analysis of different honey sample types regarding colour and floral origin the suitability of the developed method for purposes of determination food safety was confirmed.

Keywords: *macrolides, honey, HLIC-MS/MS, food safety*

G21
ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS OF LEGUMES

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Legumes such as cowpea (*Vigna unguiculata*), azuki bean (*Vigna angularis*), and soybean (*Glycine max*) are eaten worldwide. These legumes contain dietary fiber, minerals, protein, starch, and polyphenols. Epidemiological studies indicate that high consumption of whole legume products reduce the risk of cardiovascular disease and certain cancer. These effects have been ascribed to dietary fiber and phenolic compounds, including procyanidins and anthocyanins. The objectives of this study were to determine antioxidant activity and phenolic content, and to examine the comparison between antioxidant activity and phenolic conpounds such as anthocyanins and procyanidins in color legumes.
The 8 cultivars of cowpea, 2 cultivars of azuki beans, 1 cultivar of kidney bean (*Phaseolus vulgaris*) and 2 cultivars of soybeans were used as samples. Their antioxidant properties were measured by hydrophilic-oxygen radical absorbance capacity (H-ORAC) assay and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay. The total polyphenol content (TPC) of the bean extract was determined according to the Folin-Ciocalteu spectrophotometric method. Total procyanidin content were determined using vanillin and H_2SO_4 according to the method of Sun *et al* (1998) (Vanillin assay). The compounds of anthocyanins and procyanidins in the investigated legumes were determined by high-performance liquid chromatographic (HPLC).
The H-ORAC values ranged from 94 to 541 μmol-TE/g in extracts of colored legumes. The H-ORAC values was significantly higher in black cowpea extracts in other legumes ($P < 0.01$). With respect to seed color of black legumes, high correlation was found as follows: between H-ORAC values and total anthocyanin content (r=0.909), and between H-ORAC values and total procyanidin content (r=0.989), respectively. These results suggested that both of anthocyanins and procyanidins contribute to the antioxidant activity of color legumes.

Keywords: *antioxidant activity, legume, anthocyanin, procyanidin, ORAC*

G22
AUTOMATED DERIVATIZATION WITH RUGGED HPLC METHOD FOR AMINO ACID ANALYSIS

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Amino acids are gaining attention in food industry. To assess the quality of food, a reliable and accurate method is required to measure amino acids. HPLC with precolumn derivatization is commonly used for the analysis of amino acids. Some immediate drawbacks to manual offline derivatization are sources of error due to operator skill, competence, and laboratory technique. Other drawbacks include extra sample manipulation, extra time required, and increased risk of contamination. Automated online derivatization minimizes these error sources, immediately improves precision, and saves time. Thus, a rugged high-resolution HPLC method including online derivatization, can increase productivity compared to offline methods. This study presents an automated method for the analysis of pre-column derivatized amino acids using AdvanceBio Amino Acid Solution. The online injector program with OPA and FMOC derivatization decreases sample preparation time, and increases reproducibility over traditional offline methods. The HPLC method is rapid and highly reproducible, with an excellent % RSD of peak area and retention time for all amino acids, with most between 1 and 2 percent. Furthermore, we also used this technique to determine the LOD, LOQ and system suitability requirements. The method presented here was applied to analyze cell culture media and protein hydrolysate standards.
For Research Use Only. Not for use in diagnostic procedures.

G23
AN INTERNATIONAL INTERLABORATORY STUDY AS A FIRST STEP TOWARDS A HARMONIZED ANALYTICAL APPROACH TO CHLORINATED PARAFFINS

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Short-chained chlorinated paraffins (SCCP) have since their development in the 1930s been widely used as high-pressure lubricants, plasticisers or flame retardants, leading to a worldwide pollution of the environment due to unsafe handling of products or waste containing SCCPs [1]. Ever since more widespread efforts to ban the production (but not use) of SCCPs, medium chained chlorinated paraffins (MCCPs) have increased in production numbers, often replacing SCCPs in the products [2]. With the ban and/or restriction of SCCP production and use adopted by the Stockholm Convention in April/May 2017 [3], a robust but selective analysis with comparable results is needed to ensure official controls to enforce this ban.
A survey taken amongst national reference laboratories, official and private laboratories in the EU and beyond have revealed a plethora of methods currently in use or in planning stages for CP analysis. To establish a common ground and eventually ensure comparable results, the EURL for Dioxins and PCBs has started a series of interlaboratory studies focused on sensitivity, selectivity and robustness for the determination of SCCPs and MCCPs as part of their mission brief given by the European Commission. In this first round, spiked coconut fat samples were presented to the participants for analysis. Future rounds will introduce actual fat extraction and more complex sample clean-up to the requirements to eventually reach the level of analytical prowess needed to analyse food and feed samples.

[1] Tomy GT (2010) *The Handbook of Environmental Chemistry, Vol. 10,* Springer, Heidelberg, London
[2] Gluege J, Wang Z et al (2016) *Science of the Total Environment,* 573 1132–1146
[3] Stockholm Convention on Persistent Organic Pollutants, Decision SC-8/11, 26 April 2017

Keywords: *interlaboratory study, chlorinated paraffins, persistent organic pollutants, mass spectrometry*

Acknowledgement: *We would like to thank the EU Commission for the financial support of the work of the EURL for Dioxins and PCBs in Feed and Food.*

G24
DEVELOPMENT OF AN OPTIMIZED AND RELIABLE ELISA TEST FOR HISTAMINE TESTING IN FISH AND FISH PRODUCTS

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Histamine in Fish is regulated by Reg CE 1441/2007 with limits set at 100 to 400 ppm in fresh and brined fish when tested using HPLC Methodology. Since the 1990's, ELISA methodology has been recognized as a valuable and easy to use alternative method to HPLC, well suited both at plant level for a high throughput screening and in external laboratories. Rapid tests, commonly less than 1 hour, are welcomed by the seafood industry for the rapid release of these high value perishable goods.
When evaluating ELISA based technologies for seafood regulatory testing, many look at standardized proficiency tests such as FAPAS for guidance. In many cases, ELISA technologies do not score within the range required to meet accepted tolerances within the FAPAS definition of a +/- 2 z-score.
Here we will look at some possible conditions affecting negatively some ELISA results:
- Homogeneity of sample handling with a very high dilution factor.
- Additional dilutions required for targeting the EU regulation values
- Variability of matrices tested
- Direct competitive ELISA format limits number of samples per run
- Specific equipment needing metrology.
- Manual handling compared to automation

Taking these limiting factors into consideration we have developed an improved rapid ELISA test including:
- Reduced dilution factor of 1/25 covering the 0/300 ppm quantitation range
- Validation of various seafood matrices including fresh, frozen, fish meal and traditional fish products
- Indirect competitive format minimizes well to well drift, allows an increased number of samples per run
- Strict procedural and handling guidance,
- Validation of automated procedures

The performance and suitability of this new HistaSure assay for testing various matrices is demonstrated by:
- AOAC validation PTM 021402 Method regarding AOAC 977.13 onto various kind of fish
- 4 years follow-up onto FAPAS Histamine Proficiency Tests shows a 0.99 R^2 (n=14)
- Correlation R^2=0.9975 with HPLC at national reference laboratory on the range 0-200 ppm

This new rapid ELISA kit is suitable for the determination of Histamine in various seafood matrices with a high throughput in a reduced time and budget. The validation as an alternative method to the future European PR EN ISO 19343 will be conducted when it becomes effective.

Keywords: *histamine, ELISA, fish, immunoassay*

G25
IDENTIFICATION OF WINE POLYPHENOLS WITH ANTIMICROBIAL AND GROWTH-STIMULATORY ACTIVITY USING A NOVEL IN-SITU METHOD BASED ON HPTLC/MS

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During winemaking, malolactic fermentation (MLF) is a critical step for obtaining high-quality wine. Some wine parameters such as low pH, high alcohol content and some polyphenols with antibacterial activity may affect this fermentation process. Wine polyphenols contribute to the organoleptic characteristic of wines, color, astringency and bitterness. Their consumption has been associated with some health benefit like protection against cardiovascular disease. Some reports have confirmed that various wine polyphenols have an inhibitory effect on lactic acid bacteria responsible of MLF. This antibacterial activity strongly depends on the phenolic structure, dosage, and microbial strain. The main polyphenols with inhibitor effects over MLF bacteria (*Oenococcus oeni*, *Lactobacillus hilgardii* and *Pediococcus spp.*) are flavonol and stilbenes. Although, wine polyphenols may have antibacterial activity, other authors reported that some polyphenols may promote bacterial growth. The objective of the present work was to evaluate both activities identifying the bioactive compounds by High-Performance Thin Layer Chromatography (HPTLC) coupled to mass spectrometry (MS). Nowadays, HPTLC/MS are a simple and fast technique for the separation and identification of bioactive compounds present in the raw extracts such as wine. The proposed HPTLC/MS method allowed the separation of nine polyphenols presents in wine, i.e. quercetin-3-O-arabinoside, malvidin-3-glucoside, peonidin-3-glucoside, vitisin B, malvidin-3-O-(6-acetyl) glucoside, petunidin-(6-acetyl)-glucoside, malvidin-3-O-(6-p-coumaroyl)-glucoside, gallic acid and petunidin-3-glucoside. Bioassay was performed using *Lactobacillus rhamnosus* previously acclimated as target bacterium. The assay was performed with malvidin-3-glucoside, freeze-dried wine and *trans*-resveratrol. Results showed that malvidin-3-glucoside and the freeze-dried wine had growth-stimulatory activity, while *trans*-resveratrol showed antimicrobial activity. Finally, the proposed method based on HPTLC/MS contributes to the fast and simultaneous identification of antibacterial and growth-stimulatory compounds in wine.

Keywords: *HPTLC-MS, polyphenols, wine, bioassay*

Acknowledgement: *Thanks to University of Concepcion, Viña Chillán, Chilean National Commission for Scientific and Technological Research (CONICYT), Fund for Scientific and Technological Development (FONDECYT) project N° 11150921 and FONDEQUIP N°130209.*

G26
NUTRITIONAL COMPOSITION OF BRAZILIAN SORGHUM GENOTYPES FOR HUMAN CONSUMPTION

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Sorghum is one of the five most important cereal crops behind rice, wheat, corn and barley. It is a staple food grain in many semi-arid and tropic areas of the world because of its good adaptation to hard environments and its good yield of production. The interest in using sorghum in human foods has been gradually increasing in some countries because it is an excellent alternative ingredient for making gluten-free products. Moreover, some sorghum genotypes may contain high levels of nutrients and bioactive compounds including tannins, phenolic acids and anthocyanins, which are associated with several health benefits. In Brazil, sorghum is cultivated mainly for feed and there are limited nutritional composition data of different cultivars for human consumption. Thus, the aim of this work was to evaluate the proteins, lipids, fibers, ash, carbohydrates, iron and zinc contents of 11 sorghum genotypes with tannins (BR305, SC103, SC319, CMSS005) and tannin-free (BRS330, BRS332, BRS373, BRS380, BRS501, HE1167017, HE1167048). The genotypes were grown in the same experimental area of the Embrapa Milho e Sorgo, Sete Lagoas, Brazil. The protein content was quantified using an FP-528 Leco Nitrogen Analyzer (nitrogen x 6.25). The fibers were analyzed in a Tecnal EQ LCC 08 fiber analyzer and the lipids in a XT10 Ankom Fat extractor. The ash content was determined with calcination of the organic matter in a Q 318 D 24 Quimis muffle at 600°C for 2 hours and the carbohydrates were determined by the difference between the total in the sample (100%) and the content of proteins, lipids, fibers and ash. The minerals Fe and Zn were determined in an Inductively coupled plasma-optical emission spectrometer (ICP-OES) Varian 720 ES. All results were expressed on a dry matter basis. The results showed significant differences (Tukey´s test, $p<0.05$) in the contents of all nutrients among the genotypes. The protein concentration of samples ranged from 12.2 to 16.9%, lipids from 2.2 to 4.6%, fibers from 9.4 to 20.2%, ash from 2.2 to 6.5%, carbohydrates from 57.5 to 71.8%, iron from 27.9 to 53.2 mg/kg and zinc from 18.7 to 43.0 mg/kg. SC103 was the best genotype to protein, iron and zinc contents. The fiber, lipids, ash and carbohydrates concentrations were higher in the genotypes BR305, BRS380, BRS373 and BRS501, respectively. The conclusion was that grains of some sorghum genotypes may be sources of nutrients and may contribute to insure food security, especially in regions with high prevalence of malnutrition as in the Brazil Northeast Region, where the climate is semi-arid.

Keywords: *Sorghum bicolor (L.) Moench, gluten-free, food security, nutrients*

Acknowledgement: *The authors acknowledge the "Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA" and the "Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG" for the financial support.*

G27
COMPLEMENTARY APPROACHES FOR SAMPLING FLAVOUR VOLATILES IN THE TD-GCMS ANALYSIS OF HOPS AND BEER

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The brewing industry has seen significant growth in recent years, with many new producers in the market providing a range of different styles of beer. The differing aroma profiles of these products are derived from the complex mixture of organic compounds, including monoterpenes, which are a major constituent of the natural products used as key ingredients in beer production.
Many different approaches are used in brewing to effect the characteristic flavour of a particular beer, and it is important for brewers to understand the relationship between the type of hop used, the way in which it is used in the brewing process, and the flavour compounds delivered to the final product.
Traditional methods for aromas profiling include headspace sampling of dried hops, and SPME sampling of the final liquid product. In this study, thermal desorption is utilised as the sample introduction technique for GCMS analysis, and sampling is achieved by 2 different, but complementary techniques; dynamic headspace for hop samples, and high-capacity, immersive sorptive extraction for the beer itself.
Combining these high-capacity, versatile sampling approaches with TD and its inherent pre-concentration capability aids comprehensive characterisation of low concentration, aroma-active compounds and immersive sampling provides additional information to that obtained with traditionally used headspace apporaches.

Keywords: *beverage, flavour, GCMS, headspace*

G28
A FAST AND SELECTIVE METHOD TO DETERMINE PHENOLIC COMPOUNDS IN QUINOA (CHENOPODIUM QUINOA WILL) SEEDS APPLYING ULTRASOUND-ASSISTED EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Non-communicable (chronic) diseases (NCDs) possess a long duration and a generally slow progression. The four most relevant are cardiovascular diseases, cancers, chronic respiratory diseases and diabetes, being responsible of 63% of all deaths worldwide (36 million). The main risk factors for NCDs are smoking, physical inactivity, excessive alcohol consumption and unhealthy diet. Thus, in the latest time, the demand for healthy foods has shown an important increase, especially of those denominated "functional foods". Quinoa, a pseudocereal from Andean region in South America, has acquired much attention because presents a high nutritional value, it is gluten-free and it is a functional grain due its content of bioactive molecules like polyphenols and carotenoids. The objective of this work was to development a fast and selective chromatographic method to determine phenolic compounds in quinoa seeds. With this highly selective method it was possible to evaluate the presence of caffeic acid, vanillic acid, quercetin 3-ß-D-glucoside, *p*-coumaric acid, vanillin, *trans*-ferulic acid, benzoic acid, quercetin, kaempferol and myricetin. After milling, polyphenols were extracted from 0.8 g of quinoa seed sample using 8.0 mL of neat ethyl acetate (1:10 w/v ratio). Ultrasound-assisted extraction (UAE) was carried out using a 10L ultrasonic bath with a potency of 200 W and frequency of 40 kHz. Factors time and temperature were optimized by means of face-centered composite central design using the extraction yield of each polyphenols as response (n=10). After establish the individual maxima, a multiple response optimization was performed obtaining that 10 min at 39ºC was the optimal extraction conditions for all polyphenols. Polyphenols quantification was carried out by HPLC/UV using a Phenomenex Kinetex XB C_{18} column using a binary mobile phase composed of acetonitrile and 10mM ammonium formate (pH 3.0). A complete separation was accomplished in less than 18 min. Validation was performed following International Conference on Harmonization (ICH) recommendations. Calibration data fitted a linear regression model with R^2 >0.999. Repeatability (n=6) and intermediate precision (n=3) in matrix showed relative standard deviation (RSD) values lower than 2.89 and 3.61%, respectively. Selectivity was evaluated by LC/MS/MS without observe interference compounds. The proposed method was applied to determine the polyphenols profile in 10 quinoa seeds samples that belongs to different varieties (shape, color, proximate composition), finding a concentration range from 1.1 to 2.0 mg/kg. Thus, the proposed method proves to be a fast and selective alternative to evaluate polyphenols in different varieties of quinoa seeds.

Keywords: *ultrasound-assisted extraction, quinoa seeds, HPLC*
Acknowledgement: *Authors thank to the National Commission of Scientific and Technological Research (CONICYT) of the Chilean Government for the doctoral scholarship Nº Nº 21150629 as well as to projects FONDECYT Nº 1140661, 1171857, FONDEQUIP Nº 130209 and the University of Concepcion.*

G29
DETECTION AND IDENTIFICATION OF ACETYLCHOLINESTERASE INHIBITORS COMPOUNDS IN ANNONA CHERIMOLA MILL. BY HPTLC-BIOASSAY METHODOLOGY COUPLED MASS SPECTROMETRY

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Acetylcholinesterase inhibitors (AChE) compounds are considered as an important strategy in the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis. Cherimoya (*Annona cherimola* Mill.) is a fruit grown in subtropical areas with only few studies regarding the identification of its bioactive compounds. For this reason, the objective of this work was to detect and identify AChE inhibitor compounds in cherimoya by HPTLC-Bioassay coupled mass spectrometry. The enzymatic assay was optimized and performed on HPTLC silica gel 60 F_{254} plate using a mobile phase that contains 1-naphthyl acetate (1.5 mg mL^{-1}) as enzymatic substrate. After chromatographic separation, the organic solvents from the mobile phase were removed, then the plate was sprayed with an acetylcholinesterase enzymatic solution (1 U mL^{-1} in 0.05 TRIS-hydrochloric acid buffers at pH 7.8) and incubated at 37°C for 10 min. Finally, the plate was atomized with a Fast Blue B salt solution (1.0 mg mL^{-1}) to obtain a purple background which contrasts with colorless inhibition zones. The methodology was applied to evaluate bioactive compounds present in pulp extract, peel and seeds. Extracts using methanol and dichloromethane were separated with a mobile phase composed of chloroform: methanol: ethylacetate (80:14:6% v/v/v). Two bands showed inhibitory activity and a previous structural analysis by visualization reagents showing positive results for alkaloids with Dragendorff reagents. Two compounds: anonaine and glaucine, were identified by HPTLC-DAD-ESI-MS/MS with con I_{max} 269 and 299 nm, and $[M+H]^+$ *m/z* values of 266 and 356, respectively. To the best of our knowledge, this is the first report about acetylcholinesterase inhibitory capacity of these Cherimoya alkaloids.

Keywords: *HPTLC, bioassay, cherimoya, acetylcholinesterase*

Acknowledgement: *Authors thank to the National Commission of Scientific and Technological Research (CONICYT) of the Chilean Government for the doctoral scholarship N° 21141096 as well as to projects FONDECYT N° 1171857, FONDEQUIP N° 130209 and the University of Concepcion.*

G30
TLC-MS: COUPLING THIN LAYER CHROMATOGRAPHY WITH MASS SPECTROMETRY - A PRACTICAL APPROACH FOR MATRIX-LOADED SAMPLES

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A straightforward way to couple thin layer chromatography (TLC) with mass spectrometry (MS) is the TLC-MS Interface from *Camag* [1]. It is an elution-based, semi-automatic system to extract zones from the TLC plate and transfer them online into the MS. It is suitable for all thin layer materials and every eluent that can be sprayed in the ion source. The interface can be connected to any kind of LC-coupled mass spectrometer. We show how the TLC-MS interface can be used for the development of TLC-MS applications in the areas of food & beverage, pharmaceutical ingredients, cosmetic actives and peptide & protein analysis.
- separation and identification of insulin species
- investigation of UV-filters in suncream
- analysis of steroids
- determination of caffeine in energy drinks

All experiments were performed on newly developed HPTLC plates with a reduced separation layer thickness. After chromatographic separation the analytes were extracted with acetonitrile/water (95:5, v/v) and transferred online into the MS with a flow rate of 0.2 ml/min. The ionization technique was electrospray ionization (ESI) in the positive mode.

[1] H. Luftmann, A simple device for the extraction of TLC spots: direct coupling with an electrospray mass spectrometer, Anal. Bioanal. Chem., 378, 964-968, 2004

Keywords: *TLC-MS, caffeine, energy drinks, HPTLC*

G31
INVESTIGATION OF PEA PROTEINS TO MONITOR THE POSSIBILITIES TO EXPAND THE RANGE OF SUSTAINABLE FUNCTIONAL FOODS WITH HIGH ADDED-VALUE BASED ON LEGUMINOUS SEEDS

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Peas (*Pisum sativum* L.) are becoming an important vegetable source of proteins and a potential alternative to soybean in Europe. The increased acceptance of pea proteins is due to pea manifold qualities, good functional properties in food applications, high nutritional value, availability, and relatively low cost. Also, pea beans and their products are a rich source of biologically active components that may exert beneficial health and therapeutic effects. The sequencing of the pea genome has not been completed. Therefore, proteomic approaches are currently limited.
Proteomic analysis is emerging as a highly useful tool in food research, including studies of food allergies. According to the literature, protein content varies among genotypes and is influenced by environmental factors. Technological-functional properties of pea protein-based products depend on several factors including protein content and composition of starting pea bean, the purification and processing method. 15 domestic peas were submitted under protein investigation by gelelectrophoresis. We aimed to compare the different types, the parts of the legumes (shelled pea, husk, leaf), and furthermore the mature and early aged peas on the basis of protein distribution. Proteins were fractionated into albumins and globulins by Osborne-method. By *in vitro* digestion we monitored the faith of the pea proteins.
Our aim was to reveal the availability of the pea types for functional food production.

Keywords: *pea, proteins, gel electrophoresis, in vitro, digestion*

G32
INVESTIGATION OF GARLIC AND ONION CONTAINING FOOD PRODUCTS FOR A FALSE POSITIVE SULFITE RESPONSE BY LC-MS/MS

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Sulfites are a family of additives regulated for use worldwide in food products. In the United States, sulfites must be declared on the label if they are present in concentrations greater than 10 mg/kg (determined as) SO_2 because an allergic-like response has been reported in a small subset of the population upon consumption of sulfite-containing products. The current US regulatory method for sulfites, the optimized Monier-Williams method (OMW), produces false positive results with vegetables from the *Allium* (garlic) and *Brassica* (cabbage) genera due to extraction conditions that are thought to cause endogenous sulfur compounds to release SO_2. Recently, an LC-MS/MS method was developed for sulfite analysis but has only been tested with vegetable samples that are 100% *Allium* or *Brassica*. Analysis using the LC-MS/MS method showed concentrations below 10 mg/kg for the *Brassica* samples but only displayed a slight reduction in the *Allium* matrices. To get data on more representative products, three different blank matrices: potato chips, hummus, and quinoa were spiked with varying concentrations of a lab-made onion and garlic powder. It was determined that even at concentrations of 2% garlic or onion powder, the measured sulfite concentration was below the 10 ppm SO_2 labeling threshold. Higher concentrations of the powders were tested to determine how much garlic and onion would need to be added to a matrix to produce the concentrations that would result in a false positive response. Spiked recovery studies were conducted to determine if these methods would detect added sulfite. The LC-MS/MS method had recovery values of 108, 125, 116 and 107 % for water, fresh garlic, roasted garlic, and hummus, respectively. The ability to eliminate false positives will result in a greater reliability in the accurate determination of added sulfite to ensure compliance with sulfite labeling requirements.

Keywords: *sulfite, false positive, LC-MS/MS*

G33
DDPCR FOR QUANTITATIVE GMO ANALYSIS IN MAIZE, SOYBEAN AND RAPESEED

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As for many other countries, the European Union (EU) has established a legal framework to ensure that the development of Genetically Modified Organisms (GMOs) takes place in safe conditions both, minimizing the supposed risks for human, animal and environment before any GMO is placed on the market, and enabling consumers and professionals to make an informed choice about their use through clear labelling rules.
Few transgenic events (GMO varieties) are currently authorized to enter the EU market and any food or feed product containing GMOs as an ingredient in a proportion higher than 0.9% must be properly labelled. The enforcement of such regulations depends fundamentally on the ability to detect and quantify the presence of GMOs in food and feed. Theoretically both authorized and unauthorized events should be interrogated but, on one hand, the increasingly high number of transgenic events and, on the other, the lack of information about certain transgenic events, makes that task unaffordable. Despite different technical approaches developed for GMO testing in order to improve the throughput and cost-effectiveness (qPCR is considered to be the gold standard) there are still unresolved issues (*e.g.*, qPCR multiplexing is not trivial) and current routine GMO testing at a rational cost is far from being exhaustive since it is mainly based on screening tests for the presence of diverse regulatory elements and inserted genes commonly present among GMOs varieties.
Droplet digital PCR (ddPCR) technology allows direct and precise molecular quantification even at a high level of multiplexing and has been reported to be highly cost-effective and comparable to qPCR in terms of satisfying quality international recommendations for GMO quantification methods. Mainly based on the works published by the DECATHLON consortium, we have developed several multiplex ddPCR assays for the quantification of maize, soybean and rapeseed GMO crops. ddPCR multiplexed event-specific assays have proven to be useful in detecting and quantifying all GMO events authorised in EU (updated July 2017) coming from maize, soybean and rapeseed (31 transgenic events analysed in 5 ddPCR multiplex reactions). Besides, we have also successfully developed event-specific assays for all those non-authorised transgenic events for which certified reference material and confident scientific information is available (16 events covered in 3 multiplex reactions). Certified reference materials, GMO proficiency testing samples and diverse qPCR analysed samples were used to validate the accuracy of the quantification results.
The ddPCR method here presented yields reliable results in a fast and cost-effective way, helping both in testing compliance with the EU labelling regulation but also in detecting un-authorized trade of the main GMO events inside the EU.

Keywords: *ddPCR, GMOs quantification*

G34
FOOD CHEMISTRY SURVEILLANCE PROGRAMS AT THE CANADIAN FOOD INSPECTION AGENCY

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The Canadian Food Inspection Agency (CFIA) is dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada´s people, environment and economy. The safety of Canada´´s food supply is central to everything we do which is why the CFIA works from the farm gate to the consumer´s plate to protect public health. We safeguard not just the food supply, but also the plants and animals upon which safe and high-quality food depends. CFIA monitors raw agricultural commodities and finished foods for chemical contaminants and residues through four main programs: the National Chemical Residue Monitoring Program (NRCMP); the Children´s Food Project (CFP); Targeted Surveys; and Food Safety Oversight (FSO) studies. These programs involve collection of samples at various points in the food continuum, testing at accredited laboratories, follow-up of non-compliant results, and reporting of results to the Canadian public. The first, oldest, and largest of the testing programs, the NCRMP, involves inspector-based collection of samples at farms, abattoirs, warehouses, dairies, and other food processing facilites. The Program involves testing for veterinary drugs, pesticides and agricultural chemicals, metals, mycotoxins, dioxins and PCBs, and polycyclic aromatic hydrocarbons in meat, poultry, eggs, honey, maple syrup, milk and dairy products, and fresh and processed fruits and vegetables. The approximately 110 000 tests on the 18 000 samples collected generate an estimated 3.3 million results. Other monitoring programs involve samples collected at retail. The CFP focuses on foods primariy marketed to and consumed by children and infants. This sub-population is of particular concern because of their distinct consumption patterns and exposure potential due to their lower body weights. Pesticides and heavy metals are typically been analyzed but mycotoxins, veterinary drugs and bisphenol-A have also been analyzed as needed. The number of samples varies from year to year, depending on the scope of testing. The Targeted Surveys examine specific chemical hazards in finished foods. The chemical hazards fall into four categores - deliberately added chemicals, environmental contaminants, process-induced chemicals, and natural toxicants. The types of foods examined ranged from dried fruits and milled grains to multi-ingredient frozen meals. The purpose of a targeted survey is to gather baseline information and determine whether a problem exists. Typically, 14 000 - 18 000 samples are distributed over 25 surveys annually. The FSO studies involve testing of a specific chemical hazard or hazard class in a specific type of food. The areas identified for these surveys were those for which insufficient data existed to be able to determine compliance with Canadian standards with an acceptable level of certainty. An overview of the testing and a summary of the results for the 2015-16 fiscal year will be presented.

Keywords: *environmental, mycotoxin, pesticide, veterinary drug, contaminant*

Acknowledgement: *The authors wish to acknowledge the Operations and Science Branch staff of CFIA for their dedication and hard work which makes this work possible.*

G35
THE ADVANTAGES OF USING AN MRMHR ACQUISITION FOR INCREASED SENSITIVITY FOR TARGETED ANALYSIS USING A QTOF INSTRUMENT

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Introduction
One difficulty that is encountered when attempting to quantitate with a QTOF instrument using a fragment ion instead of the signal from the TOF acquisition is that the collision energy for a given fragment is not formed at an optimum collision energy. A novel feature of the X500R QTOF System is the ability to acquire specific MRM pairs just as are acquired in a triple quadrupole instrument thus improving the senstiviity of the method. This presentaiton discusses the concept of an MRMhr experiment and demonstrates the advantage of this technique.
Methods
A set of standards were analyzed using a standard data dependent scan where the parent ion of each compound is selected for fragmentation in Q1 and the ion is fragmented using a collision energy of 35 volts. A quantitation method was created using the two most intense fragment ions and an instrumental detection limit was determined. The same set of standards was re-analyzed using an MRMhr scan. The instrumental detection limit from the MRMhr data was determined and compared to the previous fragmentation data.
Preliminary Data
A limitation of traditional triple quadrupole mass spectrometers for quantitative applications is that compound identification relies on a ratio of two ions from the compound fragmentation. Interference from the matrix frequently causes this ration to be unreliable for compound identification resulting in both false positive and false negative results. The slow speed of acquiring full scan MS/MS spectra from a triple quadrupole instrument prohibits the acquisition of useful spectra for compound identification due to degraded cycle times. One of the advantages of QTOF instruments is the speed of acquiring full scan MS/MS spectra during sample analysis without the slow cycle times observed for triple quadrupole instruments. Unfortunately, quantitation is accomplished using fragments from a full scan MS/MS where the collision energy is not optimized for a given transition. The SCIEX X500R QTOF system has the unique ability to acquire MRM transitions with optimized collision energies resulting in triple quadrupole sensitivity but allows for the acquisition of full scan MS/MS for compound identification without sacrificing cycle time. This presentation will discuss the features of MRMHR and present data that demonstrates the improvement in sensitivity of MRMHR versus a fragment from a full scan MS/MS spectrum.
Novel Aspect
This is the first demonstration of using a QTOF to acquire MRM transitions with optimized collision energies

Keywords: *QTOF, MRM, MRMHR, MSMS, quantitation with QTOF*

Acknowledgement: *Paul C. Winkler; K.C. Hyland; Christopher Borton; Detlev Schleuder, Jianru Stahl-Zeng*

G36
DEVELOPMENT OF STRATEGIES FOR THE DETECTION OF ABUSE OF NATURAL STEROID HORMONES IN CATTLE USING A COMBINATION OF IMMUNOAFFINITY CHROMATOGRAPHY FOR SAMPLE PREPARATION AND GAS CHROMATOGRAPHY/COMBUSTION/ISOTOPE RATIO MASS SPECTROMETRY FOR DETECTION

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When synthetic forms of naturally occurring steroid hormones are administered to cattle, this abuse cannot be proven in urine samples by the classical confirmation methods which are based on LC-MS(/MS) or GC-MS(/MS) detection. GC-C-IRMS provides a valid confirmation method for natural hormone abuse detection. While classical protocols using solid phase extraction (SPE), liquid liquid extraction (LLE) and high performance liquid chromatography (HPLC) have been described in the past, these strategies are complex and very time consuming. In this context, immunoaffinity appears as a valuable alternative extraction tool due to its superior specificity. The first task concerns the preparation and characterization of the immunoaffinity gels for the targeted androgens, estrogens, progestagens and corticoids. The second task concerns the development of sample preparation strategies for bovine urine samples, including the use of the immunoaffinity gels before detection of the natural hormones with IRMS. During the first year, the objective was to provide proof of concept for androgen abuse detection. We prepared and characterized immunoaffinity gels for the specific purification and detection of etiocholanolone, as well as for an endogenous reference compound (ERC), 5-androstene-3β, 17α-diol. We produced antisera using two immunogens: etiocholanolone-3-HMS:BSA and epitestosterone-3-CMO:BSA. The best antisera in terms of titer, sensitivity and specificity were purified by isolating the type G immunoglobulins on Protein A column. The immunoaffinity gel for etiocholanolone was prepared by coupling 3 mg IgG per mL of sepharose gel, with a capacity of 1220 ng etiocholanolone /2 mL gel. Regarding epitestosterone, the best antiserum was chosen because of its important cross reactivity with the ERC. The epitetosterone gels were prepared by coupling 10 mg IgG/mL sepharose gel, with a capacity of 1500 ng/2 mL gel for ERC. Different sample preparation strategies were afterward evaluated, incorporating IAC cleanup steps into the existing protocol. Eventually, by using the two IAC columns in series, it was possible to eliminate all preparative HPLC-steps from the analytical protocol. The resulting method, consisting of subsequent hydrolysis, SPE, LLE, IAC and acetylation was successfully validated, and can successfully applied as a strategy for the detection of androgen abuse, using etiocholanolone as metabolite and 5-androstene-3β, 17α-diol as ERC.

Keywords: *IRMS, natural hormones, immunoaffinity*

Acknowledgement: *The authors acknowledge the Belgian Federal Public Service of Health, Food Chain Safety and Environment for the financial support.*

G37
IMPROVED UNDERSTANDING OF REPRODUCIBILITY PRECISION AND METHODS FROM PROFICIENCY TEST DATA

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Proficiency tests for quantitative analytical data are usually assessed by the z-score. The z-score equation includes the denominator term standard deviation for proficiency assessment (SDPA or σ_{PT}). Ideally, the value of σ_{PT} is objectively derived from model equations, themselves derived from large scale studies. The Horwitz equation or reproducibility estimates from collaborative trials are typical examples. What happens if a model formula is inappropriate for a particular analysis? Proficiency tests are themselves large scale sets of data so it is proposed here that the same data can be used to improve the understanding of reproducibility precision for future assessment purposes. Examples are provided from proficiency tests of total fat, HMF, mycotoxins, pesticide residues and water microbiology determinations. Furthermore, the method parameters and quality control measures reported by participants can be used to improve the understanding of what it is that laboratories actually do and whether the end result is affected. The example presented here is of recovery correction and use of internal standards in pesticide residues analysis. Consideration is given to guidance document SANTE/11945/2015 in light of studying the proficiency test data.

Keywords: *proficiency test, precision*

G38
THE USE OF A Q-TOF IN A ROUTINE CONTRACT LABORATORY

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On a Triple Quad Mass spectrometer used for targeted quantitative determination of pesticides in food and feed products the number of analytes are limited. Even with segmented MRM-traces a maximum of 400 pesticides with 2 or 3 MRM's is feasible. For a suit of 800 pesticides or more three injections are required in positive and negative mode. The common perception of a QTOF is that it has to be used as rather a screening instrument then a routine instrument for quantitative targeted analysis of pesticides. But as a scanning instrument the number of analytes in one run is only limited to extraction and chromatographic feasibility of the compound of interest. Given this with a QTOF it should be possible to reduce the amount of injections to two, one positive and one negative run. This approach will be much more efficient. At Nofalab we took the challenge to prove that The SCIEX X500-R QTOF is suitable for routine analysis of over 700 pesticides in one run with performance criteria meeting EU-regulations. Is it feasible? Not only the instrumental characteristics are in place to make such a task successful, but also the software is involved. The poster will cover the instrument capabilities (including the use of SWATH and HRMRM) and the use of data analysis to create an efficient and robust method for the analysis of pesticides in food and feed commodities.

Keywords: *TOF MS, SWATH, HRMRM, pesticide, multi residue*

Acknowledgement: *Sciex EMEA*

G39
BOVINE BILE IN THE DETECTION OF BANNED SUBSTANCES: AN OVERVIEW AND ANALYTICAL PERSPECTIVES

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European Union prohibited the use of growth promoters in food producing animals since 1988. In this context, the traditional analytical methods have been crucial in the development of monitoring residue plans mainly analysing urine samples collected at the farm and at the slaughterhouse. However, they are increasingly criticised because of the low percentages of positive results (which are assumed as unrealistically low) in the face of the high number of samples analysed in all the European territory [1]. An efficient control of abuses requires the knowledge of metabolic pathways, kinetics of elimination and tissue distribution. Several research have demonstrated that bovine bile bioccumulates steroids and resorcylic acid lactones (RALs). The first study measuring biliary concentrations after the administration of steroids in cattle has been published in 1975. Pottier and coll. [2] analysed several tissues and biological fluids after an *in vivo* experiment carried out implanting trenbolone acetate to four cows. After slaughtering, the highest concentrations of trenbolone were reached in bile followed by urine, liver and kidney. Later, other studies implanting or injecting anabolic agents to cattle demonstrated that biliary concentrations of marker residues of nortestosterone and zeranol were higher and longer detectable than in urine [3]. In consideration of all the above, a screening method able to detect simultaneously over 50 banned or not authorised substances in bovine bile using liquid chromatography coupled to high resolution mass spectrometry (LC-Q-Exactive) was developed and validated. The procedure showed detection capabilities lower than 1 µg/kg for more than 90% of investigated analytes, which belonged to seven classes: beta-agonists, corticosteroids, nitroimidazoles, RALs, sedatives, steroids and stilbenes. Two separate chromatographic runs were adopted: i) ESI negative mode for chloramphenicol, RALs and stilbenes; ii) ESI positive mode for all the other compounds. The application of multiclass methods instead of the traditional single class ones and the analysis of an alternative biological fluid can undoubtedly increase the chance to detect residues of illicit treatments.

[1] EFSA. Report for 2014 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. www.efsa.europa.eu/it/supporting/pub/923e
[2] Pottier et al. Plasma kinetics, excretion in milk and tissue levels in the cow following implantation of trenbolone acetate®. Journal of Animal Science. 1975, 41: 962-968
[3] Lega et al. Abuse of anabolic agents in beef cattle: Could bile be a possible alternative matrix? Talanta. 2017, 229: 188-197

Keywords: *cattle, bile, liquid chromatography-high resolution mass spectrometry, screening method, banned substances*

Acknowledgement: *This work received financial support from the Italian Health Ministry (Ricerca Corrente IZS UM 0012013 "New target matrices to control anabolic treatments in cattle").*

G40
DETERMINATION OF NANOMATERIALS IN PERSONAL CARE PRODUCTS

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Nanoparticles are currently present in many products of everyday use. Titanium dioxide particles as UV filters in sun screen lotions and creams, aluminium oxide in tooth pastes and iron oxide in cosmetics. In the EU, several regulations already address the presence of nanomaterials in products in the approval procedure, the safety assessment, and/or by a labelling obligation. These regulations cover e.g., biocidal products, cosmetics, medical devices, (novel) food and food additives as well as food contact materials. For instance, the labelling of nanomaterials on the ingredient list is obligatory for cosmetics (since 2013) and for food (since 2014).
Reliable analytical methods for the detection and quantification of nanoparticles in food and personal care products are thus required both for exposure assessment in the framework of risk assessment/management as well as for the enforcement of existing regulations. A number of analytical methods have been developed and validated within the EU research project NanoDefine which delivers tools for the enforcement of the EU regulations. In addition a NanoDefiner e-tool has been developed that can handle analytical data and is helpful for laboratories and regulators to decide whether a material is a nanomaterial. Examples of the developed methods and their validation and application will be reviewed.

Keywords: *nanoparticles, personal care products, sunscreen cosmetics, method validation*

G41
ORGANIC PARTICLES AS DELIVERY SYSTEMS FOR COMPLEX SOURCE OF ANTIMICROBIAL AND ANTIOXIDANT COMPONENT FOR FOOD APPLICATIONS

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Encapsulation of active component into organic particles can be used in food application to enhance taste, flavor or textures of foods. Encapsulation can also be used to improve the nutritional value of food product or as a delivery system for example for antimicrobial additives in food. The presented study was focused on preparation, characterization and application of liposomes and polysaccharides particles as transport systems for active component. Predominantly complex natural extracts with high antioxidant and antimicrobial effect were used as sources of bioactive compounds.
Lysozyme was used as an example of antimicrobial component isolated from animal sources. For particle preparation chitosan was used, which is important antimicrobial substance derived from animal polymer chitin. As antimicrobial component of microbial origin bacteriocin nisin was tested. As antimicrobial components of plant origin extracts of some medicinal plants were used.
In this work a complex study focused on encapsulation of some natural extracts into organic particles and its effect on stability, activity and bioavailability of active compounds is presented. Liposomes were prepared from soybean lecithin by sonication methods. Size of prepared liposomes was determined by dynamic light scattering, colloid stability was measured using a zeta potential. As a material for polysaccharide particles alginate and chitosan were used. Polysaccharide microparticles were prepared by gelation and cross-linking using the Büchi encapsulator B-395 Pro. Extracts were encapsulated individually or as mixtures of different origin. The efficiency of encapsulation was determined by HPLC/PDA and by spectrophotometry. Long-term stability of prepared particles and amount of released component in model/real foods and in a model physiological environment were monitored too. Antioxidant activity was determined by ABTS method. Antimicrobial activity of extracts before and after encapsulation was tested using gram-positive and gram-negative bacterial test strains. Antifungal properties were studied using the test system *Candida glabrata*. Cytotoxicity of particles was tested with MTT assay using human keratinocytes.
High antioxidant and antimicrobial effect was determined primarily in extracts from cloves, cinnamon, oregano, pepper, nutmag, star anise and astragalus. Significant enhancement of the antimicrobial effect has been achieved after encapsulation these extracts into liposome particles. The size of created particles was in range of 200 nm to 2000 μm. Particles were stable for more than two months stored in an aqueous model condition and retain their antimicrobial and antioxidant activity.

Keywords: *antimicrobial activity, antioxidants, encapsulation, liposomes, polysaccharide particles*

Acknowledgement: *This work was supported by project "Materials Research Centre – Sustainability and Development" No. LO1211 (Ministry of Education, Youth and Sport of the Czech Republic).*

G42
THYME AND OREGANO ESSENTIAL OILS: POTENTIAL NATURAL PRESERVATIVES FOR APPLICATION IN FOOD INDUSTRY

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Aromatic plants contain many phytochemicals that have been found to possess several bioactive properties. This is the case of essential oils (EO) which are known for their strong antimicrobial, antioxidant and many other properties. Because of that, and due to its recognition as eco-friendly and safety, these EOs have gathered great interest for industrial application, particularly in food industries as natural preservatives [1].
In this context, the present study evaluated the antioxidant and antimicrobial capacities of *Origanum virens* and *Thymus vulgaris* EOs, aiming their incorporation in whey protein-based active coating (WPC) for further application as natural preservatives. The two EO showed close antioxidant power, as measured by DPPH• and lipid peroxidation assays. In turn, thyme EO showed slightly better inhibitory concentrations (MICs) towards *Listeria monocytogenes* (0.3 mg/mL), *Salmonella typhimurium* (0.6 mg/mL) and *Staphylococcus aureus* (0.6 mg/mL) than oregano (0.3, 1.25 and 1.25 mg/mL, respectively).
The addition of oregano (but not thyme) EO to WPCs resulted in a decrease of coatings' moisture and water vapor permeability (WVP) in 27% and 19%, respectively, comparing to control. In turn, incorporation of thyme (but not oregano) EO caused an increase of 30% in WPC's total color variation (ΔE). Thickness, transparency and solubility suffered no alterations. Considering the importance of a low WVP for controlling water migration and consequent maintenance of moisture and texture in foods, the oregano-enriched WPC was thus selected for further application in two traditional Portuguese sausages, namely *Alheiras* and *Paínhos*.
After application, sausages were vacuum packaged and stored at 4ºC for 4 months and along this period, possible alterations in total microbial load, lipid oxidation and physicochemical parameters (texture, color, water loss, pH and titratable acidity) were monitored. Sensory analysis was carried out as well. None of the physicochemical parameters in both sausages suffered notorious alterations after EO-enriched WPC appliance, indicating that this coating does not interfere with the nutritional, color, texture and acidity profile of the sausages. On the other hand, total microbial load was found considerably lower in coated rather than uncoated sausages. Coated *Alheiras* but not *Paínhos* also revealed lower lipid oxidation. According to sensory analysis, no differences were noted between coated and uncoated sausages with exception of the aroma, which was no longer detected after cooking the *Alheiras* or peeling the *Paínhos*.
Overall, the oregano-enriched WPC shows good potential to be used as a natural preservative for controlling microbial spoilage and fat rancidification in these two sausages minimally affecting their physicochemical and sensorial properties.

[1] E. Christaki, E. Bonos, I. Giannenas and P. Florou-Paneri, *Agriculture*, 2012, 2, 228–243.

Acknowledgement: *Thanks are due to University of Aveiro, FCT/MEC for the financial support to the QOPNA research Unit (FCT UID/QUI/00062/2013), through national funds and where applicable co-financed by the FEDER, within the PT2020 Partnership Agreement. Marcelo D. Catarino acknowledge FCT for financial support (fellowship PD/BD/114577/2016).*

G43
SHELF-LIFE OF VIRGIN OLIVE OIL SEALED WITH CORK STOPPER

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Virgin olive oil is an edible oil of high economical value due to its nutritional and sensorial characteristics. Several studies have been done to study the stability of olive oil during storage and have concluded that the oxidation can be minimized through the control of temperature and restriction of light and oxygen over careful selection of packaging. However, the bottle closure is also an important issue given of the negative impact of oxidation process in virgin olive oil quality.
The aim of this study was to evaluate the shelf-life of virgin olive oil sealed with natural cork stopper.
Cork stopper compared with screw cap has the advantage of being 100% natural, reusable and recyclable and is one of the world's most versatile materials.
To evaluate the effect of bottle closure some quality parameters were performed during the storage period of 24 months, after harvest, comparing the effect on virgin olive oil quality of three types of glass bottle closures: screw cap, natural cork (covered with bee wax) and bartop cork stopper. Most of the chemical parameters (mainly oxidative index´s and phenol content) studied showed that the cork stopper has similar results to the screw cap during the storage time tested. The olive oil sealed with a cork stopper covered with bee's wax have a composition very similar to the olive oil composition at the beginning of the test
The consumer preference is more positive when the bottle seal is a sustainable one, like cork, than when screw cap is used.

Keywords: *cork, olive oil, oxidation, phenols*

Acknowledgement: *Amorim Top Series Unit by the cork stopper availability; "Herdade Tapada da Tojeira" for raw material availability. Centro de Estudos Florestais is a Research Unit funded by FCT within UID/AGR/UI00239/2013.LEAF, Linking Landscape, Environment, Agriculture and Food is a Research Unit funded by FCT within UID/AGR/04129/2013.This study was also supported by Project CENTRO-04-3928-FEDER-000009 - Beira Baixa Terras de Excelência - Comunicação, Animação e Inovação.*

G44
FAST AND RELIABLE QUANTIFICATION OF VIABLE LEGIONELLA SPP. AND IDENTIFICATION OF LEGIONELLA PNEUMOPHILA IN WATER BY REAL-TIME PCR

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Rising numbers of legionellosis outbreaks all over the world show the high demand of diagnostic tools to monitor water systems such as drinking water systems, cooling towers and air conditioning systems. However, fast action against *Legionella* is hampered by the reliance on microbial culture, which may take up to 14 days to result.
To offer a rapid, quantitative real-time PCR assay for the simultaneous detection of *Legionella* spp. and *Legionella pneumophila* in water, including discrimination of *Legionella pneumophila* serogroup 1, as this serogroup accounts for more than 90% of all infections.
Conventional PCR assays have the disadvantage of detecting DNA from live and dead cells alike. In contrast, the PCR assay is designed to quantify live cells only, employing a novel viability reagent. The process also detects VBNC cells, which are missed in bacterial culture. To aid rapid handling, a filtration unit for filtration of up to 1 liter of water has been devised. The unit houses a small sized filter that can be processed in standard microcentrifuge tubes.
Specificity of the assay was tested with a large panel of *Legionella* species as well as close relatives and bacteria of the same habitat. The assay showed perfect inclusivity and exclusivity among this wide variety of *Legionella* strains and other waterborne microorganisms.
The system also displays high sensitivity with detection of at least 10 genomic equivalents per reaction for DNA extracts and about 10 colony forming units per filtrated sample. Reliable quantification is possible to levels below 100 cfu/filter and closely matches cfu counts obtained by standard methods.
To guarantee comparable results in diagnostic routine, the new foodproof® *Legionella* Quantification LyoKit was compared against standard methods like ISO 11731.
In conclusion, this novel diagnostic approach will improve *Legionella* surveillance in water systems to prevent new infections.

Keywords: *Legionella spp., Legionella pneumophila, quantitative real-time PCR, drinking water systems*

G45
EFFECT OF GAMMA RADIATION PROCESSING ON THE ANTIOXIDANT ACTIVITY OF GINGER

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Biological activity of ginger, especially antioxidant activity, has been associated to their bioactive compounds, gingerols, mainly 6-gingerol. Foods have some key compounds, which regulate their taste, aroma and nutritional profile, even if they are present in low concentration. These compounds used to be sensitive to irradiation in high doses. The aim of this study was to evaluate the effectiveness of gamma irradiation from ^{60}Co at doses 0, 5, 10, 15 and 20 kGy on ginger (*ZINGIBER officinalle* Roscoe), particularly about its antioxidant activity. The quantification of phenolic compounds was performed by Folin-Ciocalteu method and assessing the potential of antioxidant activity by the free radical [2,2 difenil-1-pricril-hidrazil (DPPH•)] scavenging and by Rancimat® method. The 6-gingerol quantification was performed by High Performance Liquid Chromatography (HPLC). There were no significant differences of total phenolic compounds in the irradiated samples compared to control ($p>0,05$). Eventhough, no irradiated extract showed higher ability on free radical scavenging. The Rancimat® method showed that antioxidant activity index (AAI) was not significant different ($p>0,05$) between analyzed extracts, as well as 6-gingerol quantification. It could be concluded that gamma radiation processing may be a feasible alternative for ginger because it does not significantly alter its major phenolic compounds or its significant antioxidant potential.

Keywords: *food irradiation, biocompounds, ZINGIBER officinalle roscoe*

Acknowledgement: *The authors are grateful to IPEN-CNEN/SP, CNEN, FSP/USP, CNPq. and CAPES for the financial support.*

G46
USING ELEMENTAL ANALYSIS FOR DISCRIMINATION OF PINOT NOIR WINES FROM SIX DIFFERENT DISTRICTS IN AN AVA

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The application of multi-element profiling of macro, micro and trace elements in wine has been proposed as a means of establishing authenticity and typicity. Previous studies have considered the differences between countries and wine regions but there is very limited information for wines made from grapes grown within the same wine region, let alone single cultivar wines from within a wine district. Twenty-five Pinot noir wines from 6 districts within a Californian AVA were analyzed for 49 elements using a combination of inductively coupled plasma-mass spectrometry (ICP-MS) and microwave plasma atomic emission spectroscopy (MP-AES) instrumentation. All wines were from the 2016 vintage and analysis at approximately 6 months of age, with little or no barrel contact and not fined or treated for physical instabilities. Canonical variance mapping using the single elements and various ratios of elements revealed complete separation of the wines into 5 groups based on the district and tight clustering of the wines from each district within its group. The complete resolution is achieved with the first two factors which together account for 87% of the total variance. The separation between the district groups is between 3 and 5 times the dimension of the district groups. The method has potential applications in tracing authenticity and understanding the contributions of site to wine elemental composition.

G47
FLUORESCENCE POLARIZATION IMMUNOASSAYS FOR DETECTION OF SEVERAL AMPHENICOLS

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The quality and purity of food samples must be controlled and it must be in global scale with high-throughput screening (HTS). The current methods of assays are gas and liquid-chromatography mass spectrometry and immunoassays, mainly enzyme-linked immunosorbent assays (ELISA). The current trend of immunoassays is developing multi-analyte immunoassays for more than one analyte can be detected per test. Fluorescence Polarization Immunoassay (FPIA) could be the optimal method for screening and quantitative monitoring of level of contamination with toxic compounds. The FPIA is homogeneous method based on the competitive binding and the measurement of fluorescence polarization value for reaction mixture of sample (typically 10-50 µl) and immunoreagents: fluorescent-labelled antigen (tracer) and specific antibodies. The total time required for an assay is few seconds or minutes. The limit of detection is 10-100 ng/mL and could be reached lower than typical Maximum Residue Limit for toxicants. The results for development FPIAs for amphenicols: Chloramphenicol, Florfenicol and Thiamphenicol will be presented. Using different combination of immunoreagents (tracers and antibodies) different specificity of immunoassays could be received for specific detection for each amphenicol in parallels FPIAs or class-specific method based on broad specific antibody.

Keywords: *immunoassay*

Acknowledgement: *The works of Sergei A. Eremin under obtaining and studying the fluorescent tracers were financially supported by the Russian Science Foundation (grant 14-16-00149).*

G48
THE WALLOON DAIRY SECTOR UNDER THE INFINIPLEX FOR MILK (IPM) MAGNIFYING GLASS

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In Belgium, the absence of antibiotic residues is checked on each milk delivery from the farm to the industry. This control is done by means of a sample taken at the time milk is collected by the tanker at the dairy farm.
These samples are analyzed within an inter-professional laboratory, according to a protocol defined in the legislation. Analyses combine bacterial growth inhibition test and receptor test.
Sometimes, for complex samples, this protocol can however require up to ten hours of processing to get the final result. In order to simplify the control protocol, a study was conducted by means of InfiniPlex for Milk IPM (Randox Food Diagnoses). InfiniPlex for Milk is a screening test which can analyze up to 129 compounds (antibiotic, non-steroidal, anti-inflammatory drugs, anti-parasitic drugs and mycotoxins) simultaneously in 25 µL of raw bovine milk within 2 hours.
The study whose results are presented is based on positive samples which screened positive during the first screening test, and were subjected to analysis by means of the IPM. Samples were collected during the first quarter 2017 at the inter-professional laboratory in Battice, and consisted of the following groups of samples: (n=16, group I) led to a penalization, for others (n=22, group II), antibiotics were highlighted but with contents lower than the MRL and for the rest (n=37, group III), the positive result with the growth inhibition screening test could not be correlated to any antibiotic compounds.
Some samples, for which a molecule was identified, were diluted in order to evaluate the result in relation to the MRL. The IPM confirmed the presence of antibiotics with core b-lactam in 15 samples out of 16 (group I), neomycin having been detected in the last sample. In group II, a sample could not be analyzed, due to the quality of the milk, and no contaminant was detected in four samples. In group III, two samples could not be analyzed, and five samples did not contain any residue. Thirty samples contained at least a b-lactam and 1 sample showed traces of rifaximin. After dilution, the result of this sample became negative. The use of the IPM made it possible to detect the presence of non-antibiotic residues: prednisolon, nitroxynil and aflatoxine M1. These groups must be confirmed with analytical protocols.
The IPM made it possible to confirm the massive presence of antibiotics with b-lactam core in the samples coming from the dairy sector and giving a positive result to the microbiological screening test (83%). The study showed the interest of this test with broad spectrum since compounds of other natures were detected. A broader study should be carried out on the whole of milk collections distributed over one year, thus integrating the various periods of the dairy cattle (seasons, calving) and specificities of modes of breeding (organic, extensive, intensive).

G49
OCCURRENCE OF SELECTED PHARMACEUTICALS IN THE TEJO ESTUARY

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The ubiquitous presence of pharmaceuticals compounds in the aquatic environment has rendered these substances the designation of persistent contaminants. Thus, monitoring their presence in water bodies is crucial and a priority concern since they can inflict effects in non-target organisms at very low concentrations. Moreover, such effects must be investigated in order to reduce and manage the impacts of such stressors in the environment.
Estuaries are vital ecosystems, with very high productivity and providers of natural and recreational services. As such, all over the world these areas are densely populated which results in high anthropogenic pressures in return.
Therefore, assessing the presence of pharmaceuticals in such ecosystems is of paramount importance. The present study aimed to investigate the occurrence of a selection of pharmaceuticals in the Tejo Estuary (Portugal). As part of the monitoring plan, thirty sampling sites were selected and at each site water, sediment, fish, invertebrates and plants were collected with replicates (minimum 3). The selection of pharmaceuticals monitored included antiepileptic and anticonvulsant drugs, benzodiazepines, anti-inflammatories and analgesics, angiotensin receptor blockers, b-blockers and antibiotics in a total of 67 compounds. Detection and quantification was carried with a multi-residue multi -class UHPLC-ToF MS method developed and validated. Results show that most of the compounds assessed were detected, with anti-inflammatories (e.g. diclofenac), angiotensin receptor blockers (e.g irbersartan) and antibiotics (e.g flumequine, doxycycline, cinoxacine and sulphathiazole) with the highest concentrations, throughout the estuary.

Keywords: *estuary; pharmaceuticals; monitoring; UHPLC-ToF MS*

G50
QUICK EASY AND COST EFFECTIVE METHOD TO PROVE PROTEIN FORGERY

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The focus at protein counterfeiting was in 2008 when milk powder was mixed with melemine to increase its apparent protein content. Unfortunately, these protein powders made for children demanded children´s lives [1]. The traditional and most widely used protein measurement methods (DUMA, Kjeldal) are measuring nitrogen content and cannot distinguish between protein or other sources of nitrogen. Conversion of the nitrogen content into protein equivalents by factor numbers [2]. This involves the risk of identifying all nitrogen-containing compounds as the sample of proteins. Protein counterfeiters have also recognized this deficiency, and unfortunately, they take advantage of the incorrect identification of the measurement methods. [3]. Counterfeiters mixing much cheaper nitrogen-contain compounds such as amino acids (glycine) to the protein and they don't labeling it on the package Counterfeiting is brilliant because it can only be proved if it is measured with amino acid profile analysis after acidic hydrolysis. [4]. To do this usually with HPLC-UV, -FID [5] or HPLC-ESI-MS-MS [6] coupled system are used after performing post or pre-column derivatization, which are costly and often unmanageable in industrial conditions.
In this study, our aim was to develop a simple and cost effective method to prove protein forgery in the case of a food matrix for sports nutrition. Gerhart DUMA was used to determine the total nitrogen content of the samples (Accuracy: +/- 2%, linearity: 0.992, quantitative determination limit: 15.9 m/m%). NIR spectrometer was validated in reflection mode to quantify protein content. For validation, 260 protein-containing sports nutrition food (guaranteed to be not counterfeited) were used, with a protein content of 10 to 90 m/m%. Among the many function transformations, the first derivative gave the most accurate estimation function. The performance the method were determined by re-measuring samples of known concentration (accuracy: +/-2%, quantitative determination limit: 30 m/m%). Protein counterfeiting was modeled with collagen and glycine addition experiments. We used oat flakes as ballast material. According from the obtained results, it can be concluded that if the two measured results (NIR, DUMA) weight percentages differences are greater than 5%, then we are facing with a protein-forged product.
So there is a way of identifying and filtering collagen or glycine-enriched proteins.

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Keywords: *protein, forgery, DUMA-NIR*

G51
DEVELOPMENT AND VALIDATION OF A MULTI-LOCUS DNA METABARCODING METHOD TO IDENTIFY ENDANGERED SPECIES IN COMPLEX SAMPLES

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DNA metabarcoding holds great promise for species identification in complex samples such as food supplements and traditional medicines (TMs). Such a method would aid CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) enforcement officers to combat wildlife crime by preventing illegal trade of endangered plant and animal species. The objective of this research was to develop a multi-locus DNA metabarcoding method for wildlife forensic species identification and to evaluate the applicability and reproducibility of this approach across different laboratories.
A DNA metabarcoding method was developed that makes use of 12 DNA barcode markers that have demonstrated universal applicability across a wide range of plant and animal taxa, and that facilitate the identification of species in samples containing degraded DNA. The DNA metabarcoding method was developed on the basis of Illumina MiSeq data generated for 15 well-defined experimental mixtures, and for which a bioinformatics pipeline with user-friendly web interface was developed. The performance of the DNA metabarcoding method was assessed in an international validation trial by 16 laboratories, in which the method was found to be highly reproducible and sensitive enough to identify species present in a mixture at 1% dry weight content.
The advanced, multi-locus DNA metabarcoding method assessed in this study provides reliable and detailed data on the composition of complex food products, including information on the presence of CITES-listed species. The method provides improved resolution for species identification, while verifying species with multiple DNA barcodes contributes to an enhanced quality assurance.

Keywords: *endangered species, CITES, DNA metabarcoding, COI, matK*

G52
STABLE CARBON AND NITROGEN LABELLING APPROACH FOR ASSAY DEVELOPMENT OF NISIN A IN RIPENED CHEESE, PROCESSED CHEESE, MASCARPONE AND CLOTTED CREAM BY LC-LRMS, AIDED BY LC-HRMS CONFIRMATION

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Nisin A is a type-A lantibiotic (peptide) added during production of ripened cheese, processed cheese, mascarpone and clotted cream to prevent spoilage by inhibiting the growth of gram- positive bacteria. The present study presents a stable carbon and nitrogen isotopic dilution assay (SIDA) approach in tandem with 3.5kDa size exclusion membrane filter as utility by liquid chromatography-tandem mass spectrometry method for accurate detection of Nisin A in cheese and cream products. LC-LRMS (linear ion trap) was applied as primary tool for assay development, with LC-HRMS (time-of-flight-tandem MS) as confirmation technique for detection of salient precursor ions of Nisin A adducts of m/z 671.31632 ($[M+5H]^{5+}$) and 838.89358 ($[M+4H]^{4+}$), respectively. Matrix effect for individual cheese and cream products are examined at six concentration levels of 2, 4, 8, 12, 16 and 20 mg/kg. Spiking experiments in individual matrix of cheese and cream dairy products are evaluated at three concentration levels of 4, 8 and 12 mg/kg, respectively. Limits of detection and limits of quantitation are also determined in individual matrix. The role of bovine serum albumin in preparation of working standards is also examined for the first time. The availability of a rapid and accurate SIDA method becomes available for determination of Nisin A in cheese and cream products.

Keywords: *Nisin A, LC-LRMS, LC-HRMS, SIDA*

Acknowledgement: *The authors would like to acknowledge Health Sciences Authority for the financial support (OPG110210).*

G53
A COST EFFECTIVE SAMPLING APPROACH FIT FOR DIETARY EXPOSURE ASSESSMENT TO FOOD CHEMICALS OF VARIOUS SUB-SAHARAN AFRICA POPULATIONS

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Aim: In order to build capacity of national food safety authorities of Benin, Cameroon, Mali and Nigeria, this survey aims to assess the dietary exposure of populations of Sub-Saharan Africa to food chemicals.
Method: Household budget surveys released by national institutes of statistics of Benin, Cameroon, Mali and Nigeria gather food expenditure data of more than 70.000 households. These data were processed to estimate mean daily intakes of food items as consumed. We selected 2 study centers per country, including 3 densely populated coastal cities and 5 semi-arid areas located near the Sahara. Core foods fit for sampling were identified on the basis of mean daily intakes in grams per adult male equivalent per day, so as to cover at least 90% of the average total diet and more than 50% of each food group by weight. The breakdown of 12 subsamples per composite sample was determined according to 3 complementary approaches based on *(i)* food balance sheets and origins of imported food commodities *(ii)* food consumption statistics at the most detailed stratum of the food classification and *(iii)* random selection from main market places.
Results: We set up a sampling plan, which is *(i)* fit for the Sub-Saharan Africa Total Diet Study *(ii)* replicable in other developing countries. We developed a cost-effective and representative sampling approach, in order to characterize food chemical concentrations, prior to performing dietary exposure assessment.
Conclusion: In spite of a number of limitations associated to this approach, subsequent food analysis will provide a useful baseline assessment of food chemical concentrations and dietary exposure, which are likely to be used for comparison with future surveys, and to identify *(i)* priorities in terms of food control and surveillance as well as *(ii)* the need for further research, either on specific food chemicals, or on specific populations groups in a part of the World, where this type of data may not be as common as it is elsewhere.

Acknowledgement: *The Sub-Saharan Africa Total Diet Study is a Standard and Trade Development Facility funded project (STDF Project Grant 303, 2014-2018), implemented by the Food and Agriculture Organization of the United Nations (FAO) with the technical support of the Wor…*

G54
EXTENDING THERMAL STABILITY AND COLUMN LIFETIME OF WAX GC COLUMN

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Today's demanding GC and GC/MS applications mainly focus on sensitive and reproducible qualitative and quantitative analysis of more challenging analytes. WAX GC columns, based on 100% Polyethylene Glycol (PEG) stationary phase, are commonly used for analysis of a wide variety of compounds with polar functional groups. They are an ideal choice for numerous applications in quality control and method development labs. Applications in these industries often involve complex mixtures of polar compounds containing a variety of polar functional groups.
Traditional WAX GC columns have had some shortcomings. In comparison to polysiloxane stationary phases the maximum operating temperature of a WAX GC column is much lower, mainly up to 250°C/260°C. A new innovative WAX column is developed, with improved thermal stability, giving better column-to-column reproducibility over the life of the column. With its maximum temperature limits of 280°C isothermal and 290°C programmed for standard 30m x 0.25mm x 0.25µm configuration, this provides several advantages.

Keywords: *WAX, PEG, maximum operating temperature, polar compounds, column lifetime*

G55
INFLUENCE OF PROCESSING ON HONEY – NEW INSIGHTS USING NMR HONEY PROFILING™

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For many years adulteration of honey is a huge issue for the honey market. Sometimes it may occur unintentionally through feeding bees during bad weather conditions, but also by intentionally adding sugars/syrup to the final product. According to USP honey is on third rank of the most often adulterated foodstuffs.
Adulteration may occur in many different ways, for example by changing the botanical or geographical origin on, by feeding bees during nectar flow, by adding cheap syrup to honey, but also by processing of honey to remove unwanted substances like antibiotics and to reduce the water content. The resulting product does not comply with the EU honey directive (Council Directive 2001/110/EC of 20 December 2001 relating to honey) and cannot be marketed as honey.
The capabilities of NMR honey profiling™ in terms of determining geographical and botanical origins as well as adulteration with syrups has been presented before. This presentation describes how results of untargeted NMR honey profiling™ can lead to the discovery of new marker substances. In this case the marker substance finds its way into blossom honey either through adulteration with high end syrups or through treatment with ion exchange resins as part of purifying processes.

Keywords: *honey, processing, NMR, adulteration, fraud*

G56
A STUDY OF THE RELATIONSHIP AMONG THE Δ15N VALUES OF SOILS, FERTILIZERS AND PRODUCTS IN ORDER TO ENSURE THE TRACEABILITY OF THE ORGANIC HORTICULTURAL PRODUCTION FROM THE SOUTH OF SPAIN

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In the last decades the demand for organically produced foods has increased constantly and the farmers have adapted their production to those demands. The increase of declared organic agriculture productions under intensive conditions has underpinned new reflections on the interest of organic producers and the inputs that are used in those systems.
Organic products tend to retail at a higher price than the conventionally grown, mainly due to the certification costs and the yield gap. This yield gap is supplied by the premium price and the increasing demand of the organic foods. The higher profits increase the risk of fraudulent techniques in the organic food along the different levels of the supply food chain. The organic farming producers use organic fertilizers (animal manures, composts...) following the legislation guidelines (ER 834 /2007 and ER 889/2008) to maintain the soil fertility since the synthetic fertilizers are not authorized. In this sense, it is a challenge for the analytical techniques to assure the origin of the agriculture inputs and recent advances on this matter have been done using the signature of stable isotopes of nitrogen. In the case of synthetic fertilizers the isotopic nitrogen signature ($\delta^{15}N$) differs markedly from the organic ones. However, the isotopic fractionation processes undergone to obtain the organic fertilizers and the different isotopic fingerprints of the inputs used, complicate the interpretation of results between source-sink, and their applications in organic foodstuffs. The relations among the $\delta^{15}N$ (‰) from the agricultural inputs and the products obtained are not completely studied. This is one of the reasons why to date there is not a validated analytical technique to discriminate between organic and conventional foodstuffs.
The main objective of this work was to underpin this issue by studying the relationships among inputs, soils and horticultural products produced in one of the most important areas of production in Europe (Southeast of Spain). The results showed that using this new approach we could be able to follow the organic production and therefore to improve the certification mechanisms.

Proposal for a Regulation of the european parliament and of the council on organic production and labelling of organic products. Available: http://ec.europa.eu/agriculture/organic/documents/eu-policy/policy-development/report-and-annexes/proposal_en.pdf; 2014.

Keywords: *traceability, organic production, soil, fertilizers, nitrogen isotopes*

Acknowledgement: *This work has been funded by IFAPA and the European Rural Development Fund (ERDF) through the Project "Viabilidad de la relación de isótopos estables de nitrógeno (15N/14N) como metodología para la caracterización de la producción ecológica frent.*

G57
HIGH-THROUGHPUT ENZYME ACTIVITY SCREENING OF RAW MATERIAL FOR FOOD CONTROL

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It is well known, that natural occurring enzymatic activity causes various issues in food production. Raw material harvest variation can influence flour quality in baking and phase separation in juice production.
Enzymes are naturally present in all raw materials, some are beneficial, like amylases in grain, some are less beneficial, like stabilizer-degrading enzymes, which shorten the shelf-life of products. Either way, the more you know about the enzymatic composition of the raw material, the easier the processing will be.
In the baking industry, the dough quality is dependent on amylase activity. Existing methods, the falling number method for example, is over 70 years old and provides an empirical result. We have developed a direct method to analyse amylase activity quantitatively. The protocol is simple, we just add the flour directly to our chromogenic substrates and after incubation: the enzyme activity is detected using spectrophotometry.
Another example of enzyme activity in raw material is experienced in the juice industry, where glucanases are causing trouble as they break down the stabilizer carboxymethylcellulose (CMC). This decreases shelf-life and alters product appearance. By monitoring CMC degrading enzymes, it is possible to ensure high quality of the final product. We have developed a customized chromogenic stabilizer substrate to imitate the original raw material complexity. Then we added different fruit concentrates to this substrate and directly measured the degradation of the stabilizer.
With GlycoSpot enzyme screening technology, we have found a way to analyse enzyme activity in raw-material in a fast, easy and reliable way to ensure optimal food control.

G58
NEW HIGHLY SENSITIVE LATERAL FLOW RAPID TESTS FOR FOOD TOXICANT DETECTION

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Immunochromatographic strips, combining ease of analysis, low cost, and visual assessment of the results, often have inferior sensitivity to common immunochemical and physico-chemical analytical methods. We have proposed and characterized a set of new methodological solutions to overcome this limitation. The following restriction to traditional competitive immunochromatography was analysed and overcome. When analysing low molecular weight compounds, a competitive scheme is used. The registered signal is formed due to the binding of specific labelled antibodies to the antigen immobilized on the test zone. The visual detection limit corresponds to the antigen concentration that completely prevents this reaction. Small amounts of antigen prevent the binding of part of the marker and cannot detected visually. Thus, only a significant amount of antigen in a sample completely inhibits the binding of all label antibodies and provides reliable detection. This is why the sensitivity of the test is low.
We suggest a new scheme with registering labelled antibodies that bind to the sample antigen (instead of the registration of non-bound labelled antibodies by the traditional scheme). Such antibodies are knocked out of the zone containing the immobilized antigen and pass it. The next zone of the test strip (with anti-species antibodies, protein A/G, etc.) is used to account for them. The intensity of its staining increases as antigen concentration increases. The proposed scheme enables the visual detection of lower concentrations. Thus, the detection limit of mycotoxin deoxynivalenol that was achieved was 100-fold lower than traditional analysis (2 vs 200 ng/mL). Another approach that was realised was the replacement of commonly used conjugates of specific antibodies with labels with a combination of native specific antibodies and secondary (anti-species) antibodies or streptavidin conjugated to the label. The test strip that was developed is a dry chemical device that provides two-step immunochemical interactions—the formation of antibodies-antigen complexes and their detection by labelled anti-species antibodies. Using the new scheme, we developed test-strips for the detection of six key mycotoxins: ochratoxin A, zearalenone, deoxynivalenol, aflatoxin B1, fumonisin, and T2-toxin. The detection limits in the raw water–organic extracts of grains (corn and wheat) and nuts were decreased 10-20 times and reached levels of 50 pg/mL. The systems developed show high recovery values (not lower than 90%) for the detection of mycotoxins in grains. The analysis can be implemented within 15 min in non-laboratory conditions. These characteristics indicate that the proposed approaches have good prospects for improving the various immunochromatographic tests.

Keywords: *mycotoxins, rapid test, lateral flow assay*

Acknowledgement: *The work was financially supported by the Grant of the President of the Russian Federation for state support of young Russian scientists—PhD No. MK-2075.2017.4 (agreement No. 14.W01.17.2075-MK).*

G59
NEW PRODUCTS FROM PARMIGIANO-REGGIANO MANUFACTURING CHAIN: CHARACTERIZATION OF THE NITROGEN FRACTION AND STUDY OF THE BIO-FUNCTIONAL PROPERTIES

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Parmigiano-Reggiano is a worldwide known Italian, long ripened, hard cheese. Its inclusion in the list of cheeses bearing the protected designation of origin (PDO, EU regulation 2081/92) poses restrictions to its geographic area of production and to its technological characteristics.
With the aim of innovating the product from the health and nutritional point of view, using ingredients and technologies suitable with the PDO regulation, the research project Parent was funded by the Emilia Romagna region and the EU (POR – FESR 2014-2020). In the first part of the project, the production of defatted lyophilized and of low salt content Parmigiano-Reggiano was addressed.
Concerning the production of the defatted and lyophilized cheese, 5 different defatting procedures (Soxhlet, Nilsson, Rose Gottlieb, Folch and supercritical CO_2) were initially tested. The nitrogen fraction (free amino acids, non-proteolytic aminoacyl derivatives, peptides and proteins) of the obtained products was characterized and compared with the one of the whole cheese; the nitrogenous molecules were extracted, analyzed by LC-MS, identified and quantified. Soxhlet procedure was chosen for the production of lyophilized Parmigiano-Reggiano since it leads to the lowest loss of the nitrogen fraction. Above all, the preservation of the low molecular weight species was considered important, since they are the main responsible for the taste and the bio-functional properties of the cheese. Moreover, bio-functional properties were evaluated and the digestibility was tested through simulated gastro-intestinal digestion experiments.
To obtain Parmigiano-Reggiano cheese with lower salt content (25% less NaCl), but with the same organoleptic properties of the traditional one, various parameters were changed in the production process (wheel size and duration of the pre-salting and salting period), producing 13 different cheese wheels, that are being aged.
Since the salt content is relevant to the activity of the proteases responsible, during the aging period, for the degradation of caseins to yield small peptides and amino acids, its variation could have a considerable impact on the properties of the product. Therefore, the evolution of the nitrogen fraction is being monitored during the aging of the low salt content Parmigiano-Reggiano wheels. Samples were taken after 6 months of aging at 3 different depths of the wheels and the nitrogen fraction was characterized and compared with that of traditional Parmigiano-Reggiano. Other samples will be taken and characterized after 9 and 12 months of aging.
The obtained results will be considered in the selection of the best parameters to be used for the production of low salt content Parmigiano-Reggiano.

Keywords: *Parmigiano-Reggiano, proteolysis, nitrogen fraction, bio-functional properties*

Acknowledgement: *The research project Parent (POR – FESR 2014-2020) is kindly acknowledged for funding.*

G60
FOOD CROSSING DISTRICT, AN ALTERNATIVE PATH FOR INDUSTRIAL SYMBIOSIS AND VALORIZATION OF DURUM WHEAT BRAN BY-PRODUCTS

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The agri-food sector generates significant quantities of by-products and waste, the disposal of which produces negative environmental and economic impacts. The adoption of an industrial symbiosis approach to transfer and share resources between dissimilar industries, reflects recent European strategies on decoupling economic growth from environmental impacts. In the Food Crossing District project, the durum wheat bran by-products were investigated to obtain added-value products and/or other products for diverse non-food industrial sectors. The lipid matter present in five diverse fractions of durum wheat bran by-products were studied as related to shelf-life (30 days, at 25°C). The main lipid classes (triacylglycerols (TAG), diacylglycerols (DAG), monoacylglycerols (MAG), free and esterified sterols, free fatty acids (FFA)), total fatty acid (FA) composition and free acidity, were determined in each lipid extract obtained from the different by-products. The lipid content (3.6%-6.3%) significantly changed among the by-products, showing a significant increase of free fatty acids during the storage while TAG dropped. The lipid matter was mainly composed by polyunsaturated fatty acids (57.20%±0.36 – 59.43%±0.72 of total fatty acids), of which linoleic acid was the most representative. The free acidity found confirmed an intense lipase activity during storage, since the highest value (65.3%) was reached after 30-day of storage. The oil obtained from durum wheat bran by-products could be used as a functional ingredient due to its high content of PUFA and low amount of SFA, with an important contribution of linoleic acid (essential fatty acid). The results obtained will be used for the evaluation of environmental sustainability (by Life Cycle Assessment (LCA)) of the oil extraction and production at both industrial and market levels. The definition and optimization of industrial symbiosis routes will be supported by the implementation of a software tool for the collection and processing data from enterprises; this dynamic mapping of circular economies of the durum wheat chain will allow to identify possible system synergies.

Keywords: *durum wheat bran, by-products, oil, lipids, durum wheat bran oil*

Acknowledgement: *We thank the POR FESR 2014-2020 Food Crossing District project for the financial support, as well as Barilla G. and R. F.lli Spa for kindly supplying samples.*

G61
HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) FOR LC-MS/MS ANALYSIS OF CITRULLINE IN VARIOUS BIOLOGICAL MATRICES

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Citrulline is an amino acid, which has been discussed as an useful biomarker of gut health in humans and pigs.[1-3] Our goal is to develop a robust and accurate analytical method for monitoring of intestinal permeability and absorption in pig. The most interesting candidates for citrulline analysis are serum and urine. These samples represent a substantial hurdle for the sample preparation to ensure an accurate and reproducible MS quantification. Moreover, chromatographic separation must efficiently separate citrulline from arginine, which is always present in biological matrices and has almost identical MS/MS transitions. The starting point for method development is previously described detection of citrulline - based on HILIC separation.[4] We compare various HILIC and RP stationary phase chemistries in suitability for analysis of citrulline in biological samples. The final method allows minimal sample clean-up and a short measurement time.

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Keywords: *LC, tandem MS, biomarker*

G62
ELECTROCHEMICAL IMMUNOSENSOR FOR RAPID AND SPECIFIC DETECTION OF FOODBORNE PATHOGENS

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Foodborne illnesses caused by pathogens and their toxins pose serious threat for human health. Many pathogens have been identified as a cause of food-borne outbreaks but for the most of them are responsible especially *Salmonella spp., Listeria monocytogenes* and *Escherichia coli* [1,2]. Standard methods including several consecutive steps as culture enrichment, selective enrichment and plating followed by standard serological or biochemical confirmation are considered as a gold standard. Time-consuming selective plating steps could be replaced by qPCR or ELISA assays, but this strategy has led to shortening of detection time from the current 5 days to just 2 - 3 days, the positive samples must be still confirmed by traditional microbiological approach [3]. Therefore, the development of rapid (in hours), sensitive/specific and robust method for foodborne pathogens detection continues to be a challenge.
Our strategy suitable preferably for liquid samples combines immunomagnetic separation step with direct electrochemical detection of the captured bacteria cells by square wave stripping voltammetry sensor. Captured bacteria cells in a complex with magnetic beads are labelled by electrochemically active probes, specifically e.g. gold nanoparticles or quantum dots functionalized by specific IgG molecules. Square wave stripping voltammetry was used as a detection technique for analysis of metal ions released from the aforementioned probes by acid hydrolysis. Magnet located near the miniaturized disposable screen-printed electrodes attracts the labelled complexes, and released metal ions provide proper measurable signal. This analytical strategy leads to the results in a few hours.

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Keywords: *foodborne, pathogens, immunomagnetic separation, electrochemical immunosensor*

Acknowledgement: *This work was supported by the European Union´s LOVE-FOOD 2 MARKET project under the contract No. 687681.*

G63
HIGHLY EFFECTIVE TRANSFORMATIONS OF CHALCONES BY CYANOBACTERIA LEAD TO THE FORMATION OF NATURAL SWEETNESS

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Naturally occurred chalcones (1,3-diaryl-2-propen-1-ones), are biogenetic precursors of all known flavonoids, and exist mainly in hydroxylated forms, produced by hydroxylases in the biosynthetic pathways of plants. Because of the wide spectrum of their biological activities, especially health promoting properties, those substances have attracted considerable attention. Many efforts focused on finding new biologically active derivatives of chalcones, are currently taken up. Although the extraction from plants is classic and still the most popular way of obtaining the majority of flavonoids, its efficiency suffers from the low concentration of these compounds in plant tissues, and depends on environmental, seasonal or regional variations of their abundance. The second possibility - chemical synthesis, requires extreme reaction conditions and often toxic chemicals, what limits its application on an industrial scale. Biocatalysis seems to offer an interesting alternative to both aforementioned approaches, especially when it involves whole microbial cell systems that offer scalable, cheaper and more accessible strategies for efficient acquisition of derivatives of chalcones.
Due to their sweet taste and natural origin, dihydrochalcones are desirable in the food industry for the production of multicomponent, low-calorie sweeteners. In turn, antioxidant, UV-protective and pro-health properties make those substances interesting for pharmaceutical and cosmetics industries.
Cyanobacteria are known to produce phenolic compounds, including flavonoids, as natural components of cells, hence those organisms should possess an ability to perform biotransformation of such a compound. To verify this hypothesis, we decided to study such processes using naturally occurring various derivatives of chalcones as the substrates. Whole cells of halophilic and freshwater cyanobacterial strains were used to indicate the favoured routes of biocatalytic transformation of chalcones in batch cultures performed on analytical, preparative and mini-pilot scales. Identification of the products of biotransformations indicates that these processes, when carried out by whole cyanobacterial cells, are based on reduction, hydroxylation and dehydroxylation reactions. Most often dihydrochalcones – natural sweeteners, were the products of those transformations, thus our finding open the new, biotechnological way for their acquisition.

Keywords: *flavonoids, chalcones, cyanobacteria, biotransformations, dihydrochalcones*

Acknowledgement: *This work was supported by Polish National Science Centre (NCN) grant number 2016/21/N/NZ9/02310.*

G64
CREATION OF HYPOLIPIDEMIC MEAT PRODUCT BASED ON PROTEOMIC STUDIES

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Proteomic maps of *Bos taurus* and *Sus scrofa* cardiac muscle and aorta tissues (native, autolyzed and sterilized) were analyzed. Two-dimensional electrophoresis was carried out according to the method of O.; Farrell. Identification of protein fractions was performed on DE after trypsinolysis by MALDI-TOF/MS and MS/MS mass spectrometry in the positive ion mode in molecular weight range of 500-8000 Da. Obtained tryptic peptides mass spectra was analyzed using Peptide Fingerprint option in Mascot software with MH+ mass determination; search was carried out in databases of the National Center for Biotechnology Information, USA (NCBI).
More than 100 protein fraction were identified in *Bos taurus* and *Sus scrofa* cardiac muscle and aortas tissues, including major structural proteins, which were almost identical, but in *Sus scrofa* aorta tissue were identified pre- and apolipoprotein A-1 involved in the formation of high-density lipoproteins, peroxiredoxin-1 in mixture with transgelin involved in the suppression of oxidative stress, galectin-1 induced apoptosis of T-lymphocytes; in *Sus scrofa* heart tissues – fatty acid-binding protein. It was shown that detected tissue-specific proteins disintegrated during autolytic proteolysis, while only fatty acid-binding protein remained after sterilization. We hypothesized that decomposition of target proteins could led to generation of functional peptides with the same action.
According to obtained results, meat product was processed from *Sus scrofa* heart and aorta tissues. Hypolipidemic effect was studied on thirty male *Wistar rats* (380±20 g) aged approximately 1 year, which were randomly divided in 3 groups: group 1 – negative control (n=10); group 2 – positive control (n=10) and group 3 – experimental animals (n=10). Rat model of alimentary hyperlipidemia (group 2 and 3) was developed by adding cholesterol (2.0-10.0%) and fat (10.0 - 25.5%) to the standard diet and vitamin D2 injection *per os* (35,000 IU/kg b.w.). Incorporation into the animal diet of meat product led to cholesterol and triglyceride decrease in serum by 31.8% and 28.2% compared to group 2, cholesterol LDL, cholesterol non-LDL and non-HDL reduced by 21.6% and 2.4 times ($P<0.05$), respectively. In group 3 atherogenic index was by 41.3% lower than in group 2.
Meat product made of pork aorta and heart tissues demonstrated positive effects in hyperlipidemic rats due to the ability of heart and aorta tissue-specific proteins modify the lipid metabolisms and reduce the rat blood serum atherogenic index.

Keywords: *proteomics, tissue-specific proteins, pig aorta, pig heart, hyperlipidemia*

Acknowledgement: *This work was supported by the Russian Science Foundation (project No. 16-16-10073).*

G65
DETERMINATION OF AMINO ACID SEQUENCE IN PEPTIDE FRACTION FROM SUMBAWA HORSE MILK BY MASS SPECTROPHOTOMETRY

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Edman degradation has been used for amino acid sequencing since 70 years ago. However, this method will spend many chemical solvents and be through complex reaction mechanism. Mass spectrophotometry can reduce the limitation of Edman degradation by using ion source (IS) and mass analyzer (MA). Therefore, the aim of the study is to determine amino acid sequence of Sumbawa horse milk by mass spectrophotometry and Mascot software. For this research, initially the peptide fraction <3kDa was diluted by ultra pure water (UPW). Then, the solution was measured by LCMS and LCMSMS using binary gradient system, and it results were analyzed by mascot software using MS/MS ion search mode. The results showed that there were possible four peptides found in chromatogram but three peptides merely can be analyzed further based on confirmation of mass spectrum. Then, according to protein score, peptide score, and error control, one peptide (Pep-1) could be determined amino acid sequence, namely Nterminus-KVPQVSTPTLVEVSR-Cterminus with m/z ratio=1638. This sequence is similar to sequence of *Bos taurus*'s serum albumin. In conclusion, considering similarity of albumin serum sequence from bovine and horse, the sequence KVPQVSTPTLVEVSR was suggested as a part of horse serum albumin.

Keywords: *peptide, sequencing, mass spectrophotometry*

G66
DEVELOPMENT OF AN ANALYTICAL METHOD FOR THE SIMULTANEOUS DETERMINATION OF 12 RED DYES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-UV-DIODE ARRAY DETECTION

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Control of the presence of food additives is important to ensure meat quality and safety. However, the attention of the official control plans is usually more focused on identifying some food preservatives (i.e. nitrites/nitrate and sulphites) with respect to food dyes.
The addition of six red food dyes in food is admitted by European Legislation, and regarding meat products, only cochineal, carminic acid, carmines (E120), Ponceau 4R, cochineal Red A (E124) and Allura Red AC (E129) are allowed with precise restrictions (European Commission Regulation 1129/2011/EC). Furthermore, the presence of different red dyes, including some carcinogens, have been found in some types of spices used in the production of meat products (European Commission decision 2004/92/EC).
To the best of our knowledge, in literature there are no analytical methods for the simultaneous multiresidual determination in meat products of the most important red food dyes (banned and not banned), therefore, new analytical tools are needed.
For these reasons, an accurate method combining high performance liquid chromatography with UV-diode array detection has been developed in the present study. The chromatographic separation for the simultaneous identification and quantification of 12 red dyes in meat products has been accomplished by a Hypersil Gold (Thermo Fisher Scientific, 150 x 4.6 mm, i.d. 5 µm) column eluted with an optimized step-change gradient, based on a mobile phase consisting of a acetate buffer solution and acetonitrile, which has guaranteed a very good selectivity towards endogenous interfering substances. The method validation, performed by an in-house model, according to the European Commission Decision 2002/657/EC and Regulation 2017/625/EC, provided excellent results with respect to linearity ($r^2 > 0.997$), expanded measurement uncertainty (below 15%), recovery values (in the range 88.8% - 105.0%), repeatability (CV% < 18%) and sensitivity demonstrating the conformity of the proposed method with the European directives. The results of this study offer a valid support to widen the knowledge about the safety and the quality control of meat food products

Keywords: *food additives, red dyes, meat products, HPLC-UV-DAD, core shell column*

Acknowledgement: *We are grateful to the Ministry of Health, Research Programme who financially supported this project; "Development of innovative analytical methods for meat products safety and quality assurance". – Project GR-2013.*

G67
DYE RESIDUES IN AQUACULTURE PRODUCTS: TARGETED AND METABOLOMICS MASS SPECTROMETRIC APPROACHES TO TRACK THEIR ABUSE

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Chemotherapy has been applied in aquaculture for the last decades and is of growing concern because of current safety worries: environmental contaminants, emergence of resistance to antibiotics, consumer demand on healthy foods… Among these chemicals, several pharmacologically-active dyes like the pretty well-known malachite green (MG) may be administered because they are cheap and they bear interesting antiseptic and antibacterial activities. More interest was recently focused on the possible use of similar compounds to MG that would not be sought in aquaculture products. But treatment based on MG and other related dyes is now prohibited due to toxicity concerns. Then appropriate analytical approaches are needed to control their abuse. The aim of the study was to initiate an exhaustive strategy of control by implementing both targeted and non-targeted approaches. On the one hand, the strategy of reliable and high-throughput targeted mode was proposed considering all the dyes of interest. A LC-MS/MS method was validated to analyze 14 dyes belonging to different related families. An oxidative step was integrated in order to recover the parent forms for the dyes which are supposed to metabolize in reduced forms after administration to fish. On the other hand, the non-targeted approach was conducted to investigate the potential presence of biomarkers after treatment of farmed fish and selecting for this research study two specific dyes namely MG and Victoria pure blue BO. The data were further processed to study the metabolic fingerprints by means of statistical tools implemented through the Workflow4metabolomics, a collaborative research network for comprehensive metabolomics data pre-processing, statistical analysis, and interpretation.

G68
DETERMINATION OF FLUORIDE IN TEA USING A COMBUSTION ION CHROMATOGRAPHY SYSTEM

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When excessive amounts of fluoride are consumed, it can lead to dental fluorosis, bone fractures, and skeletal fluorosis. Currently, the U.S. EPA has an enforceable drinking water standard of 4.0 ppm for fluoride with a non-enforceable secondary standard of 2.0 ppm to protect children against tooth discoloration and/or pitting. Tea is an important product for China's economy. According to China national standard NY659-2003, 200 ppm fluoride is the limit for tea. The current standard test method of fluoride in tea is NY/T 838-2004. This method is an ion- selective electrode method which is lengthy, unsafe, and cumbersome.
This poster describes a simple method to determine fluoride in tea using a Combustion Ion Chromatography (CIC) system. While ion chromatography (IC) can determine fluoride in a number of samples, when used for tea in solution there are a number of early eluting organic acids that can interfere with fluoride determinations. When tea samples are prepared by combustion the organic acids are destroyed and the fluoride in the gas generated by combustion is captured in solution for determination by IC. Using the CIC system fluoride in tea is determined in 11 to 20 min, a significant time savings over the current Chinese national method. The CIC method is sensitive (MDL =1 ppm) precise and accurate. The only sample preparation required is that the tea be ground and dried. We applied this CIC method to a variety of black and green teas.

G69
EVALUATION OF CHEMICAL COMPOSITION OF WINE BRANDIES AGED USING OAK AND CHESTNUT WOOD

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During the traditional ageing process in wooden barrels the wine brandy undergoes important chemical modifications that lead to the improvement of its quality. These changes are closely related to the ageing technology, which involves the wooden barrel characteristics such as those imparted by the wood botanical species. Research made by our research team on Limousin oak wood and chestnut wood has shown that they are suitable for this purpose while conferring different chemical characteristics and sensory properties to the wine brandy.
Still, the alternative ageing technology using wood pieces in the distillate kept in stainless steel tanks is of increasing significance for the beverage industry due to the need to reduce the production costs.
Bearing in mind the interest of using simultaneously the two wood botanical species in the ageing of brandy to take advantage of their best features and to decrease the ageing costs, it becomes necessary to study its influence in both ageing technologies. This study provides innovative information on the effect of Limousin oak and chestnut wood, used simultaneously, in the chemical composition of the aged wine brandy. Ageing was performed in 650 L wooden barrels and in 3000 L stainless steel tanks with staves over a two years period.
The chemical composition of wine brandies was evaluated based on dry extract, total polyphenol index, low molecular weight extractable compounds analysed by HPLC; analytical colour and dissolved oxygen.
The differences in ageing technology and ageing period were also analysed with FTIR-ATR technology with promising results.
It is possible to conclude that the impact of the simultaneous use of Limousin oak and chestnut wood in the characteristics of wine brandies depends on the ageing technology, being a promising option that decreases the cost of the process and ensures the quality of final product.

Keywords: *ageing technology, wine brandy, wood, FTIR-ATR*

Acknowledgement: *Centro de Estudos Florestais is a Research Unit funded by FCT within UID/AGR/UI00239/2013. ICAAM is a research unit funded by FCT (UID/AGR/00115/2013). This participation was supported by Project CENTRO-04-3928-FEDER-000009 - Beira Baixa Terras de Excelência - Comunicação, Animação e Inovação.*

G70
POSSIBILITIES OF REDUCING THE CONTENT OF SODIUM IONS IN MEAT PRODUCTS

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Common salt is used as a food preservative in the production of meat products for thousands of years because of its effect on shelf-life and flavour. The insignificant effect of sodium chloride is also the solubilisation of the myofibrillar proteins in meat. It activates the functional proteins to increase water-binding capacity and hydration, which lead to increased binding properties of proteins and to the improved texture of meat products. However, reports linking excessive intake of sodium to the occurrence of hypertension, which is the major reason for reducing its content in meat products. There are various options to reduce the content of sodium. Apart from lowering the level of common salt added to meat products, there is apparently one of the most widely used approaches, which involves the partial replacement of sodium chloride by salt substitutes, in particular, potassium salts (chlorides, lactates etc.).
In this study, we examined both aforementioned approaches of lowering the level of sodium chloride and evaluated its impact on the key technological and organoleptic properties of cooked hams. Samples from the meat industry were tested using six different salt mixtures. The following technological properties were measured: pH values, cooking loss, color using reflective spectrophotometry, lipid oxidation based on TBARS and texture estimation through a Warner-Bratzler shear force test. The meat products were also subjected to sensory analysis. The samples with potassium chloride were comparable in all respects to the samples with sodium chloride. Cooking loss was increased in the samples with potassium lactate, but other organoleptic and technological properties were also similar to the standard sample. Our results suggest that sodium chloride could be partially replaced by potassium chloride and potassium lactate without negatively affecting the properties of the final product.

Keywords: *sodium ion reduction, meat products, cooked ham*

G71
ELASTIC LIGHT SCATTER AND MALDI-TOF: COMPLEMENTARY TECHNOLOGIES FOR REDUCING FALSE NEGATIVES

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The advantage of elastic light scatter (ELS) measurement is that it can determine colony number and colony identification in a reagent-free way and without destruction of the colony. Another advantage is that using advanced classification algorithms ELS can rapidly identify the number of clusters, or groups of different organisms on a plate. Typically, in the microbiology laboratory, an expert will evaluate a culture plate and review the colonies for potential pathogens. The obvious goal is to ensure that, by double confirmation from ELS and visual observation (ELS pattern & technician), any suspicious organism is selected for further analysis.
We established a comparison between ELS and MALDI-TOF methods with the purpose of using ELS as a low-cost, rapid pre-analysis technology to define the number of groups/classes of organisms on a plate and increase the probability of selecting a member of each cluster for a follow-up MALDI-TOF measurement. Since standard MALDI-TOF protocol doesn't analyze all the colonies on the sample plate, providing an pre-analysis results with most probable groups will greatly enhance the accuracy of the colony picking process. Our study evaluated the statistical likelihood of missing a pathogen colony using MALDI-TOF alone on a culture dish. We also demonstrated that in contrast to a manual evaluation of the number of groups of organisms on a culture plate (which is subjective and often irreproducible), the ELS technology employs fast, repeatable and reproducible process guided by rigorously defined mathematical criteria which can recover the most probable number of organism classes.
This study provides a strong argument for the use of a pre-analysis technology to define the most likely organisms that would be classified as pathogens, thus increasing the final probability of pathogen recognition and reducing the likelihood of reporting a false negative result caused by excluding relevant colonies from the analysis. The use of ELS before MALDI-TOF also provides a significant economic advantage by lowering the number of samples that must be tested using MALDI-TOF system while bolstering the screening accuracy with a second independent method.

Keywords: *ELS, bacterial light scatter, MALDI-TOF, bacterial identification*

Acknowledgement: *This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No.1935-42000-072-02G to the Center for Food Safety Engineering. 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. CG is a NSF Fellow (grant number DGE-1333468).*

G72
TRACE LEVEL DETECTION OF HISTAMINE AS LOW AS 0.1 MG/L IN WINE USING RIDASCREEN® HISTAMINE (ENZYMATIC)

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Frequently HPLC is used to detect histamine in wine. However, at low concentration the method is not reliable due to disturbing peaks. Also the HPLC method is very cumbersome. Therefore, the enzymatic method for wine was improved to measure histamine reliably at very low levels.
It was reported that a few susceptible consumers already reacted to 15 – 30 µg histamine per serving size of wine (200 ml). This is equivalent to a concentration of 0.08 – 0.15 mg/l histamine. Our RIDASCREEN® Histamine (enzymatic) has a claimed LOD of 0.54 mg/l which is not sensitive enough to detect these low concentrations of histamine. Therefore, we developed a sensitive application to cover these needs. The extraction procedure using RIDA® Sample Decolorant remains the same except all pipetted volumes are doubled. The enzymatic procedure was changed by increasing the calibrator/sample volume from 100 µl to 200 µl and introducing on the opposite a more concentrated buffer at a lower volume. Due to these changes the calibrator concentrations need to be changed by the user using the calibrators from the test kit. The new linear range of the test kit is from 0.2 to 5 mg/l histamine. Using this new procedure, the LoD and LoQ were decreased to 0.19 and 0.34 mg/l histamine in wine, respectively. A wine sample with a mean histamine concentration of 0.14 mg/l showed a CV of 38% while at 0.31 mg/l the CV was 8.7%. Recovery at concentrations between 0.25 mg/l and 1 mg/l is at 70% and beset with some uncertainties since the red wine used for spiking showed a mean concentration of 0.14 mg/l but a quite high CV. Nevertheless, the results clearly show that the new procedure is capable in measuring the challenging concentrations.

Keywords: *histamine, enzymatic analysis, wine analysis*

GENERAL FOOD ANALYSIS

G73
DIFFERENTIAL ANALYSIS IN PRODUCT CONTROL APPLICATIONS USING GC/MS AND COMPREHENSIVE GCXGC/MS

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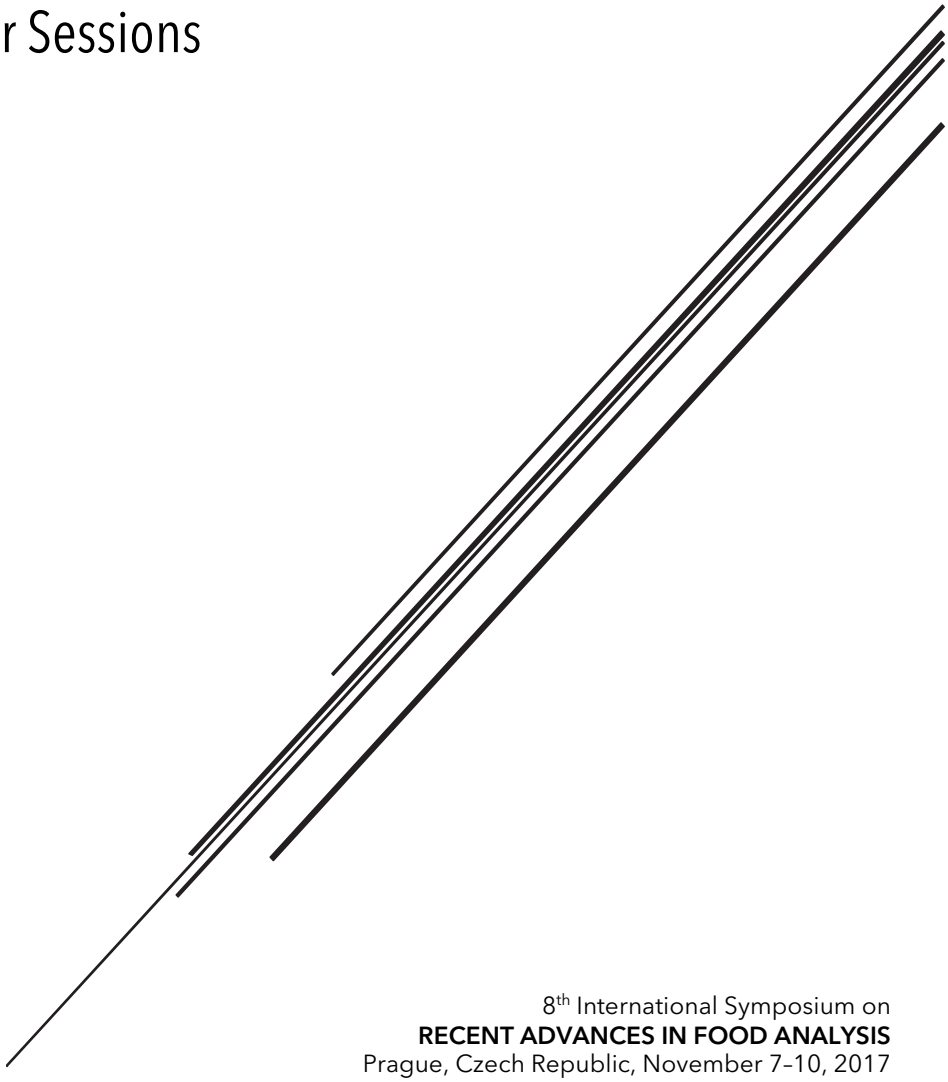
A novel GC/MS and GCxGC/MS software platform was developed for sensitive comparative or differential analysis, especially for Product Control Applications and Trouble Shooting.
Many applications in GC/MS and GCxGC/MS relate to finding differences between a newly measured Sample and a Reference Sample. These questions may typically arise in application areas like Product Control or during Trouble Shooting. Examples are: what new impurities are present in a new batch compared to a reference batch, why does this product behave different compared to the reference batch, or the comparison of samples in Food Fraud applications to detect illegally added substances. Typically for the above examples is the limited time available to solve these problems. Furthermore, most of the time only a few samples are available, which excludes the use of statistical comparison tools as applied in the field of Metabolomics.
GC-Analyzer performs differential analysis by comparison of all ions between sample and control. Using the "All Ion" approach allows for the detection of very small deviations hidden under large peaks and not seen by a visual examination. Subsequent Deconvolution and Identification is based on "Differential Ions" only, which makes it possible to even detect exactly co-eluting unknown components.
A similar procedure is used for comprehensive GCxGC/MS comparative analysis. Depending on the complexity of the data, it is possible to perform the analysis based on the TIC or using "All Ion Data Processing". It will be demonstrated that an "All Ion" data processing technique for GCxGC data outperforms a TIC approach, as there might still be many unresolved overlapping components.
In situations where no reference sample is available, the software can be used for peak detection, deconvolution and identification. Technical details of the algorithms will be explained and examples will be given from Food, Flavor & Fragrances and Base Chemistry industry.

Keywords: *GC/MS, GCxGC/MS, food control, compartive analysis, trouble shooting*

Acknowledgement: *C.C.M. van der Kroft, University of Utrecht*

MAJOR NUTRIENTS & VITAMINS

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

MAJOR NUTRIENTS & VITAMINS

H1
A KEY ROLE OF BIO-ANALYSIS IN GUIDING THIAMIN BIO-FORTIFICATION: DEVELOPMENT OF A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-FLUORESCENCE DETECTION METHOD FOR THE DETERMINATION OF THIAMIN AND DERIVATIVES IN PLANT MATERIAL

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INTRODUCTION: Worldwide, millions of people are suffering from micronutrient malnutrition. Micronutrients are vital compounds for a living organism, such as vitamins and minerals. Thiamin (vitamin B_1) plays an essential role during several metabolic reactions. An acute deficit of vitamin B_1 results in the disease Beri-Beri, which can be fatal due to neurological and cardiovascular complications. As humans are not able to synthesize thiamin themselves, they rely on the diet for its supply. However, the content of vitamin B_1 in main staple crops is below minimal requirements. Thiamin deficiency is especially a problem in developing countries, where a varied diet is lacking and people mainly rely on rice for their energy supply.
A cost-effective strategy to fight vitamin deficiencies is the improvement of the nutritional quality of staple crops via biofortification, which may be achieved via genetic modification. HET-P synthase (THI1) and HMP-P synthase (THIC), two key enzymes for thiamin synthesis, are promising candidates for metabolic engineering. Bio-analytical determination of the intermediate and end products of the biosynthesis pathway not only serves as a guidance for the effectiveness of the genetic modification, allowing the selection of the most promising rice lines, it also allows to get a better insight into (regulation of) thiamin biosynthesis.
METHODS: A method for the determination of thiamin and its phosphate derivatives in Arabidopsis *thaliana* is being developed. Arabidopsis *thaliana* is chosen as a starting point to gain more insight into thiamin regulation in plants. To quantify the analytes from limited amounts of starting material, a straightforward sample preparation procedure preceded derivatization to thiochromes, followed by an optimized liquid chromatographic separation and fluorescence detection. Optimized parameters included mobile phase A (25mM, 50mM or 100mM PO_4^- buffer, pH7 or 8.4), gradient, flow rate, column and optimal excitation/emission wavelength.
RESULTS: Following extraction of 200 mg sample with 1 ml MeOH/H2O (1:1), 400 µl was derivatized with 80 µl 30 mM $K_3Fe(CN)_6$ in 3.7 N NaOH. After evaporation and reconstitution in 400 µl H_2O, 50 µl was injected into the chromatographic system. Separation was performed using a Purospher® Star RP-8e (125 mm x 4 mm, 5 mm) column at 30°C in a 17 min gradient run, with as mobile phases, with a flow rate of 0.8 ml/min, 100mM PO_4^- buffer pH 8.4 (since this gave the best separation between phosphate derivatives) and methanol. Thiochrome compounds were detected at an excitation and emission wavelength of 370 and 430 nm, respectively.
CONCLUSION: Bio-analytical determination of thiamin, its precursors and derivatives plays an essential role in guiding the effectiveness of the biofortification strategy. Moreover, it allows us to gain more insight in the regulation of thiamin biosynthesis in plants, which is fundamental to achieve maximum thiamin levels in rice.

Keywords: *thiamin, biofortification, phosphate derivatives, plant material, HPLC-FLUO*

Acknowledgement: *Jana Verstraete would like to acknowledge the FWO Research Foundation-Flanders for granting her a PhD fellowship (application number 1S61617N).*

H2
RAPID AND SIMPLE DETERMINATION OF FAT-SOLUBLE VITAMINS IN VARIOUS TYPES OF INFANT FORMULAS BY HPLC-DAD

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The aim of this study was the development of a fast, accurate, low cost and eco-friendly method for routine analysis of fat soluble vitamins A and E in cereal and milk based infant formulas (IF). Two main pretreatment methods were hyphenated to High Performance Liquid Chromatography (HPLC) - diode array detector (DAD): (1) a generic method for qualitative and quantitative analysis of all samples and (2) a simple screening method for detecting the form of vitamins initially added into the IF (without saponification) to check each IF's label claim. Parameters such as use of a-amylase, antioxidants (L-ascorbic acid and butylated hydroxyl-toluene (BHT)), sampling, time of saponification, number and time of extractions, reconstitution volume were studied and optimized. Regarding validation, trueness, linearity and repeatability were estimated, with %RSD (n=3) of 1.8 % for vitamin A and 3.1% for vitamin E, respectively. Trueness was tested by both the standard addition method and by certified reference material (CRM, NIST 1849a) analysis.

Keywords: *retinol, a-tocopherol, HPLC-DAD, fast routine analysis, infant formulae*

H3
NUTRITIONAL QUALITY OF SELECTED FISH PRODUCTS ON THE CZECH MARKET

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The fish and the fish products are a highly valued food commodity due to their nutritional composition. The fish muscle contains high-quality and easily digestible proteins, advantageous fat composition and many important micronutrients, such as vitamin D and E and the others.
The fat content in fish and mainly in their products is very variable and may range from 0.5 to 58 %. The composition of fatty acids in fish fat depends on many factors. For this reason, there is a huge difference in composition of fatty acids between freshwater and sea fish. Fish and fish products contain significant amounts of polyunsaturated fatty acids, especially n-3 fatty acids. For this reason, they are increasingly recommended for human consumption.
This study is focused on evaluation of selected nutrients in marine fish and fish products from Czech market. The analyzed parameters are proteins, fat, dry matter, fatty acid composition and the content and composition of tocopherols (vitamin E). The aim is to evaluate the benefits of these products for human diet from a nutritional point of view.

Acknowledgement: *Financial support from the specific university research (MSMT No 20/2017).*

H4
ANALYSIS OF T-RETINOL (VITAMIN A), TOCOPHEROL ISOMERS (VITAMIN E) AND VITAMIN D BY LCMS-MS BY MICROWAVE DIGESTION FOLLOWED BY LCMS-MS DETECTION

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The analysis of oil soluble vitamins (A, E, D) is typically a laborious process requiring methylation under alkaline solutions and a series of liquid-liquid extractions before preparing the samples for chromatography. Depending on the vitamins required, there is a high demand on instrumentation and variation of chromatography (normal or reverse phase) and detection (UV, fluorescence or mass spectrometry). Due to the low levels found in food, if the analysis includes the determination of vitamin D (D2 or D3) additional clean up and concentration steps are required. From a consumer perspective there is an increasing trend in fortified products carrying vitamins claims which have to be verified by analysis. The complexity of the methodology and the need to carry out the analysis as soon as possible due to stability problems were the key drivers behind the method developed. The principle of the extraction is the same however traditional methylation using conventional heating was replaced by microwave based methylation. Development of the method was based on procedure described in the BSI Standard Publication BE EN12823-1:2014. Key improvements included a microwave samples saponification instead of saponification under reflux (over night or hot saponification) and an optimized the extraction time. The reaction takes place in sealed vessels under controlled temperature and pressure conditions. The system automatically adjusts the power to compensate for the varied samples, so that by the end of the ramp, all samples are digested similarly. The overall time to process these samples, including cool down, was 20 minutes. The benefits of the microwave saponification include the faster reaction with improved reproducibility and operation efficiency as well a stability of the analyte.
In addition the chromatography was optimised in order to achieve quick resolution of t-retinol, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, ergocalciferol (D2) and cholecalciferol (D3). The instrument used is an Agilent 1290 Infinity II LC coupled with an Agilent 6495 triple Quad LCMS. The column used is an Agilent ZORBAX Rapid Resolution HT Eclipse PAH 4.6mmx50mm, 1.8µ. The run time of the method is less than 5 minutes, allowing the laboratory to provide results for all vitamins within the same day of testing. The method has been validated for high fat products, oils, breakfast cereal, dairy and infant formulas according to ISO17025.

Keywords: *vitamins, mass spectrometry, retinol, tocopherol, vitamin D*

H5
AOAC 2016.14: A NOVEL METHOD FOR ASSESSING FRUCTANS IN INFANT FORMULA & ADULT NUTRITION

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Fructans, particularly inulin and fructooligosaccharides (FOS), are widely used in infant formulae and adult nutrition products due their scientifically proven health benefits as prebiotics and dietary fiber. Amongst others, for labeling and quality assurance purposes, accurate and selective quantification of fructans in these products is essential. Therefore, it is a prerequisite of having access to analytical techniques that guarantee selectivity, accuracy as well as detection of those compounds in low quantities. AOAC 997.08 and AOAC 999.03 are the currently accepted and most known fructan determination methods. Although these methods are generally performed, the need of several time consuming chromatographical analyses, the need of ingredient-specific correction factors, and - above all - the problematic of FOS underestimation, emerged the necessity for the development of a new method that can replace those two.
The current communication will introduce a novel method for quantification of fructans in infant formula and adult nutrition. The development of the method is the result of a Eurofins Carbohydrate Competence Centre - Nestlé RC collaboration. The method was approved by the AOAC and obtained the First Action Status of an official method: AOAC 2016.14. The advantages of AOAC 2016.14 compared to the existing methods as well as the results of the dual laboratory validation study will be outlined. A multi-laboratory trial for establishing the performance characteristics of the method is currently organized. Furthermore, the method is in the process to become also a new ISO and IDF norm.

Keywords: *fructans, HPAEC-PAD, AOAC / ISO methods, infant furmula, Inulin/FOS*

H6
DETERMINATION OF B-VITAMINS IN ENERGETIC DRINKS BY CAPILLARY ELECTROPHORESIS-TANDEM MASS SPECTROMETRY

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Vitamins are organic compounds essential for the human organism, and they need sometimes to be supplemented, because the organism is not able to synthesize them by itself. Food fortification or enrichment has been one of the main strategies used to minimize the deficiencies of vitamins. The aim of this study was to develop and validate a method for the determination of B-group vitamins in energetic drinks using CE-MS/MS. Eleven samples of energy drinks were prepared by degassing in an ultrasonic bath for 5 min, followed by electrophoretic separation in 0.05 M formic acid electrolyte (pH 2.5) using a polyvinyl alcohol (PVA)-coated capillary and detection by ESI-MS/MS. The CE-MS/MS analysis was performed on an Agilent 7100 CE system coupled to Agilent 6430 triple quadrupole mass spectrometer. The CE system was operated under applied voltage of 29 kV using 60 cm capillary length, 50 µm i.d. Analytes were loaded by injection at 50 mBar for 5 s, and the external capillary temperature was fixed at 25 °C. All acquisitions were performed using positive polarity. The MS conditions were as follows: drying gas temperature at 350 °C, gas flow at 7 L/min, nebulizer pressure at 9 psi, and capillary voltage at 3500 V. The sheath liquid (0.1% v/v formic acid in 50% v/v aq methanol) was co-injected with a syringe pump at a flow rate of 5 µL/min. Vitamins thiamine (B_1), riboflavin (B_2), nicotinamide (B_{3b}), nicotinic acid (B_{3a}), pantothenic acid (B_5), and pyridoxine (B_6) were detected using two MRM transitions for each compound in less than 6 min.
The method was validated for linearity, precision, LOD, LOQ, specificity, stability, and robustness. Calibration curves presented good linearity ($r^2 > 0.995$) over the concentration range of 0.3 – 10.8 mg/L for B_1, B_2 and B_6, 3.0 – 108.0 mg/L for B_{3a} and B_{3b} and 1.25 – 45 mg/L for B_5, with LOD and LOQ lower than 0.48 mg/L and 1.6 µg/L for all analytes, respectively. The statistical evaluation of the method was carried out by performing the intra- and inter-day precision, with RSD lower than 12%. The method was successfully used to determine B-group vitamins in energy drinks sold at Brazilian market, by standard addition and no significant difference was observed between the values found and those declared by the manufactures on the labels. The proposed method offers a good alternative for routine analysis due to its simplicity, speed (< 6.0 min) and good sensitivity and precision.

Keywords: *B-group vitamins, CE-MS/MS, energetics*

Acknowledgement: *Agilent Technologies, Inc*

H7
DETERMINATION OF TOCOPHEROLS IN EDIBLE FLOWERS PROCESSED BY IONIZING RADIATION USING HPLC

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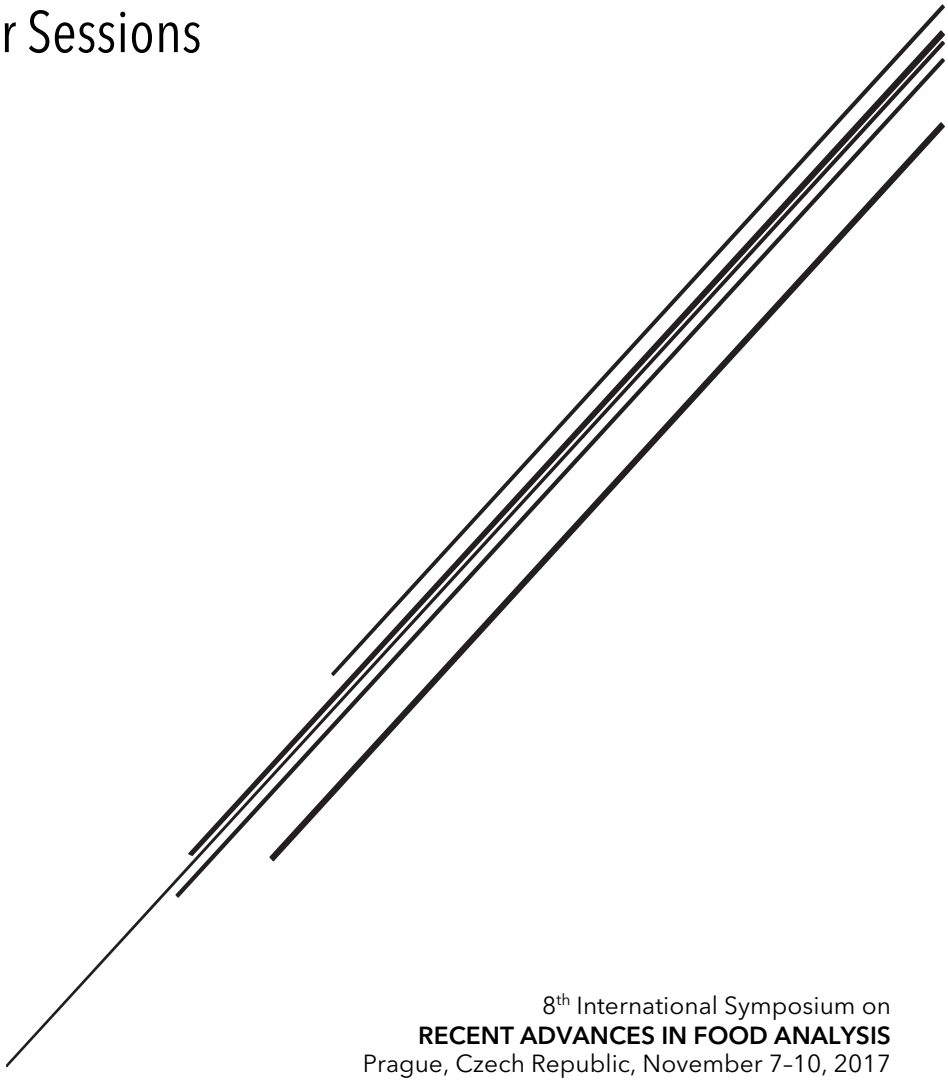
Edible flowers are used to add flavor, color, taste and visual appeal in gastronomic preparations such as sauces, salads, jelly, desserts and drinks. There are many edible flowers, rich in minerals, vitamins and other nutrients, which are very important for human nutrition. Ionizing radiation is applied for foods to improve their conservation quality, food safety, insect disinfestation and increase the shelf-life of food and is a method that can be used for the extension of shelf life of edible flowers. The purpose of this study was to evaluate the effects of ionizing radiation doses of control, 0.5, 0.8 and 1.0 kGy in edible flowers of spices *Tropaeolum majus*, *Rosa chinensis* and *Tagete patula*. High performance liquid chromatography (HPLC) was used for the determination of tocopherols (α-, β-, γ-, δ-tocopherol) with fluorescence detection (295 nm and 330 nm as preferred wavelengths) using Shim-pack NH_2 (25 mm x 4.6 mm, 5 µm) column and hexane and isopropyl alcohol (99:1) as mobile phase. In the species of edible flowers analyzed it was found alpha, gamma, beta and delta tocopherol. However, alpha tocopherol form was predominant (8.29 to x 62.79 mg/100g) for all the edible flowers species. Moreover, flowers processed by radiation have not shown significantly difference when compared to the control sample.

Acknowledgement: *CNPq and IPEN-CENEN/Sao Paulo*

METALS & METALLOIDS

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

METALS & METALLOIDS

I1
MONITORING OF ARSENIC SPECIES IN PIEDMONT RICE

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INTRODUCTION: Arsenic (As) is a ubiquitous element, which occurs naturally in the earth´s crust; it exists mainly in two oxidation states, +V (arsenate) and +III (arsenite) and their methylated derivatives, such as Dimethylarsinic acid (DMA), ArsenoBetaine (AB) and arsenosugar compounds. It is well established that the toxicity of As highly depends on the chemical form in which it is present. Drinking water, cereals, seafood and algae-based products are among the commodities with the highest levels of As. Unlike seafood, where As is dominantly present as organoarsenicals, in rice it is mainly found as inorganic species [1]. For this reason, in 2015, the European Commission has established maximum limits for inorganic arsenic in rice and rice by-products [2]. In the European Union, Italy is responsible for almost 50% of total rice production and more than 50% of rice crops are located in Piedmont, a Region in the north-western part of Italy.
MATERIALS AND METHOD: 27 raw rice samples were collected in Piedmont during the harvesting season. Total As determination was performed using an ICP-MS instrumentation, after microwave digestion with concentrated nitric acid and hydrogen peroxide. As speciation was performed using an HPLC-ICP-MS instrumentation after extraction with diluted nitric acid and hydrogen peroxide in microwave oven. Quantification limit (LOQ) was set at 0.020 mg kg-1 for total As, inorganic As (iAs) and DMA.
RESULTS: Total As concentration ranged from 0.11 mg/kg to 0.48 mg/kg, with a mean value of 0.24 mg/kg. Arsenic species identified and quantified in Piedmont rice were DMA e iAs. iAs concentration ranged from 0.070 mg/kg to 0.33 mg/kg, with a mean value of 0.19 mg/kg; DMA concentration was under LOQ in 74% of samples analyzed, while in the remaining ones, mean value was 0.033 mg/kg.
CONCLUSIONS: Total As concentration in Piedmont rice was lower than that observed in other European and extra-European countries. Only 5 of the raw rice samples analyzed exceeded maximum levels of iAs establish for husked rice by European Legislation (0.25 mg/kg).

[1] D'Amato M., Aureli F., Ciardullo S., Raggi A. and Cubadda F.; J. Anal. At. Spectrom.; 2011; 26, 207-213;
[2] Commission Regulation (EU) 2015/1006.

Keywords: *arsenic, speciation, rice, Italy*

Acknowledgement: *This work was founded by the Italian Ministry of Health (grant nr. IZS PLV 01/13 RC)*

I2
QUANTITATIVE ANALYSIS OF CHROMIUM AND ARSENIC SPECIES IN FOOD AND FOOD PACKAGING USING LC-ICPMS

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Food safety is one of the major concerns of the European population and the European Commission is aiming to assure a high level of food safety and animal & plant health within the EU through the farm-to-fork principle. This implements effective control systems for harmful substances such as pesticides, mycotoxins and heavy metals. Nowadays in food control the speciation analysis has become an important tool for the determination of elements like chromium, mercury, tin, and arsenic where simply the measurement of the total amount of the element is not sufficient. The idea is to have a clear identification and quantification of the different species for a better understanding of toxicological impacts on human health and the environment. Hot subject discussions are recently on the concentration levels of arsenic, as the inorganic arsenic species arsenite and arsenate have a bigger toxicological relevance than the organic species. Both organic and inorganic arsenic species occur naturally. The organic arsenic, which can be found in seafood, is not known to be harmful, but inorganic arsenic, which enters plants through water and soil, is toxic. Long-term exposure can cause cancer and that is why the European commission has fixed the maximum levels of inorganic arsenic in Non-parboiled milled rice at 0.2 µg/Kg and rice for the production of food for infants and young children at 0.1 µg/kg [1]. The Shimadzu LC-ICP-MS system which connects an inductively coupled plasma mass spectrometer (ICP-MS) in-line with a high-performance liquid chromatography (HPLC) system allows the high sensitive and high accuracy measurement of arsenic species in foodstuff. Analytical data are presented and advantages of the system configuration will be explained.

[1] COMMISSION REGULATION (EU) 2015/1006, 25 June 2015, amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs

I3
DETERMINATION OF INORGANIC ARSENIC IN FOOD: NOT A COMPLETELY SOLVED PROBLEM YET

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Interest in the determination of inorganic arsenic (iAs) in products for human consumption is driven by the wide recognition of its toxic effects on humans, even at low concentrations. Currently, the need for robust and reliable analytical methods is recognized by various international safety and health agencies, and by organizations in charge of establishing acceptable tolerance levels of iAs in food. Very recently, the European Union published Regulation (EU) 2015/1006 amending Annex to Regulation (EC) No 1881/2006 regarding the maximum levels of iAs in foodstuffs, especially rice and rice-based products. Furthermore, EFSA and JECFA highlighted the need for robust, validated analytical methods for the determination of iAs in a range of food items and, the need for certified reference materials (CRMs) for iAs.
Nowadays, there are several methods of extraction, as well as of measurement, for the most toxic species of arsenic, AsIII and AsV (understanding iAs as the sum of both). The difficulty is that these methods should be able to be used by most control laboratories (bearing in mind not all of them may be equipped with the newest instrumentation). That is the reason why, different methods need to be established, considering they all should be robust and comparable.
Rice and derivatives have already been extensively studied, and showed comparable results between laboratories and between methods. But this does not happen for all foods. It is being observed that, for other high-consumption foods as seafood, with a significant content of iAs, the results, using different methods published in the literature, are not comparable. For this reason, reference materials for these foods have not yet been available (for example for fishery products), nor has it been possible to report an agreed value upon in the different intercomparison exercises organized by different entities
We present results for the iAs method validation using HPLC-ICP-MS, considering foodstuff of high consumption in the human diet with presence of iAs, i.e. rice and seafood. In addition, the study shows a comparison for iAs in foods using three different analytical methods, where results showed not to be comparable for seafood. Reasons for that difference were also studied.

Keywords: *inorganic arsenic, food*

I4
PERFORMANCE OF ANALYTICAL METHODOLOGIES BASED ON ATOMIC SPECTROMETRY FOR DETERMINATION OF INORGANIC ARSENIC IN RICE

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Recommended maximum limits for inorganic arsenic (iAs) in rice and rice products have been discussed by the Codex Alimentarius Commission and established by European Union legislation in 2016 because of its recognized association with health problems. Compliance with these limits (0.2 mg kg^{-1} for polished rice) requires robust, reliable and preferably cost-effective analytical methods for routine analysis laboratories. In addition to the inorganic chemical forms (arsenite and arsenate), rice contains organic species of As, being iAs and dimethylarsinic acid (DMA) the most abundant. Unlikely what happens with the measurement of total As in food, the determination of iAs requires its differentiation from the organic forms. This may be carried out basically by: a) chromatographic separation followed by the determination of each species individually by atomic or mass spectrometry and b) generation of volatile gaseous hydrides (arsine or AsH_3) by selective reaction of the inorganic species with sodium borohydride ($NaBH_4$) in an optimized acid medium. In our work we compared the yields between a methodology based on High Pressure Liquid Chromatography coupled to Hydride Generation Atomic Fluorescence Spectrometry (HPLC-HGAFS), which allows the individual determination of the species (As^{+3}, As^{+5}, DMA and MMA) and a simpler methodology based on Hydride Generation Atomic Absorption Spectrometry (HGAAS), which allows the selective determination of inorganic As minimizing the DMA response. Both methodologies present lower investment and operating costs than HPLC-ICPMS (HPLC-Inductively Coupled Plasma-Mass Spectrometry), currently the most powerful tool available for elemental speciation. The methodology based on HGAAS presented a better performance than HPLC-HGAFS in terms of quantification limit (50 µg kg^{-1} vs 150 µg kg^{-1}), precision (RSD% 5 vs RSD% 15, for n=3) and average recovery (100% vs 85%, for n=3) at 200 µg kg^{-1} fortification level. Regarding the analysis time, the sample rate was significantly higher (50 samples vs 4 samples per hour). The achieved limit of quantification, allows the use of the HGAAS method, for iAs determination in infant food (maximum limit of 100 µg iAs kg^{-1}). The methodologies were verified by the analysis of rice Certified Reference Material ERM® - BC211 (certified values in µg kg^{-1}: iAs 124 ± 11, DMA 119 ± 13, total As 260 ± 13).

Keywords: *inorganic arsenic, analytical methods*

I5
FAST ARSENIC SPECIATION ANALYSIS OF WINES AND RICE WITH LC-ICP-QQQ

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Due to the natural occurrence of arsenic (As) in the environment, it is consequently found in food and beverages such as rice and wine. Arsenic exists in multiple forms but not all species have the same toxicity. Due to the potential health threat of some species, it is important to measure individual species and not just the total As concentration. Regulations have been proposed for the more toxic inorganic arsenic species (AsV and AsIII) in other food products around the world. Traditionally, the inorganic As values were achieved by measuring individual species using ion exchange high pressure liquid chromatography coupled to a triple quadrupole inductively coupled plasma - mass spectrometer (HPLC-ICP-QQQ) and then two inorganic forms were added together.
In this method, the same instrumentation is used (HPLC-ICP-QQQ) that has been previously used, however, instead of analyzing the inorganic As species separately, AsIII was intentionally oxidized to AsV with hydrogen peroxide prior to analysis. This allowed all inorganic arsenic to be expressed as AsV. By converting the inorganic species, this method was able to separate monomethylarsonic acid and dimethylarsinic acid from AsV in 2 mintues. This analysis time is 10 times faster than the current Food and Drug Administration methods for the speciation of As (FDA EAM 4.10 and 4.11). Furthermore, by reacting samples with O2 in the ICP-QQQ, there was a decrease in spectral interferences while increasing sensitivity. The validation results from two participating laboratories is presented to demonstrate the new method's accuracy and reproducibility in wine and rice matrices.

I6
HEAVY METALS RESIDUES IN FRESHWATER SHRIMP (MACROBRACHIUM ROSENBERGII) FARMINGS IN THAILAND

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Heavy metals are among major pollutants globally found in environmental matrices, especially inthe industrial areas. They occur either naturally or from anthropogenic activities. Heavy metals are persistent and can be bioaccumulated in living organisms including food animals. Contaminations of heavy metals have been reported in agricultural areas including paddy fields. In Thailand a large number of paddy fields and adjacent areas have usually been used for shrimp farming as an alternative culture to rice crop. This practice could result in the bioaccumulations of heavy metals in shrimp. Our study, thus, aimed to determine the contamination of heavy metals in freshwater shrimp (*Macrobrachium rosenbergii)* which is Thailand favorite shrimp culture. The shrimp samples were randomly collected from top four shrimp farming provinces. Shrimp tissues were digested and determined for heavy metals concentrations using a flame atomic absorption spectrometer (FAAS). The results showed that 8 analyzed heavy metals (Cd, Cr, Cu, Fe, Ni, Mn, Pb, and Zn) were found in shrimp tissues at different levels and also differed among provinces. Zinc concentration found was the highest (72.50 µg/g dw) whereas Cd concentration was the lowest (0.47 µg/g dw) in investigated *M. rosenbergii* tissues. In addition, the individual target hazard quotient (THQs) of Cr, Cu, Fe, Mn and Zn in tissues was lower than 1, but greater than 1 for the remainders. This indicates that consumption of this heavy metal contaminated shrimp could lead to human health risk.

Keywords: *heavy metals, Macrobrachium rosenbergii, flame atomic absorption spectrometer, human health risk*

Acknowledgement: *This research is supported by Kasetsart University Research and Development Institute, Thailand.*

I7
ARE PEOPLE WITH LOW SELENIUM INTAKE AT MUCH HIGHER RISK OF CANCER? CURRENT STATUS AND FUTURE PERSPECTIVE

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A growing interest in the biological role of selenium (Se) in terms of both protection of human health and prevention of diseases has recently been noted. Foods are the major source of Se and researchers have a vested interest in daily Se intake not only because of its deficiency or toxicity symptoms, but also in view of its potential beneficial effect contributing to the prevention of cancer and cardiovascular diseases. However, it is worth noting that the evidence for Se protective role is conflicting. The function of Se as trace element for animals and humans has been known for several decades. Selenium is an essential microelement at low levels of intake and produces toxic symptoms when ingested at level only three to five times higher than those required for adequate nutrition. In the diet, Se is largely found in animal food, and in plants, which indicates large individual differences in Se intake associated with menu composition, but also with the origin of food, which can be grown (plants) or bred (animals) on soil with different Se content. Is generally accepted that blood Se depends on dietary intake. It is recognized that some of illnesses common in man are probably due to decrease supply of the element in the food, which leads to its reduced blood and tissue levels. In recent years, most attention was paid to Se in the context of cancer incidence reduction, which was demonstrated in animal studies and human clinical trials in relation to prostate, colon, lung and liver cancers. Prospective studies of Se supplementation in cancer patients are controversial, some of epidemiological studies proved beneficial effect of Se supplementation especially on the risk of prostate cancer (PC), some others did not show significant relation between Se intake and PC cancer risk. Data from randomized placebo-controlled (35.533 men) which tested whether Se, vitamin E or both supplementation could reduce PC were carefully evaluated year by year. Study supplementation stopped 3 years before end date, and author's conclusion "Se supplementation did not benefit men with low Se status" and "vitamin E increases the risk of PCs among men with low Se status" to dispel our illusion. The environment can significantly affect human health. Environmental impacts on human health result from a complex interaction between genetic susceptibility, metabolic activity, environmental exposure and behavior and socio-economic factors. Food is clearly an important exposure route but it should not be considered in isolation since other direct environmental exposures, via air, soil and water, can be equally or more important.

Acknowledgement: *This study was partially supported by the Ministry of Science and Higher Education; Grant No. 2013/11/B/NZ7/04934.*

I8
CONTINUOUS ARSINE DETECTION USING A PELTIER- EFFECT CRYOGENIC TRAP TO SELECTIVELY TRAP METHYLATED ARSINES

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Hydride generation (HG) is a proven technique that thoroughly eliminates interfering matrix species. Post-HG speciation analysis can be fulfilled by cryogenic trapping (CT) based on boiling points of resulting arsines using liquid nitrogen (LN_2) as a coolant. In this work, LN_2 was replaced by thermoelectric effect using a cryogenic trap that consisted of a polytetrafluoroethylene (PTFE) body sandwiched by two Peltier modules. After the trap was precooled, the arsines flew along a zigzag channel in the body and reached a sorbent bed of 0.2 g 15% OV-3 on Chromosorb WAW DMCS imbedded near the exit of the trap. CH_3AsH_2 and $(CH_3)_2AsH$ were trapped; while AsH_3, that passed the trap unaffected, was detected by atomic fluorescence spectrometry. Continuous operation leading to enhanced throughput. For inorganic As, limit of detection (LOD) was 1.1 ng/g and recovery was 101.0±1.1% at 100 ng/g. Monomethylarsonic acid and dimethylarsinic acid did not interfere with 0.2±1.2% and -0.3±0.5% recoveries, respectively.

Keywords: *inorganic arsenic, arsine, cryogenic trap, Peltier effect, speciation analysis*

I9
SPECIATION ANALYSIS OF MERCURY BY DIFFERENTIAL PHOTOCHEMICAL VAPOR GENERATION AT UV-B VS. UV-C WAVELENGTH

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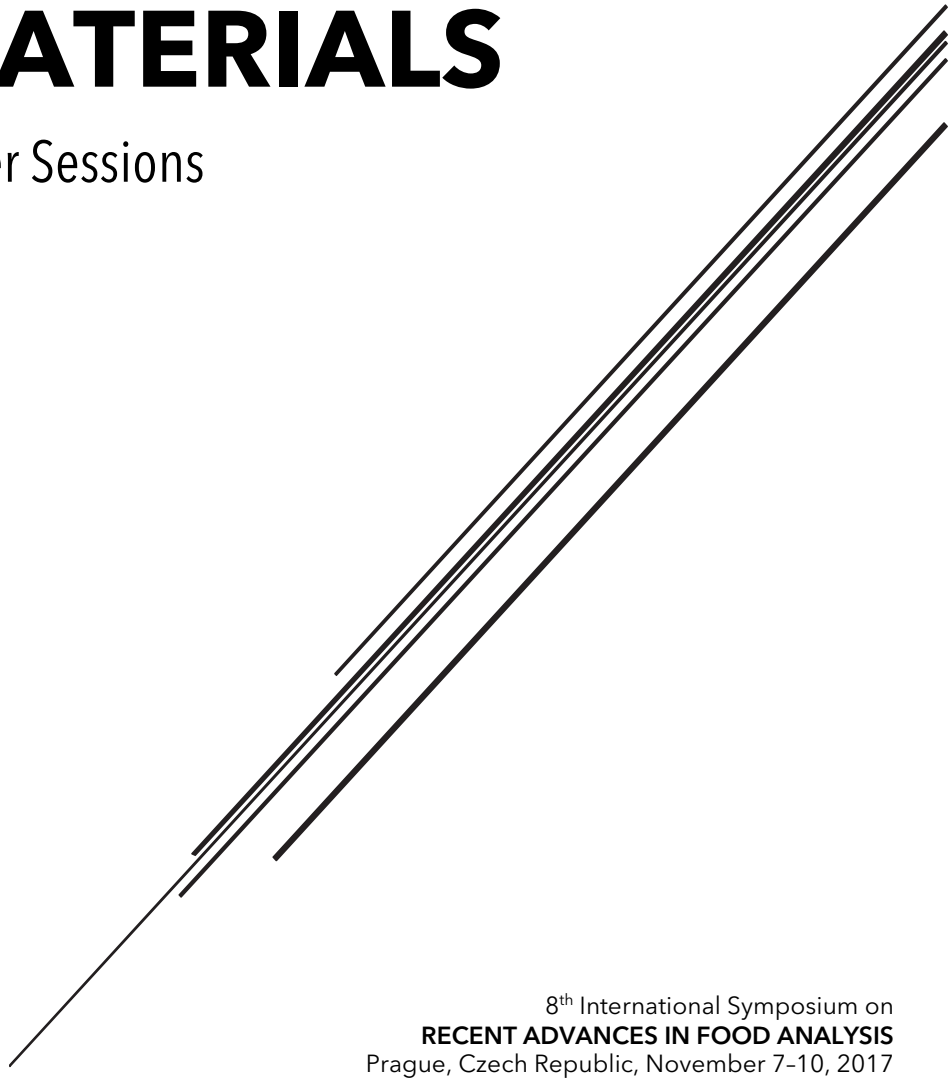
Photochemical vapor generation (PVG) is an effective sample introduction scheme for volatile mercury (Hg). Speciation of Hg^{++} and $MeHg^{+}$ was fulfilled for the first time by differential PVG under UV-B *vs.* UV-C wavelength and applied to fish oil supplements. After liquid-liquid extraction, the aqueous extract was mixed with 0.4% anthranilic acid (AA)-20% formic acid (FA) in a quartz coil, and exposed sequentially to 311 nm or 254 nm UV light. The resulting Hg^{0} vapor was detected by atomic fluorescence spectrometry (AFS). At each wavelength, the AFS intensity was a linear function of Hg^{++} and $MeHg^{+}$ concentrations, which were solvable from a set of two equations. This method achieved ultrahigh sensitivity with 0.50 and 0.63 ng mL^{-1} limits of detection for Hg^{++} and $MeHg^{+}$, respectively, and 73% recovery for $MeHg^{+}$ at 10 ng mL^{-1}. Validation was performed by ICP-MS on total Hg. Obviation of chemical or chromatographic separation rendered this method rapid, green, and cost-effective.

Keywords: *mercury, speciation analysis, photochemical vapor generation, UV vapor generation, fish oil*

MIGRANTS FROM FOOD CONTACT MATERIALS

Poster Sessions



8th International Symposium on
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MIGRANTS FROM FOOD CONTACT MATERIALS

J1
SCREENING FOR MOSH AND MOAH IN FOOD PACKAGING BY PSPE-UV/FLD-GC-FID

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Recently, the occurrence of mineral oil hydrocarbons (MOSH and MOAH) in food and food packaging has become a topic of interest. The current analytical method uses on-line LC-GC-FID and is well-developed and highly automated [1, 2], however, the required instrumental set-up is very expensive and not available in each laboratory. Additionally, the evaluation of the received data is a very challenging task.
Therefore, pSPE, which uses the fully-automated HPTLC devices, was used to analyze MOSH and MOAH on planar thin-layers in a very easy way. The method is less time and solvent consuming compared to the mentioned LC-GC-FID approach and requires a minimal instrumental set-up. In a twofold development, MOSH and MOAH were separated and focused into two sharp zones per track. Quantitation was carried out by densitometric scanning, with aromatic compounds detected by UV absorption, while aliphatic compounds were detected by the fluorescence signal on an HPTLC plate pre-impregnated with a fluorescent probe. Additionally, MOSH and MOAH zones were eluted by the TLC-MS Interface into autosampler vials and analyzed by GC to detect not only MOSH and MOAH humps, but also to identify marker substances specific for the mineral oil origin and for recycled fiber material.
Analysis of different types of paper and cardboard packages showed that most of the matrix components were efficiently separated by pSPE, clearly visible by fluorescent zones near the application position. In the subsequent GC run, pristane and phytane as marker substances for the mineral oil origin and 2,6-diisopropylnaphthalene as a marker for recycled fiber material [2] could clearly be detected.

[1] M. Biedermann, K. Grob, *Journal of chromatography. A* 2012, *1255*, 56–75.
[2] M. Biedermann, K. Grob, *Journal of chromatography. A* 2012, *1255*, 76–99.

Keywords: *MOSH/MOAH, Screening, Food packaging, pSPE-UV/FLD, pSPE-GC/FID*

J2
ANALYSIS OF FOOD CONTACT MATERIALS FROM POLYETHYLENE BAGS BY LC-QTOF-MS/MS X500R

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The production and use of food contact materials (FCMs) have been increased in the last years. Only substances included in the list of authorised additives may be intentionally used in the manufacture of FCMs. However, the control of migration products from new functionalised materials used in FCMs is a challenge for meeting food safety requirements. It is possible to detect migration of monomers that have not reacted during the polymerization, non-intentionally added substances, or other impurities.
The chemical identification of FCMs is complex, in part because of the incomplete information about the ingredients used to its manufacture. The majority of studies have focused on the development of methods using gas (GC) and liquid (LC) chromatography coupled to mass spectrometry (MS) as the suitable choice for the identification of migrant compounds. LC-MS is the choice for thermally unstable and non-volatile organic chemicals. Although, these studies have focused on the identification of FCMs using commercial or in house libraries; they also present a limitation, the degree of confidence when identification is based on unit mass resolution. High resolution accurate mass spectrometry (HRAMS) provides accurate mass measurement in both operation modes, full scan and MS/MS that increase the reliability and confidence of the results.
In this study, a migration study from polyethylene bags which are sold to store/transport purée fruits was carried out. According to the standard protocols established by EU regulation for FCM at Regulation EC 10/2011, two simulants and a contact time of 10 days at 40ºC were used. After that, the simulants were analysed by LC-QToF-MS. The acquisition mode selected was SWATH, a data independent non-target acquisition mode that divides the mass range analysed into smaller segments to perform the MS/MS mode. The screening of unknown compounds comprised retrospective analysis and data processing using open mass spectral libraries, such as ChemSpider and METLIN. This approach has provided the tentative identification of five FCMs: caprolactam, coumarandione, hexahydro terepthalic acid, bis(2-hydroxyethyl)adipate and bis(2-methoxyethyl) adipate. These compounds are plausibly toxic (they are suspected to be carcinogen, endocrine disruptors or hazardous to the aquatic environment, among others), highlighting the importance of the present study.

Keywords: *food contact materials, polyethylene, non-targeted screening, SWATH*

Acknowledgement: *The authors wish to thank the European Union's Seventh Programme for research and technological development FP7-ERANET-SUSFOOD "CEREAL Project" for the financial support given, ERA33-CEREAL-U.ALMERIA.*

J3
HYPHENATED LC-GC AND COMPREHENSIVE LC×GC WITH VUV DETECTION FOR THE ANALYSIS OF AROMATIC HYDROCARBONS IN FOOD PRODUCTS

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Mineral Oil Hydrocarbons (MOH) can find their way into food products in several different ways. They can be deliberately added, e.g. to prepare glossy coatings on sweets or fruits or to suppress dust formation of grain. They might also enter the food via contact of the raw materials with exhaust gases from energy production or transport, or by migration from packaging materials. MOH are a complex mixture of two classes of compounds: Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH). Quantification and characterization of MOSH and MOAH is important for food quality and safety reasons.
In the present contribution we describe new methods for the analysis of MOSH and MOAH in raw MOH materials used for de-dusting and foam suppression. The use of these methods for the analysis of MOSH and MOAH contaminations caused by migration from packaging materials is also described.
From the analytical perspective MOAH analysis is very complex [1]. MOAH is an enormous group of compounds and the total level of MOAH is very low. This holds for MOH products made for food applications which are extensively purified, but equally so for food contaminations. Analysis of MOAH down to the level of individual molecules is impossible. We will discuss analytical methods that enable analysis of *groups* of compounds, e.g, the groups of mono-, di- and tri-ring aromatics. By using comprehensively interfaced methods we are also able to determine distributions within groups, such as the molecular weight distributions of the various aromatic-ring groups. Multi-dimensional analytical methods allow describing the multidimensional nature of the samples in much more detail than single dimension methods. Key techniques in our coupled methods are silver-phase liquid chromatography, high temperature gas chromatography (HT-GC) and GC coupled with vacuum ultraviolet (VUV) detection. The latter, new detection method is highly sensitive and selective for aromatic species in that way making sample preparation easier.

[1] M. Biederman et al., Aromatic hydrocarbons of mineral oil origin in foods: method for determining the total concentration and first result, J. Agric. Food Chem. 57 (2009) 8711.

Keywords: *food contaminants, mineral oil, aromatic compounds, comprehensive chromatography, GC-VUV*

J4
FULLY AUTOMATED SAMPLE PREPARATION FOR THE DETERMINATION OF PLASTICIZERS IN PVC FROM FOOD CONTACT MATERIALS AND TOYS

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Food contact materials (FCM) made from PVC, such as e.g. gaskets of metal lids or cling films, may release plasticizers into the packed food. Such migration repeatedly exceeded legal limits or non-authorized plasticizers have been used. In a European enforcement campaign on migration from gaskets into oily foods in 2011, for example, legal limits were exceeded in 24% of the 308 samples analyzed [1]. The EU also banned the use of certain phthalates as plasticizers for toys and childcare products, whereas its content is limited to below 0.1%.
Plasticizers are analyzed and quantified by GC-FID or MS, if detection limits lower than 0.1% are needed. A piece of the PVC is solved in tetrahydrofurane and precipitated with ethanol. The supernatant is analyzed directly as well as after transesterification to ethyl esters. Transesterification enables the detection of epoxidized soybean oil (ESBO), epoxidized linseed oil (ELO) and polyadipates, but also confirm the identifications of the direct analysis through the transesterified products [2]. So far up to 40 different plasticizers were found and quantified in FCM and toys.
Today all steps of the sample preparation are automated by the use of the PAL RTC autosampler from CTC Analytics comprising its ability of exchanging e.g. diluting and injection tools. Automatization not only removes lab work, it also offers an automated elaboration of optimized conditions for derivatization, e.g. the transesterification. A transesterification reaction must be stopped by adding a citrate buffer after complete reaction before onset saponification. The precise reaction time is established by running a test sample under different conditions. The yield of transesterification and the start saponification is monitored by comparing an inert standard by a standard which is transesterified as well.

[1] G. McCombie, A. Harling-Vollmer, M. Morandini, G. Schmäschke, S. Pechstein, W. Altkofer, M. Biedermann, S. Biedermann-Brem, M. Zurfluh, G. Suter, M. Landis, K. Grob Eur Food Res Technol 235 (2012) 129–137.
[2] S. Biedermann-Brem, M. Biedermann, K. Fiselier and K. GrobFood Additives and Contaminants 22 (2005) 1274-1284.

Keywords: *food contact materials (FCM), polyvinyl chloride (PVC), plasticizers, transesterification, automation*

J5
NON-INTENTIONALLY ADDED SUBSTANCES IN FOOD AND FOOD PACKAGING - THE DETERMINATION OF MINERAL OIL HYDROCARBONS USING LC-GC ONLINE TECHNIQUE

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Food can get contaminated not only through direct contact with its packaging, but also along the entire production chain. So far, legislation on the migration of substances from plastic packaging into food particularly has covered the starting materials used in the plastics manufacturing. Regarding health risks related to the transfer of substances, the so-called NIAS (non-intentionally added substances) should be focused stronger; these substances are non-intentionally added during manufacturing of packaging materials. These can include impurities originating from raw materials, reaction and degradation products in manufacturing as well as impurities arising from transport and production [1]. Currently, there is much discussion on food packaging with mineral oil hydrocarbons (MOSH–mineral oil saturated hydrocarbons and MOAH–mineral oil aromatic hydrocarbons). The MOSH fraction consists of linear and branched alkanes as well as alkyl substituted cycloalkanes, whereas the MOAH fraction consists of alkylated polyaromatic hydrocarbons with up to four aromatic rings. The main focus is on the aromatic fraction, which is suspected of being potentially carcinogenic and mutagenic [2]. The proportion of the aromatic fraction is approximately 15 – 30% of the total mineral oil fraction. Undesirable mineral oil residues that, in many cases, get into products through the use of mineral oil based printing inks which are often found in food and food packagings. This effect has been increasingly detected in recycled materials but also in packagings consisting of fresh raw materials. In many products, the concentration of saturated (MOSH) and aromatic (MOAH) hydrocarbons is particularly increased. For this analysis, a MOSH/MOAH system is now available which is based on LC-GC coupling and was developed in accordance with the European norm EN 16995:2016 'Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with online HPLC-GC-FID Analysis. The advantage of the system is the configuration of two FID detectors, which allow parallel determination of MOSH and MOAH within a single run.

[1]http://www.vzhh.de/ernaehrung/368577/lebensmittelverpackungen-unbeabsichtigt-eingebrachte-stoffe.aspx
[2] R. Lorenzini, M. Biedermann, K. Grob, D. Garbini, M. Barbanera, I. Braschi, Food Addit Contam 30 (2013) 760-770

J6
METHOD OF TEST FOR PHOTOINITIATORS OF INK IN FOOD

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In order to attract consumers, multilayer print designs are usually used in commercial food package. UV-curing ink is generally used in food package printing because it has many adavantages, such as fast-drying, space-saving, environmentally friendly…etc. However, many alerts for food contamination by UV-curing ink photoinitiators arose in Europe in recent years. Therefore, food contamination of photoinitiators in UV-curing inks has attracted many attentions. In this study, a multiple-analysis method of 30 photoinitiators was established. A market survey of photoinitiators in breakfast cereal and packaged juice was also performed. For sample treatment, a QuEChERS (quick, easy, cheap, effective, rugged and safe) method for the extraction photoinitiators in breakfast cereal and packaged juice was evaluated. Chromatographic separation of two isomers, methylbenzophenone (MBP) and isopropylthioxanthone (ITX), was achieved by a pentafluorophenyl (PFP) column (1.7 µm, 100 × 2.1 mm i.d.) and MeOH: 5mM formic acid-ammonium formate (pH 4.0) in gradient elution. The average recoveries of 30 photoinitiators in breakfast cereal were between 62.0 and 120.3 % and their coefficients of variations were between 0.4 and 14.4%. The average recoveries of those in packaged juice were between 84.4 and 122.9% and their coefficients of variation were between 0.5 and 9.5%. The market survey results of five breakfast cereal and ten packaged juice were as follows: 0.013 mg/kg triphenyl phosphate (TPP) was detected in one breakfast cereal, and 2-hydroxy-4-methoxy benzophenone (BP-3), 1-hydroxycyclohexyl phenyl-ketone (Irgacure 184), methyl-2-benzoylbenzoate (MOBB) and 2,4-diethyl-9H-thioxanthen-9-one (DETX) were detected in one package juice, ranged from 0.002 to 0.153 mg/kg.

Keywords: *photoinitiator, ink, LC/MS/MS*

J7
SOURCES FOR ANTHRAQUINONE AND BIPHENYL IN COCONUT OILS

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Substituted polycyclic aromatic hydrocarbons (SPAHs) occur ubiquitously in the whole global environment as a result of their persistence and widely-spread sources. They are produced from their corresponding PAHs. Biphenyl (BP) accrues naturally in coal tar, crude oil (up to 0,4 mg/g) and natural gas (3-42 g/m^3) and it is also used as a wood preservative [1]. Anthraquinone (AQ) or 9,10-Anthracenedione is an oxidizing product of anthracene, one of the light polycyclic aromatic hydrocarbons (PAHs). Therefore, AQ is an OXY-PAH, but because it is possible to use it as a bird repellent, its residue is controlled, like biphenyl, by the Regulation (EC) 396/2005 [2].

Altogether we measured 20 different coconut oil products directly from the market and raw materials from different countries and made with different production processes. Most of the products were free of any critical findings. But three products showed significant amounts of AQ and BP. To find the source of both of these chemicals, we looked into the drying procedure used in processing. We burned the coconut shells and analyzed their smoke for AQ and BP.

Drying technique for coconut oil production is the keystep. We proved that by analyzing the ambient air after burning the coconut shells. The air sample was analysed for PAHs, OXY-PAHs, some MPAHs and BP. Exept for AQ and BP we also found other OXY-PAHs like 9-Fluorenone, 1,4-Naphthoquinone and methylated PAHs like 2-Methyl –AQ and mono- and di-methylated Naphthalene. Through this analysis of the coconut shell smoke we could prove that AQ and BP as well as other SPAHs can be developed during the combustion of organic matter during the drying process in the coconut oil production.

On another hand, the current environmental situation in Southeast Asia and Africa is the other well-known cause for the air contamination with aromatic contaminants.

[1] Boehncke et al., Concise International Chemical Assessment Document 6. Biphenyl, WHO, 1999
[2] Annexes Reg. 396/2005, Regulation (EC) 2015/401, Annex IIIA, Applicable from: 01/11/2014

Keywords: *coconut oil, biphenyl, anthraquinone, GC-MS, LC-MS*

J8
DEVELOPMENT AND VALIDATION OF A SCREENING METHOD FOR BISPHENOLS AND RELATED REACTION PRODUCTS IN CANNED FOOD

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Bisphenol a and some of its derivates (f.e. bisphenol s), are chemicals produced in large quantities, which have the potential to interact with the hormone systems of humans and animals [1][2.] Metal cans for food packaging are often coated with epoxy resin to protect the food from catalytic effects of the aluminium or iron can. The resin was identified as the source of bisphenols and derivates in the food.[3] In this study the presence of 25 bisphenols (bisphenol di- and oligomers and associated glycidyl ethers and their corresponding HCL- and H_2O derivatives) in canned food products was tested. Some of the observed substances are not well characterized and are completely unknown in relation to the potential to influence metabolic processes.[3][4]

The extraction and measurement method was developed for the quantification of a variety of bisphenols and derivates. The extraction was performed using acetonitrile as a solvent. The extract was analysed by high performance liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) was used. The combined methods of extraction and sensitiv measurement allow the use of small amounts of samples (typically 2 g), low volumes of solvent (10 ml), and short extraction times (30 min). The final screening method contains 25 different substances, including bisphenols and derivates (LOQs of 0,005 to 0,05 mg/kg) and can easily be adaptabted to the analysis of material as well as ground and surface water.

In some tested products Cyclo-di-BADGE (CdB), a cyclic product of the condensation of bisphenol a and BADGE, was detected in concentrations above the recommended threshold of the German Surveiliance Authority (The Federal Institute for Risk Assessment).[3]

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[2] JRC European Comission, Updated European Risk Assessment Report, Environment Addendum of February 2008, *4,4'-Isopropylidendiphenol (Bisphenol-A) 80-05-7*, Luxembourg: Publications Office of the European Union.
[3] Bundesamt für Risikobewertung, 15-April.2016, Stellungnahme, *Epoxidharz-Beschichtungen von Konservendosen: Stoffübergänge in Ölhaltige Lebensmittel sind möglich*, Berlin.
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Keywords: *bisphenol, BADGE, BFDGE, canned food, LC-MS*

J9
STYRENE OLIGOMERS FROM FOOD CONTACT MATERIALS (FCM) DETECTION AND DETERMINATION OF STYRENE OLIGOMERS (DIMERS AND TRIMERS) IN FOODSTUFF AND FOOD SIMULANTS USING A NEW DEVELEOPED LC-GC-MS-MS METHOD

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Polystyrene have been playing an important role in packaging material for foodstuff for a long time. It is present in several products in direct contact with the food, as for example as sheets in sausage and cheese packaging, meal trays, thermal insulating layers, yogurt cups etc. The broad application and the daily use on a massive scale of those synthetics based on styrene raises the question whether the production is compliant with Good Manufacturing Practices (GMP), also on a supra-regional level. Styrene oligomers result from the manufacture of polystyrene and remain unintentionally compounds (non-intentionally added substances, NIAS) in the final product. Therefore, they may potentially merge to foodstuff upon contact. According to article 5 (2) from the GMP regulation (EG) No. 2023/2006 [1] manufacturers must choose their starting material in compliance with previously set specifications. These guarantee that the material or the item is in accordance with the assigned requirements. The specifications have to be documented and presented to the responsible authority upon request (article 7 (3)).
These inspections do not only include educts and products but also all possible reaction products (NIAS, not intentionally added substances) as for example the styrene oligomers and examine them regarding genotoxicity.
Research by the CVUA-MEL on polystyrene oligomers revealed that styrene oligomers may migrate into food simulants. The migration is temperature-dependent and rises upon increasing lipophilicity of the simulants. In vitro experiments from literature show that oligomers isolated from polystyrene (mainly dimers and trimers) are not genotoxic. From that, a migration value of 50 µg/kg foodstuff can be derived for styrene oligomers. Styrene dimers and trimers are classified as Cramer class III which represents a tolerable uptake of 90 µg/person per day. (Statement 023/2016 by the BfR on the 21st of April 2016).
Method: The method is based on a LC-GC-MS/MS measurement which reliably determines styrene oligomers at very low concentrations (detection limit 1–60 ng/kg equivalent to 0.001–0.06 µg/kg) in foodstuffs and food simulants, especially in oils and fatty foodstuff. After separation of fatty components (mainly triglycerides) and other possible matrix components using a normal phase chromatography, the dimers and trimers of the polystyrene are fractionated. Subsequently, using an interface, the fraction is transferred online to the gas chromatograph. Here, the chromatographic separation and quantification of the analytes using a triple quad detector is performed. Direct coupling of the GC-QqQ system to the NPLC allows for a very high sensitivity. Furthermore, no time-consuming sample preparation to separate the matrix/the food simulants (oils, e.g. sunflower oil) is required.

Keywords: *NIAS, food contact material, LC-GC, migration, styrene oligomers*

Acknowledgement: *The authors thanks Axel Semrau Gmbh & Co KG for supporting this research project.*

J10
TARGET AND NON-TARGETED MASS SPECTROMETRY TO DETECT IAS AND NIAS IN FOOD CONTACT MATERIAL

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According to the regulations on Food Contact Materials [1-2], it is necessary to monitor packaging material components that could migrate into food, thus compromising its quality and safety.
In particular, substances that could migrate in food are divided in:
Intentionally Added Substances IAS (additives with functional properties as stabilizers, plasticizers, lubricants, antioxidants, etc. and or additives from adhesives, varnishes, printing inks, etc. and/or residual monomers and catalysts);
Non-Intentionally Added Substances NIAS (Substances derived from polymers or additives degradation and/or impurities in raw materials and/or reaction products and/or other contaminants)
Mérieux NutriSciences developed GC and LC Targeted and Non-Targeted Mass Spectrometry Methods to analyze both IAS and NIAS according to existing regulations, recommendations and expert opinions (ILSI Europe Guidelines) [3].
Through Non-Targeted Screening Approaches based on HRMS and MS/HRMS, Mérieux NutriSciences can detect and identify NIAS whose toxicological effects on human health are still unknown and whose limits might be restricted in the future.

[1] Regulation (EC) no 1935/2004
[2] Regulation (EC) no. 10/2011
[3] ILSI Europe current Guidelines, "Guidance on Best Practices on the Risk Assessment of Non-Intentionally Added Substances (NIAS) in Food contact Materials and Articles"

Keywords: *NIAS, IAS, high resolution mass spectrometry, food contact material, food packaging*

J11
IN SIMULACRA STUDIES ON BIOTRANSFORMATION PRODUCTS MORE TOXIC THAN SUDAN I-IV AND PARA-RED DYES

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Azo-dyes selected for the tests (Sudan I-IV and Para-red) are banned for use in food and feed, nevertheless their presence is still detected in spices, foods containing spices, eggs and animal tissues [1]. They may be transformed by intestinal bacteria into carcinogenic aromatic amines [2]. Because the possible products of their metabolism exert more toxic effects than the dyes themselves, we decided to study this phenomenon using combined abiotic and *in vitro* methods.
At first, the electrochemical analyser, capable of performing the oxidation and reduction reactions was used for the simulation of metabolic processes (10 µg/ml dyes in 0.1% formic acid in acetonitrile reduced on glassy carbon electrode). Then, the degradation process of the dyes by intestinal bacteria with four different bacteria strains was studied: *Bacillus spp., Clostridium spp., Enterococcus spp.* and *Escherichia spp*. The cultures were grown in conventional media and their subsamples were collected in intervals of 0 to 48 hours. They were extracted using acetonitrile and 40% ammonium acetate and analysed by LC-MS/MS technique developed for the purpose of this study [3].
Using the EC-MS method we have already identified four metabolites of Sudan I: acetanilide, 1,2-napthoquinone, 2-napthol and aniline; also four metabolites of Sudan II: 1-amino-2-napthol, 1,2-dihydroxynapthalene, 1,2-napthoquinone and 2-napthol and four of Sudan III: 1-amino-2-napthol, 1,2-dihydroxynapthalene, 1,2-napthoquinone and aniline. For Para Red, which is structurally similar to Sudan I, using EC-MS we have found two metabolites: 1,2 hydroxyphenylazo-2-napthol and 1,2-napthoquinone.
Using the *in vitro* tests we have so far identified aniline as a metabolite of Sudan I produced by *Clostridium spp.* and *Enterococcus spp.* and 1-amino-2-napthol as a metabolite of Sudan II produced by *Clostridium spp.* and *Enterococcus spp.*
Apart of the identified metabolites there are signals of analytes masses of: 151, 120 and 121 g/mol, which will be confirmed in our further studies.
It may be concluded that both techniques used are suitable for mimicking the formation of toxic metabolism products of Sudan dyes which may be formed in nature by different strains of bacteria.

[1] Chen *et al.*; *J. Chromatogr. B*, 2013, 939, 45–50.
[2] Xu *et al.*; *Anaerobe* 2010, 16, 114–119.
[3] Pietruk *et al.*; *Różne oblicza toksykologii*, Pulawy 19-22 September 2017.

Keywords: *azo-dyes, Sudan dyes, azo-reduction, in vitro, EC-MS/MS*

Acknowledgement: *This study was financed by the National Science Centre, competition PRELUDIUM 9 No 2015/17/N/NZ7/04097: In simulacra studies on the reduction of azo-dyes and identification of their carcinogenic metabolites.*

J12
SPME-GC-HR-TOF-MS BASED SEARCH FOR OFF-FLAVOR COMPOUNDS IN CHOCOLATE ADVENT CALENDARS

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Identification of compound(s) responsible for food off-flavor often represents a challenging analytical task, since these compounds might have a very low odor threshold. It is rather difficult to identify them, especially when present in a complex mixture. For this purpose, head-space solid-phase microextraction sampling followed by gas chromatography - mass spectrometry (SPME-GC-MS) employing time of flight (TOF) mass analyzer seems to be the best option.
Within our study, we employed both low resolution TOF and high resolution (HR-TOF) MS. Non-target screening in a set of advent calendars was performed with the aim to access aroma quality. Statistical compare - peak alignment tool was used and data matrix was then processed using multivariate statistical analysis. Surprisingly, styrene, a possible human carcinogen, migrating from plastic packaging was discovered at a significant level in one of the chocolate samples, dedicated for children. Unknown off-flavor compounds in chocolate from one of the calendars were identified using HR-TOF MS. Acrylates, responsible for an unpleasant smell of chocolate were identified in green painted parts of wholesale cardboard box. According to obtained results, SPME-GC-(HR)TOF-MS proved to be a reliable tool for non-target screening and unknown identification.

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and specific university research (MSMT No 20-SVV/2017)".*

J13
APPROACHES FOR THE DETERMINATION OF NON-INTENTIONALLY ADDED SUBSTANCES (NIAS) FROM FOOD CONTACT MATERIALS: DETECTION, IDENTIFICATION AND QUANTIFICATION

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This presentation will discuss different approaches for the non-targeted analysis of chemicals by chromatographic techniques coupled to high resolution mass spectrometry. Strategies, difficulties and challenges will be discussed and illustrated through examples of non-targeted analysis applications for the detection of unknowns in food contact materials and food. These will include:
- Considerations for sample extraction, data acquisition and acquisition quality controls
- Approaches and difficulties in handling and interpreting large and complex datasets
- Challenges in the identification and quantification of unknown substances.

Different approaches used to assist on the identification of unknown substances will be discussed e.g. comparison against databases of potential structures, use of fragmentation data, comparison to modified formulations.

Keywords: *NIAS, food contact materials*

J14
BISPHENOL A: METHOD VALIDATION AND SPECIFIC MIGRATION OF BRAZILIAN COATINGS

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Introduction: Bisphenol A (BPA) is used in the production of epoxy resins for internal and external coating of food and beverage cans. It is also used in the production of polycarbonate resins. However, BPA in the composition of feeding bottles for infants up to 12 months is prohibited. BPA has been identified as an endocrine disruptor and has been evaluated by health authorities worldwide. The limit of migration of BPA is 600 µg/kg established by European and Brazilian legislation.
Objective: The focus of this study was to validate a method of specific migration of BPA into food simulants using UHPLC-MS/MS and evaluate different coatings in Brazilian market.
Materials and Methods: Agilent 1290 Infinity LC System with Agilent 6400 Series Triple Quadrupole System. The simulants studied were: distilled water, 3% w/v aqueous acetic acid, 50 % v/v aqueous ethanol and olive oil. The limits of detection/quantification were obtained by injecting seven different solutions. The linearity was determined by standard solutions ranging from 0 to 6,000 µg/L. The recovery was evaluated using three different concentration in triplicate samples of food simulants spiked with BPA (90 for aqueous simulant and 300 for fat simulant, 600 and 1,200 µg/L).
Results and Discussion: The limits of detection/quantification obtained were: distilled water (0.76 µg/L /4.33 µg/L), 3% w/v aqueous acetic acid (0.20 µg/L /3.57 µg/L), 50 % v/v aqueous ethanol (0.95 µg/L /4.41 µg/L) and olive oil (4.40 µg/L /95.7 µg/L). The linearity of the analytical curve was studied using standard solution in six concentrations ranging 0-300 µg/L and 300-6,000 µg/L for aqueous simulants and 0-6,000 µg/L for fat simulant. The coefficient of determination (r^2) calculated by linear regression presented values greater than 0.998. The recovery obtained after 40ºC/ 10 days in conc. 90 µg/L for aqueous simulant was 71-115% and in conc. 300 µg/L for olive oil was 110%, in conc. 600 µg/L and 1,200 µg/L for all simulants was 82-103%. At least eight samples of coatings were evaluated in Brazilian market in specific migration of bisphenol A and only one was out of the permitted limit of BPA specific migration.
Conclusion: The method validated can be applied to study specific migration of BPA from plastic materials and coatings used for internal coating of cans in all food simulant and takes 2 minutes for BPA's analysis. The limits of detection/quantification obtained for UHPLC-MS/MS are suitable for the limit of specific migration of BPA established in the legislation and assist the health authorities to monitor the presence of BPA and the consumers´ exposure.

Keywords: *bisphenol A, specific migration, coating, UHPLC-MS/MS, validation*

Acknowledgement: *Agilent Technologies and São Paulo's State Government*

J15
EFFECTS OF STERILIZATION, STORAGE CONDITIONS AND CAN DENTING ON THE MIGRATION OF BISPHENOLS AND METAL TRACE ELEMENTS INTO CANNED FAVA BEANS AND OKRA

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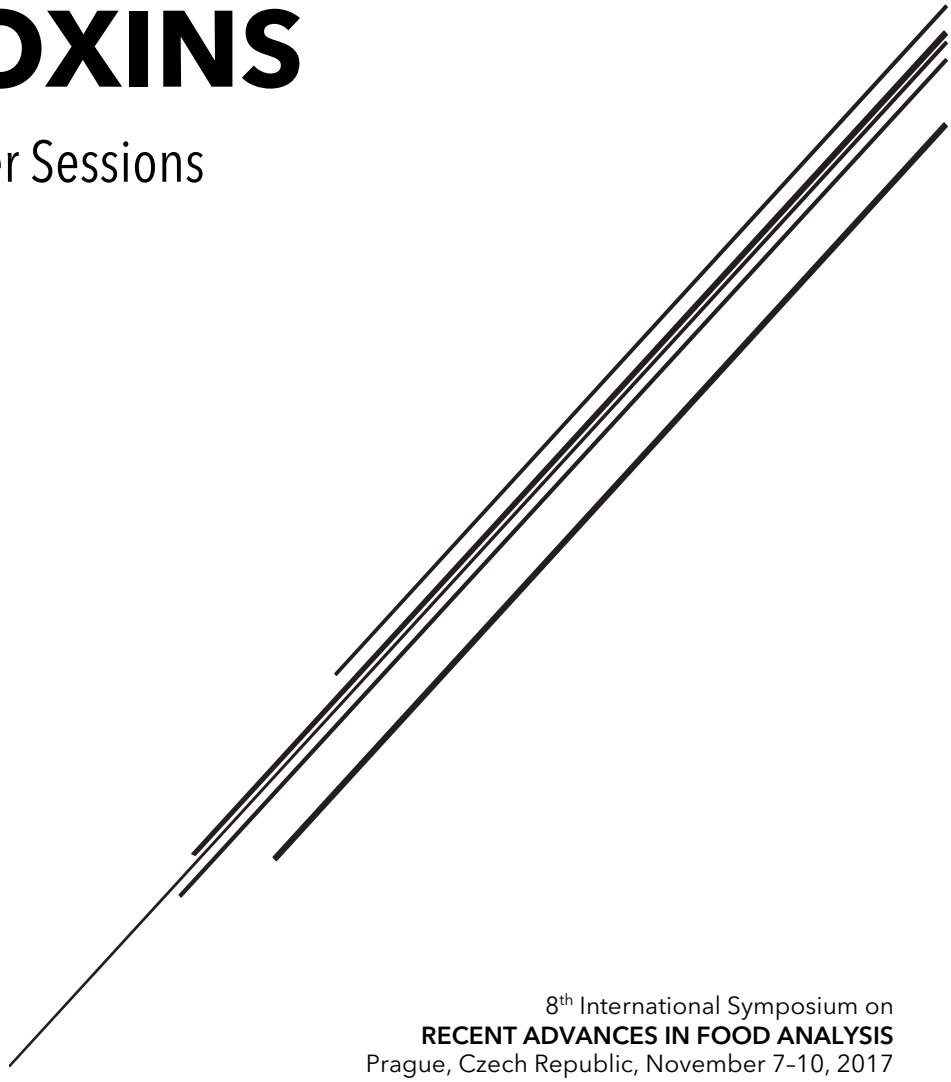
Tinplate cans have been used for preserving food for over a century. Most of the time, they are coated with organic polymer for the sake of limiting direct food/metal contact and metal corrosion. Yet, such tinplate cans may represent an important source of organic and inorganic contaminants due to food/coating interaction. In particular, the organic coating may release endocrine disrupting chemicals such as bisphenol A and bisphenol A di-glycidyl ether which are monomers used in the synthesis of cans inner epoxy resins. In the meantime, metal trace elements can be released from the alloy substrate, so that their levels may increase in canned food over storage. Likewise, the migration of these contaminants mainly depends on the coating (nature and process), the chemical properties of foodstuffs, as well as the heat processing and storage conditions.
Migration of organic or inorganic contaminants has been extensively reported in canned food and simulants. Yet, data relative to canned food from the Lebanese market are scarce, which is surprising since Lebanese cuisine is highly appreciated regionally and recognized globally with considerable annual exportation of canned food. In addition, canned foods are largely consumed by the Lebanese population. Therefore we report here migration data for two typical Lebanese canned vegetable products, namely okra and fava beans, which greatly differ in their pH and water content. The novelty of our work lies also in the simultaneous study of both types of contaminant (organic and inorganic) in the canned food.
We report here the effect of four parameters (sterilization, storage time and temperature, can denting) on the migration of both unreacted free monomers from the epoxy-phenolic coating (bisphenol A and F, bisphenol A and F diglycidyl ether and their derivatives) and trace metallic elements (Fe, Sn, Pb, Cd, Ni, Zn, Cu). Raw foods were collected before sterilization while canned samples were taken after sterilization and stored for several months at different temperatures (5°C, ambient, 40°C). Some samples of both food types were dented and stored at room temperature. For the analysis of coating based monomers, 0.2 g of lyophilized samples were soaked for 12 hours and extracted twice with methanol, then purified using solid phase extraction. UHPLC coupled with fluorescence detection was used for quantification and with MS detection for compounds confirmation. For the analysis of trace metallic elements, 0.35 g of lyophilized samples were mineralized with 3 ml nitric acid (65-69%) in open block digestion system for one hour at 95°C, followed by multi-element analysis using atomic absorption spectrometry. Our results show a significant difference in the levels of most organic and inorganic contaminants, and parameters influencing these levels, between okra and fava beans. Sterilization effect was most significant on accelerating the migration of organic contaminates but not on inorganic contaminants.

Keywords: *migration, bisphenols, metal trace elements, canned food*

MYCOTOXINS, MARINE & PLANT TOXINS

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

K1
FUMONISINS IN MAIZE (ZEA MAYS L.): EFFECT OF PARTICLE SIZE ON THE ANALYTICAL PERFORMANCE

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Fumonisins are secondary metabolites produced primarily by fungi that belong to the genera *Fusarium*, which are highly prevalent in maize crops. The relevance of these mycotoxins on human/animal health prompted the European Community to introduce maximum permissible limits in foods and feeds. Some authors have documented the problematic of the fumonisin analysis, in relation with the different experimental parameters applied during extraction, the presence of fumonisin derivatives and the possible analyte-matrix interaction [1-3]. Therefore, the understanding of mycotoxin behavior during both processing and analysis is a worldwide topic of interest due to the high economic and health impact.
The purpose of the study was to investigate the interactions between the analyte and the matrix, as well as to study the effect of the sample particle size on the extraction procedure. Four naturally contaminated maize samples were included in the study. Six different fractions, with particle size ranging between and, were obtained by five ISO certified sieve shakers. Each fraction as well as the initial sample underwent composition analysis and fumonisin determination.
While no significant difference in composition was detected among the fractions, a strong inverse correlation was found between particle size e fumonisins recovery, with an overall increase up to 400%. Although several studies are reported in the literature on the distribution of fumonisin among maize fractions and most of them reported that fumonisins are mainly concentrated in the finer fractions of maize [4-6], none of them is exhaustively addressing the effect of the particle size on the analytical performance. In our study, since fractions do not significantly differ in composition, higher fumonisins recovery in finer fractions is likely due to a better extraction. The matrix surface and its accessibility to the solvent is, indeed, higher in finer fractions. Therefore, possible interactions of the analyte with the matrix are likely better disrupted. Starting from this observation, the possible interaction between fumonisins and maize and the effect of the particle size on the solvent extraction were described using chemical and physical approaches.
The data reported within this study may offer an interesting insight into the hidden fumonisins analytical issue, thus providing a technical basis to support risk management and regulatory bodies in order to revise legislative limits.

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Keywords: *fumonisins, particle size, maize, solvent extraction*

K2
ON THE MITIGATION OF MYCOTOXINS CONTENT: THE STRANGE CASE OF AFLATOXIN B1 AND LACCASES FROM TRAMETES VERSICOLOR

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Aflatoxins are mutagenic, genotoxic and carcinogenic mycotoxins produced by *Asperigillus spp.* primarily contaminating cereals, maize, oilseeds and nuts. Aflatoxin B1 (AFB1) has been classified as a class-I carcinogen by IARC being among the most harmful natural food contaminants in terms of acute and chronic toxicity. Beside health concerns, AFB1 causes significant losses in terms of veterinary costs and managing of incompliant food and feed batches. Therefore, the mitigation of AFB1 in food and feed is critical for facing the forthcoming challenges in view of the sustainability and global trade. Indeed, reducing the contamination in food and feed ultimately ameliorates the health and welfare of humans and animals. Also, the implementation of strategies for recovering contaminated batches may concretely allow the weaker markets to compete on the global trade. A wide number of strategies for the mitigation of mycotoxins content are currently under consideration. The strategies relying on microbial/biochemical detoxification of incompliant products have the potential advantage of reducing and/or reusing waste. The enzymatic transformation is among the most promising tools for the mitigation *in situ* and the laccase enzymes seem effective in degrading AFB1. However, whether or not they can be used for mitigating AFB1 in real matrices is under discussion basically because of the unclear mechanism of degradation. The suspected need of third party molecules to trigger the reaction (i.e. mediators) and the possible low specificity rise major issues. Focusing on the laccases from *Trametes versicolor*, this work aimed at investigating the role of mediators and gaining insights on the specificity of reaction. AFB1 was incubated with laccases and degradation was monitored in a 5-days kinetic by using LC-MS/MS and LC-Fluo/UV. The degradation rate was found negligible when mediators were missing. Conversely, the almost complete degradation was observed when ABTS was added to the reaction, sustaining the role of mediators in the degradation process. Although ABTS cannot be considered in a real usage on food and feed, an aqueous maize extract succeeded in triggering the degradation pointing to the presence of "natural" mediators in matrices susceptible to AFB1 contamination. Laccases were found able to degrade AFB1 in naturally contaminated maize as well, supporting a valuable use on real matrices. Some hints on the reaction specificity were derived as no degradation was observed for other co-incubated natural compounds (e.g. rutin and quercetin). The tentative identification of degraded products was done combining MS and spectroscopic analysis.

Keywords: *aflatoxin B1, laccase, mycotoxin mitigation, trametes versicolor, enzymatic degradation*

Acknowledgement: *We would like to acknowledge Prof. Chiara Dall'Asta for the fruitful contribution in defining the experimental design and in discussing results.*

K3
FREE AND HIDDEN FUMONISINS IN YELLOW AND WHITE WHOLE GRAIN MAIZE (ZEA MAYS) AND CORRESPONDING MILLING FRACTIONS

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Maize (*Zea mays*) is one of the most spread crop because of its importance in food and feed industry. The purpose of the study was to determine the occurrence of free (FB1 and FB2) and hidden fumonisins (HFB1 and HFB2) in yellow and white whole grain maize and in the corresponding milling co-products. This crop is known for its susceptibility to fungal contamination able to produce toxins (i.e. mycotoxins natural occurring toxic secondary metabolites of fungi that may be present in food products). During cereal grain processing a large number of co-products (germ and outer layers) are generated. These fractions are mainly directed to the feed industry or used as ingredients for gluten free food products. The concentrations of fumonisins naturally contaminated maize samples were analyzed using liquid chromatography coupled to mass spectrometry. Fumonisin contamination trend (inner to outer parts) was found to be similar in both maize varieties and corresponding co-products. FB1 was found as the most abundant mycotoxin in all samples of both maize varieties and the FB1 to FB2 ratio of 1:4 was found. This is consistent with other studies [2]. The amount of total fumonisins measured based on the hydrolyzed forms (HFB1+HFB2) was 1.4-7.8 times greater than the amount of free fumonisins (FB1+FB2). The concentration of hidden fumonisins was calculated by subtracting the levels of free fumonisins from the total fumonisin levels. The levels of hidden fumonisins were calculated to be 1.0-5.4 times greater than the level of free fumonisins. A strong positive correlation (R^2=0.97) was observed between free fumonisins (FB1+FB2) and total fumonisins (HFB1+HFB2). The fumonisins contamination found in maize and co-products directed to human consumption was below the law limit. Nevertheless, these results show that the risk of exposure to fumonisins is likely underestimated if only free fumonisins are considered. This is particularly important for the "sensitive" or frequent consumers, such as people with gluten related disorders. Or else where the incidence of esophageal cancer is historically highly documented, such as parts of China and northern Italy [3]. Moreover, these results contribute to extend the available data on fumonisins in food/feed corn-based products.

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Keywords: *fumonisins, maize, milling co-products*

Acknowledgement: *Marco Spaggiari received a PhD grant by Regione Emilia-Romagna, under the scheme POR-FSE/2016.*

K4
DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AS SAMPLE TREATMENT FOR THE DETERMINATION OF AFLATOXINS IN FUNCTIONAL VEGETABLE MILKS AND ENRICHED MILKS BY HPLC WITH FLUORESCENCE DETECTION

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The consumption of plant-based milks has increased in recent years due to their nutritional attributes, being considered as functional foods. These products may contain different residues and contaminants, such as mycotoxins, if contaminated raw materials are used. However, by the moment these products have received little attention in regulation in relation to the control of hazard agents. Among them, it is possible to find aflatoxins, highly toxic secondary metabolites of some *Aspergillus spp.* that occur mainly in cereals, seeds or nuts, used for the production of vegetable milks. In this work, dispersive liquid–liquid microextraction (DLLME) has been proposed as sample treatment for the determination of aflatoxins B1, B2, G1 and G2 in different types of plant-based milks (oat, rice, coconut, almond and birdseed milk) and milk-based products enriched with nuts and cereals (oat, almond and walnut) using high performance liquid chromatography (HPLC) coupled to on-line photoinduced fluorescence detection (PI-FLD), avoiding the use of derivatization reactions to increase sensitivity. Matrix-matched calibrations were set for the different studied matrixes, obtaining satisfactory linearity, with correlation coefficients >0.994 for all the aflatoxins. The precision (repeatability and intermediate precision), expressed as RSD, was lower than 9.7% and recoveries ranged between 82-104%. In addition, limits of quantification ranged between 0.03-0.48 μg kg^{-1} for the four aflatoxins, allowing the determination of these compounds below the maximum level established by European Commission in different commodities, used as raw material in the considered functional beverages.

Keywords: *aflatoxins, functional vegetable milks, enriched milks, DLLME, HPLC*

Acknowledgement: *The authors gratefully acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project ref: AGL2015-70708-R). AMH thanks the "Erasmus Mundus - Al Idrisi II program" for a doctoral grant (2013 – 2401 / 001 – 001).*

K5
METABOLOMICS FOR ALTERNARIA TOXINS AS A TOOL FOR RISK ASSESSMENT OF MYCOTOXINS

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Among food contaminants, *Alternaria* toxins play an important role as "emerging" mycotoxins. Fungi of the genus *Alternaria* are ubiquitous plant pathogens on crops, herbs and vegetables and cause harms on leaves, stems, flowers and fruits. *Alternaria* species produce up to seventy secondary metabolites, some of which are characterized as mycotoxins, inducing adverse effects in animals. Among these mycotoxins, alternariol and alternariol monomethylether indicate genotoxic effects in mammalian cells *in vitro*. Altertoxins are mutagenic and induce cell transformation, whereas tenuazonic acid is known to be orally acute toxic to chicken and mice [1].
As only few toxicity data on *Alternaria* toxins are available, the European Food Safety Authority (EFSA) applied the threshold of toxicological concern (TTC) approach to estimate the relative risk due to dietary exposure of humans for these toxins. There is no tolerable daily intake (TDI) set for any *Alternaria* toxin because of missing toxicological data [1]. For further assessments, reliable and precise quantification methods for mycotoxins produced by *Alternaria* fungi in different food matrices are essential.
We developed and validated a sensitive LC-MS/MS method for the quantitation of *Alternaria* toxins in grain, grain products and herbs. For the quantitation of alternariol, alternariol monomethylether and tenuazonic acid stable isotope dilution assays with ^{13}C- and ^{2}H-labelled analogues as internal standards were applied. Tentoxin was quantified using matrix calibration. Limits of detection and quantification ranged between 0.02 and 0.45 µg/kg and between 0.06 and 1.4 µg/kg. Recoveries were 97 to 113%, respectively. Intra- and interday precisions were determined for a comprehensive validation and varied from 4 to 5% as well as 6 to 10%.
For a non-targeted metabolomics approach, different *Alternaria* isolates were analyzed by means of ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Good sensitivity and ultrahigh-resolution of FT-ICR-MS allowed a much deeper insight into the *Alternaria* mycobolome (metabolome of the fungi). We applied different software tools for accurate data handling and visualization purposes.

[1]: EFSA on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. EFSA Journal 2011;9(10):2407.

Keywords: *alternaria toxins, SIVA, LC-MS/MS*

Acknowledgement: *We gratefully acknowledge the support by the Faculty Graduate Center Weihenstephan of TUM Graduate School at Technical University Munich, Germany.*

K6
THE SEARCH FOR BIOMARKERS INDUCED BY CHRONIC MICROCYSTIN-LR EXPOSURE

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The growing worldwide presence of toxin-producing cyanobacteria has become an increasing concern. Also known as blue-green algae, these photosynthetic prokaryotes can be found in lakes, ponds and rivers across the globe. Where eutrophication has occurred, cyanobacteria proliferate and form a threat to human and animal health by producing toxic secondary metabolites. These include microcystins, which are hepatotoxins produced by *Microcystis*. The most toxic microcystin variant is microcystin-LR (MC-LR). Once ingested, MC-LR is transported to the liver and binds to protein phosphatases 1 and 2A, blocking their active sites. This leads to inhibition of protein phosphatase activity and prevents the dephosphorylation of target substrates, leading to hyperphosphorylation of proteins. MC-LR also induces cytoskeletal disorganization, apoptosis, genotoxicity and is potentially carcinogenic. As a result, the World Health Organisation (WHO) has set a provisional tolerable daily intake (TDI) for MC-LR at 0.04 µg per kg body weight. However, data from epidemiology studies implicate hepatotoxicity in humans chronically exposed to MC-LR amounts similar to the TDI.
The main route of exposure to microcystins is through consumption of contaminated drinking water. In addition, they have been shown to bioaccumulate in fish, snails and crustaceans, though they can also enter the human food chain through crops that have been irrigated using contaminated water. Microcystins have been found in lettuce, cabbage, radish, rocket, dill and parsley.
The effect of MC-LR exposure has been investigated in the proteome, metabolome and transcriptome in rodents and fish. While these studies give valuable insights into the effects of MC-LR across different biological systems, it is difficult to translate the results into a human scenario because of the experimental designs applied. Indeed, most –omics studies administered MC-LR via intraperitoneal injection, used high toxin concentrations, or both.
In this study, pigs were chronically exposed to low MC-LR concentrations by oral gavage. The dosing concentrations were kept low to mimic "real-life" human exposure conditions as accurately as possible. Serum samples were subjected to proteomic and metabolomic analysis, whereas liver samples were additionally analysed at the transcriptome level using RNA sequencing. Our systems biology approach elucidates the effect of low-dose MC-LR exposure by integrating data from different biological systems, which will lead to a better understanding of the effect of chronic MC-LR exposure. Preliminary results suggest that low MC-LR concentrations have little effect on the serum and liver metabolome. Further analyses will reveal whether other –omes and organs are affected.

Keywords: *microcystin, systems biology, proteomics, metabolomics, toxins*

K7
STUDY OF MATRIX EFFECTS IN THE DETERMINATION OF ALKALOIDS IN CEREALS AND CEREAL PRODUCTS BY UHPLC-HRMS. SELECTION OF A REFERENCE MATRIX

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The occurrence of matrix effects is an important issue to take into account in the method development of quantitative analysis when using liquid chromatography coupled to tandem mass spectrometry for food analysis. Commonly, the use of standard addition approach, isotopically labelled internal standard (I.S.), matrix matched standards calibration and/or dilution of the final extracts ensure the correction of this issue.
Unfortunately, there is no commercially availability of isotopically labelled I.S. for the analysis of ergot alkaloids, then, other strategies have to be applied. On the other hand, the standard addition methodology is very time-consuming, increases significantly the number of injections, and the preparation of standard curve extracts for all samples or matrices included in the same batch is a very tedious and unrealistic procedure in a routine control laboratory.
Then, in order to minimize the matrix effects, the methodology applied in the Public Health Laboratory of Valencia for the analysis of ergot alkaloids (ergotamine, ergocristine, ergometrine, ergocryptine, ergosine, ergocornine and the –inine forms) in cereals and cereal products includes dilution of the final extraction solvent and quantification through matrix-matched calibration.
For practical reasons, due to a wide variability of cereals and cereal products analysed in one batch, an extensive matrix effect evaluation was carried out to select an appropriate matrix reference to be use as unique matrix-matched calibration curve. Oat, rye flour, breakfast cereals (with and without chocolate), pasta, bread, commercial toasts and breadsticks were investigated.
The study was conducted based on the strategy developed by Dzuman et al. (2014) and León et al. (2016). Absolute matrix effects were assessed by comparison of the matrix-matched standard calibration slopes with the solvent standard calibration slopes built with three concentration levels for each matrix (SME_{abs}%).
To evaluate the suitability of each matrix to be used for matrix-matched calibration purposes, the relative matrix effects (ME_{rel}%) were also evaluated. The ME_{rel}% was calculated for each analyte and each matrix. An acceptable value of ME_{rel}% from 75% to 125% was established.

Keywords: *mycotoxins, ergot alkaloids, matrix effect, LC-MS/MS*

K8
FATE OF AFLATOXIN M1 FROM MILK TO DIFFERENT KINDS OF ITALIAN CHEESE

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Lactating animals may be frequently exposed to feedstuff contaminated by aflatoxin B1 that is excreted into milk after hydroxylation, forming a more polar compound named aflatoxin M1 (AFM1). Based on the result of toxicological studies, AFM1 has been classified as a class 2B –possible human carcinogen– by the International Agency for Research on Cancer (IARC). For this reason, the European Commission has fixed a maximum limit of 0.050 µg/kg in milk (Commission Regulation (EC) 1881/2006) whereas in derived products, limits need to be assessed based on the effects of production processes. In fact, AFM1 interacts with the casein fraction and consequently can be transferred into cheese after cheese-making process.
In Italy, depending on the regional and local tradition, hundreds of different kinds of cheese are produced and consumed. According to a decision taken by the Italian Ministry of Health in 2013, whenever the producer is not able to provide a coefficient to correlate AFM1 concentration from milk into cheese, clearly supported by scientific data, provisional general coefficients can be adopted for the definition of maximum limits of AFM1 in cheese products. For hard cheese (like *parmigiano*) the adopted coefficient corresponds to 5.5, for soft cheese the coefficient is 3.
In this work, we present the results of different studies aimed at the evaluation of AFM1 transfer from milk to four different kinds of cheese: *mozzarella, asiago di allevo*, *caciotta*, *taleggio*. For this purpose, 300 l of milk, artificially contaminated by AFM1 at a concentration close to the maximum limit (0.0455 ± 0.0006) µg/kg, were used to produce a minimum of five shapes of each kind of such cheeses. Portions from each shape were analysed to achieve an average contamination level per each kind of cheese.
The analysis consisted in an extraction of AFM1 by a solution of sodium citrate 7%, which disrupts and dissolves most of the solid matrix and solubilizes the analyte; after dilution with water and purification by immuno affinity chromatography, the final extract is analyzed by HPLC coupled to a fluorimetric detector.
The results show that the average AFM1 content in cheese is in the range 0.011 – 0.022 µg/kg and the corresponding concentration coefficients vary between 2.4 and 4.9, depending on the considered kind of cheese.

Keywords: *aflatoxin M1, milk, cheese*

K9
CERTIFIED REFERENCE MATERIALS OF BIOTOXINS

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One of the main needs related to foodstuffs is the determination of their exact composition and the presence of contaminants, due to their influence in human health. To assure the reliability of the analyses performed is essential using validated methods and certified reference materials. These types of materials are invaluable tools for chemical applications due to their main properties: homogeneity, stability and metrological trazability. In certain fields related to food analysis these materials are scarce or do not exist, making impossible the exact quantification of samples. One example of this situation are the biotoxins related to certain foodstuffs, either mycotoxins or marine and freshwater toxins. These compounds are plenty families of analogues, with similar or different structure and various effects in human organisms. Some of them have been completely characterised and have a known structure, other are being discovered continuously. This situation makes the production of certified reference materials of the known structure compounds a very important issue, either for their quantification as for the investigation needed for the complete elucidation of the unknown compounds and their properties and effects.
In this context Laboratorio CIFGA has established a system for the production, purification, characterization and distribution of these materials according to ISO Guide 34 (now modified by ISO 17034). In our installations producer microorganism are grown and the result materials are purified following various established protocols. Purified compounds (with purity higher than 95%) are analyzed by quantitative NMR and stored in appropriate temperature conditions. After homogeneity and stability studies, compounds are characterized by an expanded uncertainty associated to a concentration value and the corresponding certificates of analysis are issued. Finally, distribution of the materials is performed assuring correct temperature conditions and times of transit, to maintain their characteristics. Following these processes various certified reference materials of mycotoxins and marine and freshwater toxins have been obtained. The quality of these materials is guaranteed by the accreditation as Reference Material Producer granted by ENAC, obtained last year for the marine toxins listed in the corresponding technical annex.

Keywords: *certified reference materials, marine toxins, freshwater toxins, mycotoxins, accreditation*

Acknowledgement: *The work leading to these results has received funding and/or support from Xunta de Galicia (Program Agrobiotech Innovación, through GAIN, Axencia Galega de Innovación, in collaboration with Galician Technology Park), ENISA (public company depending on Ministerio de Economía, Industria y Competitividad), CDTI and Technological Funds-FEDER 2014-2020 supported by Ministerio de Economía, Industria y Competitividad (ITC-20161072 MYCONTROL), AEI (Agencia Estatal de Investigación) and Ministerio de Economía, Industria y Competitividad (Program Emplea 2016) and Universidade de Santiago de Compostela.*

K10
ANALYSIS OF MYCOTOXINS IN FOOD MATRICES USING THE NEW ULTIVO TRIPLE QUAD LC/MS

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Mycotoxins are produced by fungi that can grow on various crops both before and after harvest. Due to their toxicity, mycotoxin levels are monitored in foods to minimize the risk of ingestion. Mycotoxins need to be detected and accurately quantified at low levels across various regulated food matrices, as each matrix poses different challenges for detection. In this method, mycotoxins were spiked into corn flour, peanut and black pepper extracts, which were created using Agilent QuEChERS kits and a novel modified lipid removal sorbent. A fast, robust, and precise method was developed for the detection of 12 commonly regulated mycotoxins, using the new Agilent Ultivo triple-quadrupole mass spectrometer. The targeted mycotoxins were separated using a 3.0 x 150mm, 1.8µm, C18 reversed-phase column, with mobile phases consisting of water and methanol, each modified with 0.1% formic acid, 5mM ammonium formate, and 0.5mM ammonium fluoride. Quantitation limits were 20x lower than the maximum levels defined by the European Union Commission Regulation (EC) No 1881/2006 and No 105/2010 for most the analytes in each matrix, highlighting the excellent sensitivity of the new instrument. Two MRM transitions were monitored for each analyte using dynamic MRM for optimal dwell time. Excellent method repeatability demonstrated the robustness of the Ultivo system, with relative standard deviations (RSD%) of <10% at the lowest level of quantitation. The combination of the clean-up of matrix interferences, chromatography, and the newly developed triple-quadrupole allows for the sensitive and robust detection of mycotoxins.

Keywords: *mycotoxins, Ultivo*

K11
FATE OF FUSARIUM MYCOTOXINS DURING PRIMARY AND SECONDARY PROCESSING OF CEREALS

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Fusarium spp. are widespread plant pathogens that can cause mycotoxin contaminations in cereal-based foods. Important *Fusarium* mycotoxins cover for example fumonisins, zearalenone, and the type B trichothecene deoxynivalenol, which show different acute and chronic toxicological effects on humans. In the European Union (EU), maximum levels are set by law for certain mycotoxins to protect consumers' health [1]. The legal limits depend on the mycotoxin, the cereal, and its grade of processing and they typically refer to the product "as is".
The poster illustrates potential effects of processing on major *Fusarium* toxins in selected food chains and compares those with the legal obligations in the EU. For that, data from the literature regarding the impact of milling and of common secondary processing procedures was collected. To estimate the underlying processing factors based on the product "as is", dilution or concentration effects were taken into account. In contrast, the effect of cleaning (which strongly depends on the quality of a batch) was not considered, because the EU maximum limits for *Fusarium* mycotoxins in unprocessed cereals also apply to cleaned grains. For deoxynivalenol and zearalenone, specific legal limits are defined for cereals, excluding rice, and their corresponding products. Regarding fumonisins, maximum limits are laid down for the sum of fumonisin B1 and fumonisin B2 in maize and maize-based foods.

[1] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Consolidated version of 01.04.2016 including amendments.
http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:02006R1881-20160401

Keywords: *deoxynivalenol, fumonisins, maize, maximum limits, wheat*

Acknowledgement: *The work was done in preparation for tasks of the MyToolBox project. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 678012.*

K12
COMPARISON OF MULTI MYCOTOXIN CLEAN-UP OPTIONS PRIOR TO LC-MS/MS DETECTION

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Frequently in mycotoxin analysis there is a need to analyse for combinations of mycotoxins as dictated by the susceptibility of the commodity to specific fungal infection and the regulatory limits which apply. As more analysis is being carried out routinely there is a need for faster and more cost effective analysis.
With LC-MS/MS there a number of options available to the customer –
Direct injection
Clean-up with solid phase extraction columns
Clean-up with immunoaffinity columns
R-Biopharm Rhône's immunoaffinity columns offer a versatile solution for multi-toxin analysis whereby the immunoaffinity columns can be used in tandem with one another to cover the regulated mycotoxins applicable to a particular food matrix. AOF MS-PREP® and DZT MS-PREP® immunoaffinity columns were tested in tandem to determine the applicable mycotoxins (total aflatoxin, ochratoxin A, fumonisin, deoxynivalenol, zearalenone, T-2 and HT-2) using a single extraction.
Alternatively, solid phase extraction columns like PuriTox Total Myco-MS can be used as a cheaper clean-up option prior to LC-MS/MS detection.
A range of cereal matrices were analysed using both the immunoaffinity column and SPE methods and results were compared to those obtained with direct injection.

Keywords: *deoxynivalenol, zearalenone, fumonisin, aflatoxin, immunoaffinity*

K13
COMPARISON OF COMMERCIALLY AVAILABLE TRICHOTHECENE SOLID PHASE EXTRACTION COLUMNS PRIOR TO LC-MS/MS DETECTION

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Trichothecenes are a large family of chemically related mycotoxins produced by various *Fusarium* moulds. When analysing for a number of *Fusarium* mycotoxins, LC-MS/MS will enable multi-toxin detection and as a result has mainly superseded GC-MS detection.
Solid phase extraction columns contain a mixture of solid adsobents to help reduce interfering components from the sample prior to injection thus making them and ideal and cost effective clean-up tool for simple matrices like cereals.
A wide number of solid phase extraction columns are available for the analysis of trichothecenes in cereals and in this study a number of products and formats are compared.
Matrix effects with maize and animal feed were assesed using the R-Biopharm PuriTox Trichothecene and TRICHOTHECENE P columns and were compared to an alternative commericially available column. In addition, spiked maize samples were analysed and recoveries determined using the PuriTox Trichothecene columns and again compared with a similar commercially available column for the analysis of trichothecenes. Lastly, spiked animal feed samples were analysed using the TRICOTHECENE P columns and again compared to an alternative product.
Overall, the results demonstrate that the solid phase extraction columns from R-Biopharm are suitable for the analysis of tricothecenes by LC-MS/MS with results being comparable to other products on the market

Keywords: *TRICOTHECENE P, PuriTox, columns*

K14
INVESTIGATION OF MYCOTOXINS IN DIFFERENT BEERS WITH THE MYCOTOXIN SCREENING SYSTEM USING SEVERAL CLEANUP TECHNIQUES

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Mycotoxins, especially aflatoxins, can be produced by fungal infestation during or after harvest of grain and can therefore end up in beer, brewed from malt. In addition to being acutely toxic, they are also known to be carcinogenic. To ensure food safety, manufacturers of food and beverages have to strictly manage risks from such contaminants and sensitive methods to assay mycotoxins in complex matrices are essential.
Different beer types were investigated with the Mycotoxin Screening System. The system offers an automated analysis including the ready-to-use results and reports. Furthermore, several sample cleanup columns were tried for the mycotoxin analyses in beer. The mycotoxins most commonly tested in malt products were extracted and analysed in several spiked and non-spiked beer samples. The combination of fluorescence and photodiode array (PDA) detection provided detection limits for all compounds of interest ≤ the EU maximum residue limits. [1,2,3]

[1] EU: Commission Regulation (EC) No 1881/2006 of 19 December 2006 (consolidated version 2010-07-01). Setting maximum levels for certain contaminants in foodstuffs.
[2] EU: Commission Regulation (EC) No 165/2010 of 26 December 2010 amending Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins.
[3] EU: Commission Regulation (EC) No 105/2010 of 5 February 2010 amending Regulation (EC) No 1881/2006. Setting maximum levels for certain contaminants in foodstuffs as regards ochratoxin A.

Keywords: *mycotoxins, aflatoxins, HPLC, sample cleanup, beer*

K15
MYCOTOXINS – AN INTERESTING AND CHALLENGING GROUP TO ANALYZE USING QTOF TECHNOLOGY

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Mycotoxins are a class of compounds that are produced by moulds and fungi. They are carcinogenic, neurotoxic, harmful to the immune system and to the development of an embryo. Due to this high toxicity to vertebrates, and the high impact on the economy, it is important to monitor the concentrations of mycotoxins in food and feed. Therefore, European guidelines were established where MRLs were defined (EG no. 1881/2006) to protect humans from intoxications.
At the moment more than a hundred of mycotoxins are known, with Aflatoxins (B1, B2, G1, G2), Zearalenon, DON, T2-Toxin, HT2-Toxin, Ochratoxin A and Fumonisins being the ones causing the highest concern.
A fast, easy and reliable LC-MS method was developed to identify, verify and quantify numerous mycotoxins. Identification was done on the fullscan precursor ion while verification was performed using so called diagnostic ions, such as adducts or fragments from *b*road*b*and *C*ollision *I*nduced *D*issociation (bbCID). A QTOF-MS (compact, Bruker Daltonics) was used for detection.
Detection limits and LOQs down to 0.2-10 µg/kg were reached and proven in a proficiency testing with maize samples. They comply with MRL regulations and can therefore be used in routine analysis.

Keywords: *QTOF, LC-MS, mycotoxins*

K16
A SURVEY ON OPIUM ALKALOIDS IN POPPY SEEDS AND BAKERY PRODUCTS IN THE NETHERLANDS

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The seeds of the poppy plant (*Papaver somniferum* L.) are used in food, such as bakery products, toppings for dishes, in fillings of cakes and desserts, and to produce edible oil. The seeds themselves hardly contain any opium alkaloids, but they can be contaminated with the opium alkaloids from the latex as a result of poor harvesting practices, insect damage or use of poppy seeds arising as by-product from pharmaceutical production of opium alkaloids using *P. somniferum* L. Consumption of poppy seeds contaminated with opium alkaloids can result in detectable amounts of free morphine in blood as well as measurable concentrations in urine, sufficient to interfere with drug abuse testing. It can also lead to adverse health effects, especially in babies, infants, the elderly and people with severe health issues (EFSA, 2011). Currently, there is no harmonised European legislation on opium alkaloids in poppy seeds for food purposes, although Hungary has national maximum regulatory limits for opium alkaloids in poppy seeds: morphine (30 mg/kg), codeine (20 mg/kg), thebaine (20 mg/kg) and for the sum of morphine and noscapine (40 mg/kg). In 2005, BfR, the German Federal Institute for risk assessment, derived a provisional reference value of 4 mg/kg for morphine in poppy seed for use in food, which since then is used as action limit in Germany, but not for legislative purpose.
A straightforward LC-MS/MS method for the determination of six opium alkaloids (morphine, codeine, thebaine, papaverine, noscapine and narceine) in poppy seeds was developed, with a LOQ of 0.1 mg/kg. The "dilute-and-shoot" method was based on extraction with acetonitrile/water/formic acid and analysis by LC-MS/MS using a pH 10 carbonate buffer. The method was in-house validated and used for the quantification of opium alkaloids in 41 samples of poppy seeds and bakery products collected in the Netherlands and Germany in 2015.
Morphine was detected in all samples. Opium alkaloids were detected in 35 poppy seed samples in the range 0.2 mg/kg to 240 mg/kg morphine, and from LOQ (0.1 mg/kg) to 340 mg/kg codeine, 100 mg/kg thebaine, 5.2 mg/kg noscapine, 3.4 mg/kg papaverine and to 1.7 mg/kg narceine, respectively. The concentration of morphine exceeded the German guidance value of 4 mg/kg in 66% (recalculated for only seeds) of the poppy seed samples, while 25% of the poppy seed samples were not compliant with the Hungarian legislation. The ready-to-eat poppy seed-containing bakery products did not contain opium alkaloids above the guidance values in Germany or the legal limit in Hungary.

Keywords: *opium alkaloids, survey, poppy seeds, bakery products, LC-MS/MS*

Acknowledgement: *The authors gratefully acknowledge the Food Inspectors of the Netherlands Food and Consumer Product Safety Authority, for collecting poppy seed samples. This study was carried out in WOT project-02-001-018 'Method development and surveys on plant toxins' at RIKILT Wageningen University and Research, on behalf of and funded by the Netherlands Ministry of Economic Affairs.*

K17
EXPOSURE OF POLISH CONSUMERS TO BACTERIAL, FUNGAL AND PLANT METABOLITES THROUGH MILK/MILK PRODUCTS

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A number of 47 raw milk samples were collected to study its possible contamination with natural impurities. Samples were collected the same day from milk producers, which are supplying one of the biggest companies in Poland. The pooled samples came from different farms located in Lubelskie district. The milk was further intended to produce different dairy products sold entire Poland.
A multiresidue method developed by Sulyok *et al.* (data unpublished) was adopted to milk analysis and validated. Analytes were extracted from milk using liquid-liquid extraction with acetonitrile/water/formic acid 79:20:1 (v/v/v) and no further clean-up procedure. Raw extracts were analysed using LC-MS/MS technique: QTrap 5500 MS/MS system (Sciex, Canada) coupled with 1290 series UHPLC system (Agilent Technologies, Germany). Analytes were separated using Gemini® C_{18}-column 110 Å (150 × 4.6 mm, 5 µm) (Phenomenex, USA) in an elution gradient mode using mobile phases containing 5 mM ammonium acetate (methanol/water/acetic acid 10:89:1 (*v/v/v*; eluent A) and 97:2:1 *(v/v/v*; eluent B).
The results of the screening of milk samples for the presence of 710 bacterial, fungal and plant metabolites demonstrated its contamination with *Fusarium* metabolites, mainly enniatin B and beauvericin with a prevalence of 40% and 60%, respectively, whereas apicidin, culmorin, piscarinin A and Cyclo (L-Pro-L-Val) were detected in a smaller fraction of samples. The determined concentrations processes nor pose a health risk for consumers.

Keywords: *mycotoxins, milk, LC-MS/MS*

Acknowledgement: *Funded by KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015*

K18
DETERMINATION OF TRACE LEVELS OF MICROCYSTINS IN AQUEOUS SAMPLES BY DIRECT INJECTION LC-MS/MS

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Introduction
Harmful algal blooms are a growing concern for potable water providers. A new UCMR set of regulations will require the analysis of microcystins using methods 544 and 545. This presentation demonstrates the performance of an LC/MS/MS analysis for trace level microcystins.
Methods
Water samples were injected directly into an LC/MS/MS using electrospray in the postive ionization mode and schedule MRM.
Preliminary Data
Microsystins (MCs) are toxins produced by freshwater cyanobacteria or blue-green algae. Because of the health risks associated with MCs, it has been added to the Fourth Unregulated Contaminant Monitoring Rule which will be promulgated in 2018 by the U.S. Environmental Protection Agency (US EPA). Here, we have developed a highly sensitive method to analyze trace amounts of MCs in water. The method presented includes Solid Phase Extraction coupled to liquid chromatography-tandem mass spectrometry.
Novel Aspect
This presentation demonstrated the suitability of direct injection for trace level microcystin analysis

Keywords: *mycrocysteins, algae toxins, SPE, sMRM, drinking water*

Acknowledgement: *Paul C. Winkler; Craig Butt; April Quinn-Paquet; Jianru Stahl-Zeng; Ashley Sage*

K19
MYCOTOXIN ANALYSIS IN 10 SEC? USE OF LASER DIODE THERMAL DESORPTION ION SOURCE COUPLED WITH TANDEM MASS SPECTROMETRY (LDTD-MS/MS) IN DETERMINATION OF DEOXYNIVALENOL AND ZEARALENON IN ANIMAL FEED

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The mass spectrometry coupled with novel interfaces able to direct analysis of the sample became popular alternative for separation methods. Direct analysis of samples does not include chromatographic separation, and thus reducing the time involved in sample preparation and analysis and reducing costs by eliminating the need for chromatographic columns and mobile phase solvents.
One of the approaches is Laser Diode Thermal Desorption Ion Source (LDTD) introduced by Phytronix. The aim of this study was to evaluate LDTD-MS/MS technique in determination of two important mycotoxin: deoxynivalenol (DON) and zearalenone (ZEN) in animal feed.
The feed samples (solvent standard, blank feed sample, spiked blank samples and certified reference material) were prepared with two sample preparation procedures. First was based on sample sample extraction with acetonitrile: water: formic acid solution. Second procedure was expanded with clean-up step with immunoaffinity columns.
The 5 ul of extracts were diluted with desorption solution and 5 ul of sample were spotted on LazWell plate and evaporated to dryness. The spots were analyzed on LDTD interface coupled with Sciex Qtrap 5500 mass spectrometer. The laser pattern was 10 s while mass spectrometer worked with MRM mode (two transition per one analyte). Deoxynivalenol (MRMs: 297/247, 297/203) and zearalenone (319/185, 319/187) were used in positive mode.
The results show sufficient sensitivity of the technique for both analytes (LOD for DON=50 µg/kg, ZEN=10 µg/kg) and good repeatability of the signal. The carryover effects were negligible. The linearity of the signal was on the satisfactory level (r>0.99). The repeatability of the results was characterized by relatively low CV (<25%). Based on the data collected during quantitative analysis, the LDTD–MS/MS overall results demonstrated good precision and accuracy.
The results show that LDTD-MS/MS can be interesting alternative in the mycotoxin analysis and potential for further applications in this area.

Keywords: *mycotoxin, deoxynivalenol, zearalenone, laser diode thermal desorption, feed*

Acknowledgement: *Funded by KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015. Acknowledgment for Bioanaltic Gdańsk (Poland) for technical support.*

K20
INCIDENCE OF MYCOTOXINS, PATHOGENIC FUNGI AND INJURED GRAINS IN CORN STORAGE IN FAMILY FARMERS LOCATED IN THE CENTRAL REGION OF MINAS GERAIS

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The corn is a cereal that has significant importance in human and animal feeds. Small farms in the state of Minas Gerais, Brazil, adopt corn storage in spikes, in small cribs, that allow the attack of pests and the spread of toxigenic fungi, which may result in the production of mycotoxins. Mycotoxins are secundary fungal metabolites that can cause serious damage to human and animal health, besides great economic losses, because they make the grains unfit for consumption. This study evaluated the mycotoxins incidence (total fumonisins and zearalenone), injured grains and the fungi prevalence in 44 maize samples collected during two consecutive years in 11 family farmers located in the Central Region of Minas Gerais. The pathology test of grains was carried out employing the method of freezing filter paper and the percentage of injured grains was determined visually. The fumonisins was determined in fluorimeter, after purification in immunoaffinity columns and zearalenone in high efficiency liquid chromatography associated with mass spectrometry. The samples had a low incidence of injured grains with levels between 0,03% and 0,75% and between 0,02% and 0,14% in the first and second year of the study, respectively. There was a prevalence of fungus of the Fusarium genus, which presented up to 100% of occurrence, followed by *Penicillium*, *Aspergillus* e *Stenocarpella*. All corn samples analyzed were contaminated with fumonisin, with levels ranging from 31 to 4650 µg kg^{-1}. Results showed that 43,18% of the samples were contaminated with levels above the 1000 µg kg^{-1}, limit established by the European Union for human consumption. However, none of the samples were above the maximum limit established by the Brazilian mycotoxin regulations for unprocessed maize (5000 µg kg^{-1}). Only traces of zearalenone were found in the analyzed samples. The results showed a high incidence of fungi and mycotoxins, although the majority of the samples showed levels of mycotoxins below the maximum limit according to the Brazilian legislation. Therefore, it is suggested the adoption of good agricultural practices and adequate storage, to minimize the contamination of the grains, aiming at good sanitary quality of the corn.

Keywords: *fumonisins, zearalenone, fluorescence, fusarium*

Acknowledgement: *CNPq, Embrapa, Emater, Fapemig, UENF, UFMG.*

K21
HISTAMINE IN FISHERY: MULTI-YEAR SURVEY IN NORTHERN ITALY

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Histamine is a biogenic amine that can be produced by bacterial decarboxylases in scombroid and other fish. Its formation is directly correlated with the concentration of histidine in fish tissues and the level of microorganisms in the product. Histamine is potentially toxic: an intake ranging from 70 to 1000 mg can cause in consumers the scombroid poisoning, characterized by symptoms such as rash, urticaria, nausea, vomiting and diarrhea and often misdiagnosed as Ig-E mediated fish allergy. In the EU histamine poisoning is one of the most common illnesses caused by fish and fishery products and its presence in fish is controlled by EU legislation under the Regulation EC 2073/2005. This paper assesses the histamine content in fresh fish and fishery products collected from the northern Italian area of Piedmont in the years 2016-2017, in the frame of official control for food safety.
Histamine was firstly detected using the Histamine ELISA Kit (Tecna S.r.l., Trieste, Italy). Secondly, samples resulted positive at the ELISA test were analyzed for histamine quantification by quantitative DAD-HPLC. Tests were performed at the Food Control Laboratory and Chemical Laboratory of the Istituto Zooprofilattico Sperimentale Piemonte, Liguria and Valle d'Aosta (Turin, Italy): all methods are validated and accredited by the Italian Accreditation Body 'ACCREDIA', according to ISO 17025.
Totally, 653 samples of fish were analyzed by the ELISA screening method. The collection plans that produced the most part of samples are respectively represented by Border Control (n=324) and Official Control in the frame of EU Regulation 2073/2005 (n=225). Both kinds of sampling were performed according to the rule of 9 units comprising the samples in accordance with European food safety criteria for histamine. The above mentioned 324+ 225 samples derived from 61 different fish batches collected by food safety Competent Authorities. Other 24 batches were collected during foodborne outbreak investigations: in these cases, it was not possible to apply the rule of 9 units comprising the samples and, in total, 104 samples were analyzed.
Among the 653 (85 fish samples) performed analyses, 62 (11 fish samples) resulted positive and were further analysed by quantitive DAD-HPLC. Histamine presence was confirmed in 61 units (10 fish samples) in concentrations varying from 119±14 mg/kg to 4337±607 mg/kg. A fish sample collected in the frame of a FBO investigation resulted negative (< 20 mg/kg).
According to EU food safety legislation, for fish species associated with a high amount of histidine the maximum average histamine content must not exceed 100 mg/kg (ppm); two samples of the nine units comprising the samples may have a level higher than 100 mg/kg but lower than 200 mg/kg and, finally, no sample may have a level exceeding 200 mg/kg.

Keywords: *histamine, fish, official control*

K22
ANALYSIS OF TROPANE ALKALOIDS IN TEA

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Tropane alkaloids (TA) are secondary plant metabolites synthesized by a wide variety of plants, e.g. black henbane (*Hyoscyamus niger*), jimson weed (*Datura stramonium*), field bindweed (*Convulvus arvensis*), or deadly nightshade (*Atropa belladonna*). Parts or seeds of these plants are toxic, but can be co-harvested accidentally and may end as undesirable substances in food or feed. Products most affected are cereals, oilseeds, legumes as well as green, black, and herbal teas.
Poisoning related to TA, for example after consumption of contaminated foods, can cause delirium and hallucinations and ultimately can even lead to death from respiratory arrest. By now, both research and analytics were focused on atropine and scopolamine. Although only atropine and scopolamine have legal requirements in cereal-based foods for infants and young children (Commission Regulation (EU) 2016/239), also black, green, and herbal tea are frequently affected by contamination from TA. Furthermore, a recent study reported higher contents of TA in black and herbal tea than in cereals or other foodstuffs. In addition, besides atropine and scopolamine also other TA were detected in many samples [1]. Due to these findings it might be no longer sufficient to focus only on atropine and scopolamine.
Therefore, a method for the analysis of different TA in black, green, and herbal tea was developed. After a quick acid extraction the extracts could be measured directly by LC-MS/MS. Besides atropine and scopolamine the method includes the compounds anisodamine, norscopolamine and convolvine which were found to be present in many tea samples.

[1] Mulder PPJ, De Nijs M, Castellari M, Hortos M, MacDonald S, Crews C, Hajslova J and Stranska M, 2016. Occurrence of tropane alkaloids in food. EFSA supporting publication 2016:EN-1140

Keywords: *plant toxins, food contaminants, tropane alkaloids*

K23
CO-OCCURRENCE OF REGULATED MYCOTOXINS IN SERBIAN CORN FLOURS MARKETED IN 2016

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Mycotoxins are a vast group of fungal secondary metabolites characterized by very versatile molecular structures and effects on living organisms. They are considered as chronic dietary risk factors for humans, with potential to exert negative health impact after ingestion through contaminated food. Corn is susceptible to contamination by numerous fungal genera that have ability to biosynthesize various mycotoxins, depending on climate conditions. Co-occurrence of mycotoxins on corn is confirmed in many studies throughout the world.
The aim of the present study is to evaluate co-occurrence of regulated mycotoxins in corn flours for human consumption, produced and marketed in Serbia in 2016. A total of 32 samples obtained from the retail stores was analysed for the presence of aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON) and fumonisins B1 and B2. Analyses were carried out by HPLC with UV (DON) or fluorescence detection (OTA, ZEA) using *o*-phthalaldehyde precolumn derivatization (FB1, FB2) or UV postcolumn derivatization (AFs), after clean-up on immunoaffinity columns with monoclonal antibodies.
Regarding occurrence of individual mycotoxins, percentage of positive samples was 38% for AFs, 28% for OTA, 75% for ZEA, 38% for DON and 91% for fumonisins. Presence of mycotoxins from all five analysed groups was recorded in 16% of the samples. Fumonisins were practically ubiquitous and hence omitted in further presentation of the results, but have to be remembered. Following percentages of the samples with combinations of mycotoxins were recorded: ZEA, DON and AFs 22%; ZEA, DON and OTA 19%; ZEA, AFs and OTA 25%; DON, AFs and OTA 19%; ZEA and DON 25%; ZEA and AFs 38%; ZEA and OTA 28%; DON and AFs 22%; DON and OTA 19%; AFs and OTA 25%.
Apart from the recorded presence, concentration levels of mycotoxins are of immense importance. It should be noted that concentrations above the half of the respective maximum allowed levels (MLs) were noted in 16% of the samples regarding AFB1 (9% for total AFs) of which 9% exceeded the ML (0% for AFs); in case of OTA respective percentages were 9% and 3%, ZEA 3% and 3%, while DON and fumonisins have not reached half of their MLs in any of the samples. It is interesting to note that one of the samples with all mycotoxin groups represented exceeded the MLs for both AFB1 and ZEA, and another one for AFB1.
In risk assessment process, adverse health effects of the mixtures of mycotoxins are one of the major challenges. The fact that mixtures are not only result of mycotoxins co-occuring in one food item, but could also be formed in organism by consumption of different food items, further complicates the assessment. Results presented in this work contribute to the data base of mycotoxins co-occurence in corn flours in Serbia, of special interest for population consuming ethnic meals and people on gluten-free diet.

Keywords: *mycotoxins, corn, flour*

K24
DEVELOPMENT AND CHARACTERISATION OF A NEW MYCOTOXIN SCREENING ASSAY FOR GRAIN VIA GRAIN DUST

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Mycotoxins are secondary metabolic products of fungi and possess toxic or carcinogenic effects on vertebrates. Often grain is naturally contaminated with several mycotoxins. While fusarium toxins like zearalenone (ZON) and deoxynivalenol (DON) are common in Northern Europe, aflatoxin B1 (AflaB1) naturally occurs in warmer climatic regions. Nevertheless, through global trade AflaB1 generally became problematic in particular for feed and corn mills and the grain processing industry. In addition to those toxins, ochratoxin A (OTA) is either formed on the field in moderate climate or during grain storage worldwide.
Current mycotoxin detection methods show some disadvantages: either lack of sensitivity or long analysis times and need of laboratory equipment. Furthermore, both rapid and laboratory methods still need comprehensive manual sample preparation. The combination of polymer-based microspectrometers with microfluidic disposable elements for a fast on-site analysis in food production is the core of a project named "KOMBISPEC", in which these problems are approached.
The main focus lies within the development of a fast, affordable and reliable device that can be integrated in grain process operations. Four of the most economically relevant mycotoxins (ZON, OTA, AflaB1 and DON) will be simultaneously detected by means of a fluorescence immunoassay (FIA). Constituting the method's core piece, the FIA is integrated in a single-use laminar flow chip. The advantage of this fluorescent system lies within its fast detection and potential for multiplex-applications.
Antibodies for mycotoxin detection were characterized and established. First of all, the FIA compounds' concentrations were optimised for an indirect and competitive assay design. The assays' limit of detection and quantification was determined, fulfilling the regulatory limits. Moreover, incubation times were shortened and the effect on signal intensity was analysed. Hence incubation times could significantly be reduced.
In addition to that, different polymers were evaluated as possible chip material. Polystyrene that is used as standard material for microtiter plates, cyclo-olefin polymer, and cyclo-olefin copolymer were tested, respectively. Besides the assay development, the sampling and aqueous extraction protocols were adapted. First analysis of grain dust samples showed promising results for the FIAs' application as part of the KOMBISPEC system.

Keywords: *fluorescence immunoassay, grain, mycotoxins, rapid method, on-site testing*

Acknowledgement: *This work was supported by the Federal Ministry of Education and Research of Germany (BmBF) within "KOMBISPEC" (funding code 13N13943).*

K25
APPLICATION OF WATER EXTRACTION AND HILIC CHROMATOGRAPHY FOR DETERMINATION OF DEOXYNIVALENOL AND DEOXYNIVALENOL-3GLUCOSIDE IN FEEDSTUFFS

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Deoxynivalenol (DON) and its modified forms produced by *Fusarium spp* is widespread worldwide mycotoxins commonly occur in feedstuffs and is ranked as one of the most serious threat for animals. Cereal crops exposed to DON infection are capable of detoxifying this mycotoxin through plant metabolism to deoxynivalenol-3-glucoside (DON-3G). This modified mycotoxin could be hydrolyzed and back to its toxic precursor (DON) during mammalian digestion. Hence the occurrence of the DON-3G can lead to underestimation of the total DON amount in animal feed. For this reason both DON as well as its conjugate form should be still monitored in animal feed using sensitive analytics methods. One of the recent trends in analytical chemistry is development more environmental friendly methods ("green" analytical chemistry) where toxic reagents (eg. acetonitrile or methanol) are limited or replaced by nontoxic (eg. water, ethanol). Because DON and DON-3G have polar structure it is possible to extract from sample using water. A more lately approach to achieve sufficient chromatographic separations of polar compound is use hydrophilic interaction chromatography (HILIC) instead of commonly applied revers phase liquid chromatography (RPLC).
This study describes development a new method based on non-organic extraction and HILIC chromatography for the separation of DON and DON-3G. During method development extraction solvent were optimised as well as clean-up with immunoaffinity columns (IAC; DON TEST ™). Water as a extraction solvent gave the best recovery for both analytes. In case of clean-up two approach for elution has been checked: elution with methanol and with hot water (90° C) with previous incubation in 90°C. Second way gave worse peak shape for DON-3G compare to elution with methanol. Chromatographic separation was carried out with a Luna HILIC (Phenomenex) and detected with MS/MS technique (Shimadzu, Japan). The run time was 8.5 min, the column oven temperature was 25°C and the flow rate was constant 0.2 mL/min. The mobile phase was composed of water and acetonitrile and the effect of water content in mobile phase on the retention of DON and DON-3G was observed within the range from 8% to 20% (v/v). Limit of detection (LOD) and quantification (LOQ) were similar for both analytes and were for DON 3.3 and 11.0 µg/kg and for DON-3G 2.11 and 7.03 µg/kg, respectively. The method was applied for the determination of DON and DON-3G in 20 feedstuffs. All of samples were contaminated with DON, DON-3G constitute 25% of the parent compound. The results indicate that the method HILIC LC-MS/MS can be considered as an alternative to RPLC-MS/MS method and could be adopted for further evaluation of animal exposure on DON and his conjugate.

Keywords: *DON, HILIC, LC-MS/MS, feedstuffs*

Acknowledgement: *Funded by KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

K26
SENSITIVE MULTI-MYCOTOXINS ANALYSIS WITH A SINGLE SAMPLE PREPARATION BY LC-MS/MS

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There are various substances that can threaten the food safety, such as pesticides, mycotoxins. LC-MS/MS analysis is a prevailing technique for the detection of these substances in food. Mycotoxins are especially frequent contaminants of agricultural products, and brewers are concerned that they can give serious damages to consumers, for example liver cancer, nephritis, pulmonary edema and so on. This is the reason why most countries have adopted regulations to limit exposure to mycotoxins, while the regulated mycotoxins and value differ with countries. The toxicity and potential health hazards induced by mycotoxins demand the need for sensitive, robust analytical methodologies. This research provides a LC-MS/MS system for quantitative screening of 25 mycotoxins and includes a multi-mycotoxin sample preparation column to cover worldwide regulations.
Ground wheat flour (or rice flour) samples were mixed with water/acetonitrile, and shaked. Aflatoxins, zearalenone, ochratoxin A, deoxynivalenol, fusarenon-X, T-2, HT-2, neosolaniol, fumonisins, 3-Ac-DON, 15-Ac-DON were spiked to the sample when needed. After filtration on glass fiber paper, extracts were diluted with aqueous acetic acid solution. This solution was then loaded into the spin purification column (Shim-pure MYCO, Shimadzu Corp.) and vortex-mixed before centrifugation. The extract was evaporated to dryness with nitrogen gas. Reconstituted samples were then analyzed using a triple quad mass spectrometer (LCMS-8060, Shimadzu Corp.).
Mastro PFP2 (Shimadzu GLC Corp), ametal-free column, can separate 3-Ac-DON and 15-Ac-DON. These mycotoxins are isomers and have same product ions. Multi-rinse mode which is needle rinse program improve peak shape of fumonisins. Under these conditions, this study shows that a single extraction with spin column clean-up can be used for the analysis of several types of mycotoxin in one LC-MS/MS measurement. This approach is cost effective and customer can use wide range of mycotoxins.

Keywords: *mycotoxins, LC-MS/MS, sample preparation, multi-residue*

K27
DETERMINATION OF MORPHINE ALKALOIDS IN POPPY SEEDS

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Czech Republic belongs to the most important producers of poppy seed used for food purposes worldwide. Czech poppy is of first quality and has good reputation around the world. Recently, it has been begun to mix with imported cheaper pharmaceutical poppy, which contains higher number of alkaloids significantly decreasing the poppy quality. Czech legislative limit for sum of morphine alkaloids (morphine, codeine, thebaine) on the surface of food poppy seeds is 25 mg/kg. Adulteration of the food poppy with the pharmaceutical varieties is one of the reasons why fast and sensitive method for determination of morphine alkaloids is needed.
Three different extraction methods were tested: QuEChERS, QuPPe and method published by Sproll. Alkaloid recoveries from spiked blank matrices were in the range from 70 % to 120 % for all methods. QuEChERS showed lower recoveries than others. Method by Sproll was finally chosen for extraction of morphine alkaloids because of the best recovery and repeatability.
Extracts were analysed by UHPLC-MS/MS Agilent 6420. Two columns were tested: Obelisc R (100 mm x 2.1 mm x 5 µm) and ZORBAX Eclipse Plus C18 (50 mm x 2.1 mm x 1.8 µm). Both columns were suitable for analysis of morphine alkaloids, however, UHPLC column ZORBAX Eclipse Plus C18 provided higher efficiency and shorter run times compared to Obelisc R. Moreover, it allowed separation of morphine and codeine which coeluate on Obelisc R. The validation was finally carried out using ZORBAX Eclipse Plus C18 column.
Validation was performed at two different levels in five replicates. There were no negative poppy seeds suitable for spiking of low levels (LOQ) so another oil seeds, particularly rapeseeds, were used for validation experiments. Recovery of spiked samples was in the range from 83% to 113% and repeatabilities better than 21%.
Screening of morphine alkaloids was carried out on 10 samples of poppy seeds. It was found out that repeatabilities of real samples were often highly variable. It indicates that samples of unmilled poppy seeds were not sufficiently homogeneous and even after 2 hours of agitating no improvement in homogeneity was observed. Thus, weighing of larger sample portions and analysis in duplicates would be recommended for routine analysis.

Keywords: *morphine alkaloids, poppy seeds extraction, UHPLC-MS/MS*

K28
OCHRATOXIN A IN ORGANICALLY AND CONVENTIONALLY PRODUCED WINES IN CROATIA

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Ochratoxin A is a product of fungal secondary metabolism, from the genera *Aspergillus* and *Penicillium*. If the increased concentration of Ochratoxin A enter the organism it can show some unwanted effects. The main effect is nephrotoxicity, but it can also lead to neurotoxic, immunotoxic, teratogenic or cancerogenic effects. The aim of this work was to assess the impact of organic wine production on the levels of Ochratoxin A in samples collected from the most important Croatian winegrowing regions. Eleven locations with organic wine production were selected in different winegrowing regions of Croatia. The wine varieties under study were Syrah, Zweiglet, Cabernet Savignon, Merlot, Plavac mali, Malvazija, Traminac, Grenache rose, Trebiano Toscano. High-Performance Liquid Chromatography (HPLC Analysis) with flourescence detection was used for determination of Ochratoxin A in both organically and conventionally produced wines. Phenomenex Luna Column (2 × 150 mm, 3 µm) was used for chromatographic separation. The mobile phase A consisted of 0.2 % formic acid and the mobile phase B of acetonitrile. Separation was performed using gradient mode 65-9 % B (0-6 min); 9 % B (6-8 min); 5 % B (8-18 min), respectively. Mobile phase flow rate was 0.2 mL min^{-1} and column temperature was 4°C. Excitation and emission wavelengths for OTA detection were 247 nm and 480 nm, respectively. Wine samples were purified by solid-phase extraction (SPE), using immunoaffinity columns. The analysis results show that all the wine types produced in Northern Dalmatia, Istria and continental Croatia meet the requirements of the European Union by having the maximum OTA levels ten times lower in comparison to maximum residue limit (MRL). The majority of ochratoxin A positive wines were from conventional wine producers; however, the total number of positive samples was too low to draw any specific conclusions. There were no significant differences concerning ochratoxin A content between organically and conventionally produced wines.

Keywords: *ochratoxin A, wine, high-performance liquid chromatography (HPLC), flourescence detection (FLD)*

K29
OPTIMIZATION OF AFLATOXIN M1 RECOVERY LEVELS IN YOGURTS USING ELISA BY PH ADJUSTMENT AND MATRIX NORMALIZATION BUFFERS

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Introduction
There have been many problems with aflatoxin M1 recovery levels in yogurt samples (yogurts or yogurt drinks) using most commercial ELISAs, due to matrix effect and unstable pH. The only alternative is the organic solvent extraction, evaporation and reconstitution that lead to high dilution factor, not acceptable sensitivity and unsatisfactory recovery levels.
Aim
The aim of this work was the improvement of sensitivity and recovery levels of AFM1 in yogurt samples avoiding the common, tiresome and time-consuming steps.
Methods
Aflatoxin M1 in yogurt samples was determined by a direct competitive ELISA (Bio-Shield M1 ES, B2096, Lot B2096040) from Prognosis Biotech Ltd. This ELISA kit is the first specific product to determine AFM1 in yogurt samples. Taking into consideration the physical and chemical properties of yogurt samples, the method uses a simple neutralization (automated pH adjustment) and dilution normalization. In order to avoid any matrix effect, the yogurts and yogurt drinks are diluted 3 and 2 times respectively. Thirty aflatoxin-free yogurt samples were chosen from the global market and after spiking with different concentrations of AFM1, the recovery levels were determined.
Results
The ELISA requires only a sample predilution with a buffer. Combined with the neutralizing effect of this buffer, each sample has an acceptable recovery rate. The sensitivity of AFM1 also remains high giving the maximum range of quantification.
Conclusion
This ELISA is a valuable tool in the Aflatoxin M1 analysis of yogurt samples, since it optimizes the recovery levels, leading in an effortless and rapid method.

Keywords: *ELISA, aflatoxin M1, milk*

K30
AN INNOVATIVE SYMMETRIC LATERAL FLOW SYSTEM FOR THE QUANTIFICATION OF AFLATOXIN M1

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Introduction
Aflatoxin M1 in milk is considered to pose certain hygienic risks for human health and as a result there is an established EU limit 0.05μg/kg. Aflatoxin M1 can be detected by lateral flow rapid tests and quantified by ELISA, with a total procedure time at least 15 and 90 minutes respectively. The EU limit constitutes an obstacle for high sensitivity because the immunochemistry of immunochromatography technology is limited. There have been also lateral flow devices that tend to make one more step forward in the semi-quantitative analysis. However, ELISA remains the most reliable method for Aflatoxin M1 screening quantification.
Aim
The aim of this work was the comparison between a new innovative immunochromatography assay and ELISA for the quantification capability of Aflatoxin M1 in milk samples.
Methods
An indicative lateral flow strip consisted of high affinity monoclonal antibodies, conjugated to colloidal gold, a chemically modified hapten test line and an anti-species control line. The anti-Aflatoxin M1 was produced after mice immunization, preparation of hybridomas, clones screening and protein G purification. This is a competitive immunoassay and needs only 10 minutes without any sample preparation. The concentration of Aflatoxin M1 was determined by a novel quantification system using a high technology scanning device. All the samples used in this immunochromatography assay, also used in ELISA (Bio-Shield M1 ES, B2096, Lot B2096040, Prognosis Biotech Ltd).
Results
The combination of the high affinity antibodies and the advanced quantification system contributed to a high range standard curve. According to scanning device findings, the type of graph and consequently the recovery of all the results were almost identical to ELISA method levels.
Conclusion
This innovative lateral flow device constitutes the most accurate tool in the rapid quantification of Aflatoxin M1 in milk samples, providing results comparable to the results of a quantitative ELISA.

Keywords: *ELISA, aflatoxin M1, rapid test, dairy, symmetric*

K31
INNOVATIVE HIGH SENSITIVE ELISA FOR THE QUANTIFICATION OF AFLATOXIN B1 IN BABY FOOD

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Introduction
Most controlling government agencies worldwide have regulations regarding the concentration of aflatoxins permitted in human foodstuffs. Aflatoxin B1 in baby food products is considered to pose certain hygienic risks for babies' health and as a result the EU limit is 0.1μg/kg (0.1ppb). On the other hand, there have been many problems with Aflatoxin B1 recovery, LOD and LOQ levels using most commercial competitive ELISAs for baby food. The accuracy and rapid determination of Aflatoxin B1 presence in baby food is of paramount importance and it is essential that a baby food immunoassay should use high-affinity specific antibodies.
Purpose
The purpose of this work was a new way to improve the LOD, LOQ and recovery levels of Aaflatoxin B1 in baby food matrices avoiding the pre-column treatment.
Methods: Fifty grams of the above samples were extracted with 70% methanol and after filtration the Aflatoxin B1 was determined by a direct competitive ELISA (Bio-Shield B1 Baby Food, B3196, Lot B3196006, Prognosis Biotech Ltd) without pre-column treatment. The microplate of this ELISA was coated with a high-affinity monoclonal antibody produced after mice immunisation, preparation of hybridomas, clones screening and protein G purification. The immunoassay had two incubation steps (in contrast with other tests), the first incubation of 20% extract and 80% HRP-free buffer mixture followed by washing steps, the second incubation of aflatoxin-HRP for followed by washing steps and the addition of chromogen for 5 minutes followed by the addition acidic solution.
Results
The immunoassay requires only 20% of extract and the sensitivity remains at increased levels giving the maximum range of quantification. Due to increased dilution normalization of matrix and the use of two incubation steps, the recovery levels were excellent. The levels of sensitivity and LOQ were according to the EU limit.
Significance
This ELISA constitutes the one and only immunoassay that can quantify Aflatoxin B1 in baby food. It constitutes a valuable tool since it optimizes recovery levels, leading in an effortless and rapid immunoassay.

Keywords: *ELISA, aflatoxin B1, baby food*

K32
ANALYSIS OF MYCOTOXINS AND PESTICIDE RESIDUES IN COMPLEX MATRICES BY LIQUID CHROMATOGRAPHY AND HIGH RESOLUTION TANDEM MASS SPECTROMETRY

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Natural toxins and residues of agricultural chemicals belong among the most frequent contaminants of food crops and foods in general. Their determination often represents a challenging task, especially when occurring either at low concentrations or in rather complex matrices varying in chemical composition such are herbal-based dietary supplements, spices, etc. Utilization of generic isolation approaches and chromatographic techniques, predominantly liquid chromatography, with mass spectrometric detection enabling simultaneous determination up to hundreds of analytes preferably in a single run represents the modern trend in determination of such substances. Specifically, ultra-high liquid chromatography systems hyphenated to tandem mass spectrometers equipped with several mass analyzers combining the advantages of each analyzer finds application in this area.
Within this study, an in-house analytical method based on QuEChERS-like extraction and ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (U-HPLC–HRMS/MS; Dzuman et al., 2015, Anal. Chim. Acta 863, 29–40) was modified and optimized to improve the chromatographic separation efficiency a detectability of 322 pesticide residues, 56 mycotoxins and 11 pyrrolizidine alkaloids. The method update comprised the following steps: (i) extension of the method scope by 68 new pesticides (total 457 analytes), (ii) selection of a new core-shell analytical column, optimization of (iii) LC gradient program and (iv) collision energy (NCE) for all analytes since the original method was based on generic stepped NCE settings. In the last phase, the method applicability was tested on a wide set of 420 samples of tea and spices originating from Thailand. Generally, high co-occurrence and content of pesticide residues and mycotoxins was observed in the analyzed material.

Keywords: *mycotoxins, pesticide residues, complex matrices, ultra-high performance liquid chromatography, high resolution tandem mass spectrometry*

Acknowledgement: *This work was supported by the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and by the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

K33
SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED WITH MASS SPECTROMETRY: NOVEL APPROACH FOR AFLATOXINS ANALYSIS IN HIGH FATTY MATRICES

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Due to their high toxicity, thus impact on human and animal health, aflatoxins (natural toxic secondary metabolites produced by microscopic filamentous fungi - *Aspergillus* species) represent one of the most important mycotoxins groups, which may contaminate agricultural commodities either on the field, during the storage and/or food processing. They are commonly occurring in improperly stored commodities such as e.g. peanuts, sesame seeds, sunflower seeds, tree nuts, wheat, and a variety of spices, coming especially from countries with warm climate of Africa, South Asia, Southeast Asia, America etc. Aflatoxins are also carcinogenic compounds (classified to group 1 according to the IARC) and therefore their content in food and feed must be controlled. For these reasons, aflatoxins are regulated by relevant authorities worldwide.
Enormous demand for aflatoxins determination in food crops and products thereof has occurred in the recent decades. For the control aflatoxins in these matrices, a wide range of analytical methods has been developed until now. Currently, a reverse phase liquid chromatography coupled with mass spectrometry (RPLC-MS) is generally preferred technique. In case of high fatty matrices such as e.g. nuts, almonds and seeds, the presence of triacylglycerols (TAGs) in the final extract represents a problem due to their strong retention in the RPLC system. Within this study, a novel strategy based on supercritical fluid chromatography coupled with high resolution mass spectrometry (SFC-HRMS), is demonstrated to overcome this issue. Since carbon dioxide, representing non-polar mobile phase (normal chromatography), is used in the SFC-HRMS system TAGs are easily eluted.
Analytical procedure for the determination of aflatoxins B1, B2, G1 and G2 has been developed. Peanuts (and walnuts) have been selected as representatives of high fatty matrices (more than 60% of fat), with relatively often incidence of these aflatoxins. For the purpose of validation, aflatoxins were quantified in the obtained extracts by QuEChERS based approach. The recoveries obtained for both tested spiking levels were in the range 75–81% (RSDs 1-5%) at 20 µg/kg and 87–88% (RSDs 3%) at 4 µg/kg for peanuts. Similar results were achieved for spiked walnuts, in the range 79–82% (RSDs 2-3%) at 20 µg/kg and 86–111% (RSDs 1-3%) at 4 µg/kg.
Satisfactory accuracy for all measurements was achieved by employing matrix-matched calibration. The experimentally established limit of quantification (LOQ) was 2.5 µg/kg for all analytes, which is in compliance with EU legislation Reg. (EU) 1881/2006 and Reg. (EU) 165/2010.

Keywords: *aflatoxins, supercritical fluid chromatography, mass spectrometry, fatty matrices*

Acknowledgement: *This work was supported by the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and by the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

K34
SCREENING OF BACTERIAL, FUNGAL AND PLANT METABOLITES IN SHEEP'S MILK FROM THE FIRST SPRING MILKING

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Seasonality of reproductive cycle in sheep is associated with the season and the day length, as a recurring reproductive cycle is an endogenous rhythm [1]. Sheep are short-day breeders, with milking period dependent of secretion of melatonin and prolactin [1]. Due to the recently increased interest in sheep's milk products which are available seasonally since the spring, we decided to study the presence of natural contaminants in sheep's milk. We began the milk analysis in April, when the sheep were already grazed on a pasture, to study the possible contamination of milk products sold to tourists during the season.
A multiresidue method for natural contaminants was adopted to milk analysis. Twenty individual mothers from two herds, located in Bieszczady Mountains, were milked and samples were analysed using two methods. At first, liquid-liquid extraction with acetonitrile/water/formic acid 79:20:1 (v/v/v) and no further clean-up procedure was applied. Further, the same milk samples were directly loaded onto the AFL-M1 immuno clean-up columns, eluted with methanol and analysed to screen the samples for the presence of aflatoxin M1 at the level of 50 ng/kg. The analytes' recoveries varied between 70-120 % for 80% of the analytes, with the coefficient of variation below 20%.
The results revealed that 10 out of 20 milk samples were contaminated with only one mycotoxin – enniatin B, at a very low contamination levels up to 0.0042 µg/kg (LOQ 0.0015 µg/kg). Thus, it may be concluded that there is no risk of high levels of mycotoxin exposure to consumers *via* the sheep's milk products.

[1] Molik E., Zięba-Przybylska D. In *Current Topics in Lactation*, 2017

Keywords: *mycotoxins, milk, enniatin B, sheep, LC-MS/MS*

Acknowledgement: *Funded by KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

K35
NEW METHOD OF ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH ORBITRAP MASS SPECTROMETRY FOR ANALYSIS OF VARIOUS ALTERNARIA TOXINS AND THEIR CONJUGATED FORMS IN TOMATOES AND TOMATO-BASED PRODUCTS

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Alternaria ssp., also known as black molds, belongs to a genus of worldwide occurring fungi known to produce a variety of potentially dangerous mycotoxins which can contaminate the food chain. Several tens up to hundreds of known secondary metabolites are produced by *Alternaria* species, however, the molecular structures of only a few of them have been elucidated so far. In addition to the free *Alternaria* toxins, also their conjugated forms originating during the plant detoxification processes have been emerged. Within this study, a new analytical method based on ultra-high performance liquid chromatography (U–HPLC) coupled with high-resolution tandem mass spectrometry (HRMS/MS) employing Q-Exactive™ Plus (hybrid quadrupole-orbitrap) was developed for targeted screening of more than 70 different *Alternaria* metabolites and their conjugates in tomatoes and tomato-based products. For the purpose of analytical method optimization, extracts of tomatoes with visible black *Alternaria* molds were used (commercial availability of pure analytical standards is limited). As concerns the sample preparation, QuEChERS–like extraction together with simple aqueous extraction were compared. In this method, a reverse-phased analytical column Acquity UPLC® HSS T3 (100 mm × 2,1 mm, 1,8 µm) was used and two different aqueous buffers with or without addition of formic acid were tested. As a 'strong' mobile phase, methanol was utilized. Within tuning the detection conditions, different ionization modes, ion source settings (auxiliary / sheath gas flow, spray voltage, heater temperature, capillary temperature, S-lens value) and detection parameters (maximum inject time, automatic gain control) were tested with respect to final intensity of particular analytes. Identification of *Alternaria* metabolites was realized through the measurement of the exact masses and elemental composition calculation. As the confirmatory criteria, the agreement of isotopic patterns and detection of specific fragment ions in high resolution, were taken. The poster presents the results of screening of the above discussed analytes in tomatoes and tomato-based products from the Czech market.

Keywords: *Alternaria mycotoxins, masked mycotoxins, tomato based products, U–HPLC-HRMS/MS*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

K36
CHALLENGES IN ANALYSIS OF PYRROLIZIDINE ALKALOID ISOMERS AND RELATED N-OXIDES IN PLANT MATERIAL

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Pyrrolizidine alkaloids (PA) and related N-oxides are secondary plant metabolites and can, for instance be found in plants of the *Asteraceae*, *Boraginaceae* or *Fabaceae* family. PA are liver-toxic and some of them are suspected of being carcinogenic or mutagenic. Nonetheless, PA containing plants can be co-harvested easily and may end as undesirable substances in food or feed, especially tea, food supplements and cereals.
Despite the health risk, no threshold value was established, but the ALARA (As Low As Reasonably Achievable) principle is applied. For evaluation of a contamination, the EFSA Panel on Contaminations in the Food Chain (CONTAM) recommended the analysis of 17 PA by LC-MS/MS [1], even though over 600 different PA are known. In addition, the toxicological relevance of the different PA has not been clarified to date.
In the context of this wide variety a big challenge in LC-MS/MS analysis of PA is the differentiation between target compounds, isomers and interfering signals which are typically present in PA containing samples like herbal teas or other plant materials. For instance intermedine and lycopsamine – which are among the 17 – can easily be misinterpreted with the isomeric compounds indicine, rinderine or echinatine. A summation of these isomeric compounds to the so-called lycopsamine type PA is common practice in routine analysis but could be inaccurate if retention times and response factors are unknown due to the lack of reference standards. In order to avoid false-positive or false-negative results a suitable chromatography and consideration of isomers is essential. Nonetheless, the sample evaluation is demanding and should only be applied by experts with experience in PA analysis.
The poster illustrates challenges in LC-MS/MS analysis of PA, especially those related to the presence of isomeric compounds. Furthermore, approaches were discussed in order to reduce the risk of a false identification of PA and related N-oxides.

[1] EFSA Panel on Contaminations in the Food Chain (CONTAM), Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA Journal 2017; 15(7): 4908

Keywords: *pyrrolizidine alkaloid isomers, 17 monitored PA, challenges in LC-MS/MS analysis*

K37
SENSITIVE AFLATOXIN M1 DETECTION IN MILK AND ALL KINDS OF MILK PRODUCTS: A NEW, SIMPLE METHOD FULFILLING GLOBAL MARKET NEEDS

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Global feed supply-chains and higher incidence of aflatoxins have led to increasing need for aflatoxin M1 (M1) tests in milk and milk products. Regulations for M1 exist in >60 countries. Most legal limits for M1 in milk are at 50 ng/kg (EU) or tenfold higher at 500 ng/kg (USA, LATAM, ASEAN). For the determination in milk and milk powder a European Standard (EN ISO 14501:2007) exists. The method bases on defatting of milk or reconstituted milk powder, clean-up by immunoaffinity chromatography (IAC), and determination by HPLC fluorescence. However, the method can hardly be used for more complex milk products like cheese, fruit yoghurt, or ice cream. Furthermore, the limit of quantification (LOQ) of 80 ng/kg of M1 in milk powder is too high for market needs in some EU countries. Hence, labs use additional methods based on organic solvent extraction, followed by evaporation and dilution with buffers prior to IAC clean-up. Low LOQ is achieved by evaporation of IAC eluate and dissolution in smaller volumes prior to HPLC. Both evaporation steps are laborious, time consuming, and error-prone.
In a best-practice program within the Eurofins group methods for M1 determination in 13 labs around the world were to be optimized and harmonized. As centrepiece of the analysis the Tecna *IClean* C IAC column was chosen. The column fulfilled all requirements of ISO 14501 in terms of recovery rate, provided sufficient capacity even for high-contaminated samples, and was offered at best price. The goal was to develop one single method for all kinds of milk products. A new sample preparation was developed tailored to sample variability and the selected IAC. The method bases on a solvent-free extraction approach developed for M1 ELISA (Anfossi et al., 2008, J. Agric. Food Chem., *56*, 1852ff.). Sodium citrate was used to better extract and stabilize milk proteins in the sample solution. In this way, water based extraction of M1 from cheese, butter, or fruit yoghurt became possible. An evaporation step prior to IAC was not necessary. After proper defatting, high concentrated solutions of milk products smoothly passed the IAC column. Enrichment was sufficient to measure the eluate directly without evaporation and solvent change. In this way an easy-to-handle sample preparation for all kinds of milk products was achieved. As result of changing the IAC column and sample preparation HPLC methods had to be adapted. Fast separation from interferences was finally achieved on a phenyl-hexyl phase.
The method is validated for milk, infant formula, cheese, yoghurt, butter, milk cereal, and chocolate dessert. It is linear over a wide range from LOQ of 2 ng/kg for milk and 10 ng/kg for milk products up to 600 ng/kg of M1 in milk or 2500 ng/kg in milk products. Over the complete range recovery rates were >70%. Low coefficients of variation were shown for intra- ($CV_R \leq 7\%$) and inter-laboratory reproducibility ($CV_R \leq 21\%$). Except one, all participating labs passed a proficiency test.

Keywords: *aflatoxin M1, solvent-free extraction, immunoaffinity chromatography, HPLC-FD, milk products*

Acknowledgement: *This work was supported by the Eurofins Operational Best Practices Programme (OBP) in the Cross Company Team (CCT) Contaminants and Residues.*

K38
MULTI-CLASS MYCOTOXIN ANALYSIS WITH SELECTIVE LIPID REMOVAL USING CAPTIVA EMR-LIPID

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Sample preparation of fatty foods for multi-class, multi-residue analysis often requires a lipid removal step to improve method performance, maximize sensitivity, and keep the instrument clean. Mycotoxins are chemically diverse classes of compounds and the removal of lipids using current cleanup technologies can give insufficient cleanup and indiscriminately retain some analytes. Captiva EMR-Lipid is a next generation sorbent that overcomes the limitations of traditional cleanups by providing highly selective lipid removal for fatty samples without analyte retention. Captiva EMR-Lipid is available in 3 mL and 6 mL cartridges and offers a simple, pass-through cleanup of extracts from QuEChERS, protein precipitation, and other organic extractions. This work describes the validation of a QuEChERS based method for 13 representative mycotoxins in hard cheeses followed by cleanup with Captiva EMR-Lipid and analysis with LC/MS/MS. The method demonstrates excellent extraction and cleanup efficiency from fatty cheese samples, delivering high recovery (70-120%) and precision (%RSD <20%) for target analytes. Lipid removal efficiency and comparisons with traditional cleanup methods are also shown. Captiva EMR-Lipid pass-through cleanup cartridges are an attractive option for laboratories looking for a simple workflow that can improve method performance for multi-residue analysis through cleaner samples.

K39
THE NEED AND COMPLEXITIES OF MEASURING COMBINED EXPOSURE O NATURAL TOXINS: THE AFLATOXIN B1 AND MICROCYSTIN-LR CASE STUDY

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Human co-exposure to natural toxins through food is inevitable. Although many countries have regulations in place to protect consumers, toxicological risk assessments have traditionally been performed for single contaminants rather than evaluating multiple compounds. As a result, vital data namely, additive, synergistic and antagonistic effects is not available for consideration by the relevant authorities. This has huge implications in terms of human health and international trade for both developing and developed countries alike.
Aflatoxin B1 (AFB1), a frequent contaminant in many crops is known to cause liver cancer. While strict maximum limits protect those in developed countries, it is known that the poorest people in Asia, Latin America and in sub-Saharan Africa are exposed to dangerous levels of the toxin. Another widely known natural hepatotoxin, Microcystin-LR (MC-LR) is produced globally by freshwater cyanobacteria. Various studies have intimated that it may play a causal role in hepatocellular carcinoma and IARC have classified it as possibly carcinogenic to humans. As with AFB1, those most at risk of exposure are from poor areas in Africa, Asia and South America where natural freshwater bodies provide the only source of drinking water.
Using High Content Analysis, multiple cytotoxicity endpoints were measured for the individual toxins as well as mixtures in human hepatocellular carcinoma cells (HepG2), Madin-Darby bovine kidney epithelial cells (MDBK) and human epithelial colorectal adenocarcinoma cells (Caco-2). Cell number (CN), nuclear area (NA), nuclear intensity (NI), mitochondrial mass (MM) and mitochondrial membrane potential (MMP) were evaluated. Significant cytotoxic effects were observed for AFB1 above the regulatory limit in all cell lines. CN dropped 17.3% ($p \leq 0.001$) and 13.5% ($p \leq 0.05$) in HepG2 and MDBK cells respectively. Increased NA was detected in HepG2 (17%, $p \leq 0.001$), Caco-2 (10.1%, $p \leq 0.001$) and MDBK cell lines (6.6%, $p \leq 0.01$) while increases of 12.3% ($p \leq 0.001$) in HepG2 and 8.3% ($p \leq 0.001$) in Caco-2 cell were observed for MM. The binary mixture (AFB1 500ng/ml and MC-LR 250ng/ml) revealed greater cytotoxicity in HepG2 affecting CN (decrease of 11.3%, $p \leq 0.05$), NA (increase of 8.2%, $p \leq 0.01$) and MM (increase of 10.6%, $p \leq 0.01$). MM increased in Caco-2 cells (5.4%, $p \leq 0.05$) and NA in MDBK cells (3.7%, $p \leq 0.05$). Synergy was evident for NI in HepG2 cells for AFB1 500ng/ml and MC-LR 250ng/ml mixture. Antagonistic effects were observed for NA at these concentrations in both HepG2 and MDBK cell lines and for CN in the HepG2 cells, antagonism was observed for all concentration mixtures tested, including below regulatory limits.
The antagonism demonstrated between exposure to AFB1 and MC-LR is clear evidence of the complexity around trying to regulate for human exposure to multiple contaminants. The HCA approach has proven itself to be an invaluable tool in helping provide such complex information.

Keywords: *aflatoxin B1, microcystin-LR, co-exposure, high content analysis*

K40
TETRODOTOXINS IN SHELLFISH: AN EMERGING ISSUE IN THE NETHERLANDS

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Tetrodotoxins (TTX) are potent neurotoxins which block the sodium channel, depending on the dose intoxications range from numbing of the tongue till paralysis or even death. TTX was previously associated only with specific fish species from tropical areas. For example, TTX intoxication is well known from the Japanese delicacy fugu which originates from puffer fish which if prepared wrongly can be lethal. In Europe the detection of TTX was mainly related to puffer fish entering the Mediterranean Sea via the Suez Canal. However previously unexplained marine biotoxin mouse bioassay results from this region revealed after LC-MS/MS analyses the presence of TTX in shellfish. After the discovery of TTX in shellfish in the Mediterranean a survey was conducted in the United Kingdom which also revealed TTX in shellfish. This event led to the decision of the Netherlands to conduct a survey on TTX and since 2015 and onwards TTX was discovered in shellfish. TTX is currently incorporated in the routine monitoring and production sites are monitored with the same frequency as the other EU regulated marine biotoxins. For most of the marine biotoxins the producing organisms and bloom dynamics are known from historical knowledge, for TTX this is not the case yet. Therefore, industry together with the government are funding a multidisciplinary research project on TTX in the Netherlands. Questions to be answered are related to the producing organism, climate and hydrology dynamics, economic and social impact, toxicology, occurrence and distribution of the toxins. To get an insight in the TTX issue it is of importance to understand the distribution within the Netherlands but also between species and where the toxin is located in the shellfish.
For official monitoring recently, the European Food Safety Authority derived an acute reference dose [(ArfD) 0.25 µg TTX /kg bw] from which a safe concentration in shellfish was then calculated. It can be stated that consumption of 400 g of shellfish meat with a concentration below 44 µg TTX-eq/kg will not result in adverse effects in humans (not exceeding the ArfD). The level of 44 µg TTX-eq/kg is currently being used as the official limit in the Netherlands for closing shellfish production areas.
In order to comply with this limit in official monitoring and to perform the research an LC-MS/MS method was developed. Beside TTX also paralytic shellfish poisoning (PSP) toxins, i.e. saxitoxin, were included. Both groups are extremely polar and not retained in reversed phase chromatography. Therefore, a Hydrophilic Interaction Liquid Chromatography (HILIC)-MS/MS method was developed, validated and applied for the detection of the various hydrophilic marine biotoxins. In 2016 the maximum level detected in oysters was 253 µg TTX/kg, well above the established safe level of 44µg TTX-eq/kg. In the presentation the development of the HILIC-MS/MS method as well as the application in both research and routine will be presented.

Keywords: *tetrodotoxins, marine biotoxins, emerging issue*

K41
BIOTECHNOLOGICAL SYNTHESIS OF DEOXYNIVALENOL-3-GLUCOSIDE AND ITS FULLY LABELED 13C ANALOGUE WITH HETEROLOGOUSLY EXPRESSED ENZYMES

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The formation of masked mycotoxins is a major detoxification strategy of crops. Usually, a glucose molecule or a sulfate is conjugated to the mycotoxin. Although these masked toxins do not further harm the plant, their toxicity to humans and animals might re-emerge with the cleavage of the added masking molecule in the gastrointestinal tract of mammals during digestion. Toxicological data on masked mycotoxins are scarce, and current results and knowledge on the real risks and effects of these compounds are insufficient.
Deoxynivalenol-3-glucoside (D3G) is known as the most important masked metabolite of deoxynivalenol (DON) and can be found mainly in wheat, maize and barley. High levels of D3G have already been described in cereals and barley malt and therefore as well in beer samples. As this toxin is not synthesized by *Fusarium* itself, the analytical reference material can not be produced by a simple fermentation.
Usually D3G was obtained by isolation from infected plants, a rather tedious procedure leading to low yields and co-isolation of numerous undesired compounds from the plant. To improve the production of D3G for the use as analytical standard we followed a completely new strategy. A heterologously expressed enzyme, a glycosyltransferase from rice (OsUGT79), was used for the conjugation of the glucose molecule to DON. In our two steps approach UDP-glucose was first synthesized by applying a heterologously expressed sucrose synthase (AtSUS1) from *Arabidopsis thaliana* using sucrose and UDP as substrate and in the second step linked to DON via the glycosyltransferase.
With this novel procedure it is now possible to obtain very high yields of D3G and also of fully ^{13}C isotope labeled D3G, by using ^{13}C labeled deoxynivalenol and ^{13}C sucrose for synthesis. ^{13}C-D3G and ^{13}C-DON was then used to develop a stable isotope dilution assay for the accurate determination of DON and D3G in cereals. After extraction, a mixture of both internal standards was spiked into the cereal extract, which was consecutively measured by LC-MS/MS. DON and D3G could easily be separated under reversed phase conditions, while the internal standards showed perfect co-elution with the respective mycotoxins. Matrix effects in wheat and maize were effectively compensated using this internal calibration.
The above described developments show a completely new biotechnological approach for producing analytical standards and subsequently allow the highly accurate determination of contamination levels with D3G and DON in cereals for a proper risk assessment.

Keywords: *biotechnological synthesis, masked mycotoxins, deoxynivalenol-3-glucoside*

K42
PYRROLIZIDINE ALKALOID ISOMERS: ANALYTICAL CHALLENGES AND SOLUTIONS

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Pyrrolizidine alkaloids are plant toxins which are produced by several thousand plant species and which may contaminate food such as honey or herbal teas. Nowadays, pyrrolizidine alkaloids are usually analysed by LC-MS/MS and typical methods cover up to 28 substances (including the N-oxide forms of the alkaloids).
Some of these analytes are isomeric, being either structural isomers or stereoisomers. These isomers share some or even all MS/MS transitions and thus chromatographic separation is essential to differentiate between them. However, in many methods only partial resolution is obtained, complicating exact quantification. A fine-tuned chromatography allowed baseline separation of all isomeric pairs (intermedine/lycopsamine, senecionine/senecivernine and the corresponding N-oxides) on a C_{18} column.
In some honey samples it was observed that the peak for lycopsamine showed MRM ratios which strongly deviated from those of the standard, suggesting a co-elution of further isomers. Detailed investigations proved that up to two additional stereoisomers of lycopsamine (echinatine and rinderine) were co-eluting with the parent compound. These isomers, however, differed significantly in their mass spectrometric fragmentation behaviour compared to lycopsamine. Various HPLC columns and conditions were tested until a complete baseline resolution of all stereoisomers was achieved. Analysis of real-life samples showed that some contained mainly echinatine and rinderine and that a summary quantification of the isomers based on a lycopsamine standard gave far too low concentrations.
It can be concluded that a careful differentiation between the pyrrolizidine alkaloid isomers is required to obtain a true picture of contamination levels in food products.

Keywords: *pyrrolizidine alkaloids, isomers, LC-MS/MS, separation*

K43
SIMULTANEOUS QUANTIFICATION OF MYCOTOXINS IN MILK

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Mycotoxins, toxic metabolites produced by fungi, can contaminate vegetal products and can reach biological fluids, such as milk, when animals are fed with them. Also, some mycotoxin metabolites, such as aflatoxin M1, can be carried over into milk. Mycotoxin can reach man through milk and affect human health. The International Agency for Research on Cancer has classified aflatoxin B1 and naturally-occurring mixtures of aflatoxins as human carcinogens (group 1) and ochratoxin A as a possible human carcinogen (group 2B) [1]. The rumen is supposed to be a barrier against mycotoxins; however, low levels of some of them have been found in milk samples worldwide [2]. Aflatoxin M1 is the most studied mycotoxin in this matrix, and taking into account its occurrence and toxicity, the European Commission has set a maximum permitted level for this mycotoxin in milk of 0.050 mg/kg [3].
Analytical methods for mycotoxin determination in milk are needed for performing surveillance, carry-over studies, and for carrying out better human health risk assessment; however, it is an analytical challenge. Milk is a difficult matrix to work with due to its complex composition. On the other hand, a great number of mycotoxins with very different physicochemical characteristics are known. Finally, due to the low levels that are expected, low detection limits are needed. Ruminants are usually exposed to multiple mycotoxins because their diet is prepared from different raw materials, likely contaminated by different fungi species; therefore, multi-detection methods are particularly relevant.
We will review the reported analytical methods for mycotoxin determination in milk worldwide, especially the ones that are capable of detecting several mycotoxins simultaneously. Also, we will present two LC-MS/MS-based methods for the quantification of 22 mycotoxins in milk: nivalenol, deoxynivalenol, deepoxy-deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, neosolaniol, diacetoxyscirpenol, fusarenon X, T-2, HT-2, aflatoxin B1, aflatoxin B2, aflatoxin M1, aflatoxin G1, aflatoxin G2, fumonisin B1, fumonisin B2, fumonisin B3, zearalenone, ochratoxin A, ochratoxin B and s. Sample treatment is based on extraction with acidified acetonitrile. Mycotoxins were analyzed by means of LC-MS/MS (ESI). Validation of the methods has been based on the following parameters: limits of detection and quantification, linearity, accuracy, precision, recovery, stability and matrix effects. A detection limit of 0.025 µg/L was achieved for AFM1.

[1] International Agency for Research on Cancer http://monographs.iarc.fr/ENG/Classification/
[2] Presence of mycotoxins in animal milk: A review. Flores-Flores, M, Lizarraga, E, López de Cearin, A and González-Peñas E. Food Control 53 (2015) 163-176.
[3] Commission regulation (EC) No 1881/2006 of 19 December 2006: Setting maximum levels for certain contaminants in foodstuffs

Keywords: *mycotoxins, milk, LC-MS/MS, AFM1*

Acknowledgement: *We thank the "Programa de Investigación Universidad de Navarra" (PIUNA) and "Gobierno de Navarra" (Project number 0011-1383-2016-000013 PC007 DOTCO) for financial support. M. Flores-Flores wishes to express her gratitude to the "Asociación de Amigos de la Universidad de Navarra" (ADA) for the grant funding.*

K44
AFLATOXINS CONTAMINATION IN IMPORTED NUTS FOR DIRECT HUMAN CONSUMPTION: THREE YEARS (2013-2015) OF OFFICIAL CONTROL RESULTS IN ITALY

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Aflatoxins (AFs) are the most toxic group of mycotoxin and secondary metabolites of various species of Aspergillus that can occur in all agricultural commodities under appropriated field or storage conditions. These molecules can cause important health problems and have high potential toxic effects. To detect the presence of aflatoxin B1 (AFB1) and total aflatoxin (AFT), the use of the Enzyme Linked Immuno Assay (ELISA) as a screening test was evaluated and validated in order to analyze imported nuts intended for direct human consumption. The detection limit of ELISA methods was 1 ug/kg and the recovery from nut exceeded 90% for both AFB1 and AFT. The percentage of AFs positive samples (only pistachios and almonds), taken during the three years from 2013 to 2015, under the national programs of official control, amounted to 9% for B1 and 10.5% for AFT, and were confirmed by HPLC (High Performance Liquid Chromatography). The results demonstrate that the aflatoxins levels in pistachios exceeded even more than five times the maximum permitted limits set by European Commission in Reg 165/2010 and referred to the edible part of the tree nuts. The higher incidence of AFs in imported shelled pistachios is probably due mostly to an easier aflatoxin contamination following the fact that pistachios hulls with intact cuticles are more resistant to the *A. flavus* colonization.

K45
BIOSYNTHESIS OF THE TOXIC METABOLITE ASPERGILLIC ACID IN ASPERGILLUS FLAVUS

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Aspergillus flavus can colonize important food staples and produces aflatoxins, toxic and carcinogenic secondary metabolites. *In silico* analysis of the *A. flavus* genome revealed 56 gene clusters encoding for secondary metabolites. We are interested in how these metabolites affect fungal development, survival, and virulence. We are particularly interested in *A. flavus* metabolites that are produced during infection of maize seed. RNA-Seq analysis of all predicted *A. flavus* secondary metabolic gene cluster 'backbone' genes during maize kernel infection showed that in addition to the aflatoxin cluster polyketide synthase (PKS) gene, *aflC*, one of the earliest genes expressed was the uncharacterized Cluster 11 nonribosomal peptide synthetase (NRPS) gene, *asaC* (AFLA_023020). We focused on seven genes in Cluster 11, which encode the putative NRPS as well as ankyrin domain protein (AFLA_023000), desaturase/hydroxylase (AFLA_023010), P450 oxidoreductase (AFLA_023030), MFS transporter (AFLA_023050), hypothetical protein (AFLA_023060) and C6 transcription factor (AFLA_023040). LC-MS analysis of extracts from knockout mutants of these genes showed that they were responsible for the synthesis of the previously characterized antimicrobial mycotoxin aspergillic acid. Extracts of the NRPS knockout showed no production of aspergillic acid or its precursors. Knockout of the P450 oxidoreductase afforded a pyrazinone metabolite, the aspergillic acid precursor deoxyaspergillic acid. The formation of hydroxyaspergillic acid was abolished in the desaturase/hydroxylase knockout. The antimicrobial properties of aspergillic acid are attributed to its ability to chelate iron as defense against competing organisms. Iron chelation, which generates the red pigment ferriaspergillin, could also be used offensively, as a virulence factor aiding the fungus in colonizing maize kernels.

Keywords: *mycotoxin, aspergillic acid, Aspergillus flavus*

K46
BIO-CONTROL INTERVENTIONS FOR THE REDUCTION OF MYCOTOXIN CONTAMINATION IN HIGH-VALUE AGRICULTURAL COMMODITIES

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Mycotoxin contamination of agricultural food products can pose serious health and economic concerns. There is also increasing emergence of fungicide resistant fungal strains. Therefore, the development of rapid identification, detection, and effective intervention methods are imperative. Our research unit addresses food safety issues of high-value commodities by: (1) developing and implementing control measures to reduce, eliminate, or detect contamination of toxin producing fungi of tree nuts, (2) elucidating principles of microbial ecology and develop biological control measures to inhibit pathogenic and toxigenic microorganisms, and (3) identifying naturally-occurring chemical compounds that enhance the efficacy of established anti-fungal interventions. We recently identified two natural compounds that help to overcome fungicide resistance of pathogenic *Aspergillus* sp. and *Penicillium* sp. One compound has volatile properties that can remotely inhibit fungal growth as well as weed seed germination, a known reservoir of pathogenic fungi in orchards. The second compound has heat-sensitizing anti-microbial (fungi and bacteria) properties, which can drastically reduce fungal contamination of agricultural commodities and prepared foods. This research will benefit growers and consumers of high-value commodities by reduced levels of pathogen or toxin contamination through environmentally friendly, sustainable bio-control measures.

Keywords: *mycotoxins, fungicide resistance, Aspergillus*

Acknowledgement: *U.S. Department of Agriculture- Agricultural Research Service, National Program 108 CRIS project 2030-42000-039-00D*

K47
DETECTION OF TETRODOTOXIN IN SHELLFISH USING A SENSITIVE PLANAR WAVEGUIDE IMMUNOSENSOR

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Tetrodotoxin (TTX) is a potent neurotoxin emerging in European waters with suggestions that it may be linked to increasing ocean temperatures but may also be due to increased targeted surveillance. Its detection in seafood was previously performed as a consequence of using the Association of Analytical Communities (AOAC) mouse bioassay (MBA) for paralytic shellfish poisoning (PSP) toxins as TTX is not monitored independently in Europe. As an AOAC-accredited high-performance liquid chromatography (HPLC) method was accepted by the European Union as a first action screening method for PSP toxins a separate method of analysis was required. In this study, a nanoarray planar waveguide biosensor was developed and validated for the detection of tetrodotoxin (TTX). The device is compact with a low footprint reader and a simple user friendly 10 min analysis protocol. This technique offers the in situ analysis of tetrodotoxin in shellfish to meet the required EFSA recommendations of 44 µg TTX/kg of shellfish meat with an LOQ between 0.1 and 25 µg TTX/kg. The applicability to natural samples was investigated through the study of matrix effects, toxin recovery, repeatability and reproducibility and a comparison of contaminated samples by LC-MS. Limited matrix effects were observed, with recovery at the IC50 value of 20µg/kg greater than 90% and %CV less than 20%. The comparison of the method with LC-MS for contaminated mussel and oyster samples has shown the compatibility and feasibility of this immunosensor to be able to support monitoring programs and research activities for evaluation of the toxin occurrences and levels of exposure.

Keywords: *tetrodotoxin, shellfish, immunosensor, planar waveguide, diagnostic*

K48
CONTRIBUTION OF THE LOT-TO-LOT VARIATION TO THE MEASUREMENT UNCERTAINTY OF LC-MS/MS BASED MULTI-MYCOTOXIN DETERMINATION

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In the recent years, the LC-MS/MS based multi-analyte approach has been demonstrated to be a powerful technique for the simultaneous determination of mycotoxins in food and feed [1]. Quantification of mycotoxins is increasingly based on the analysis of diluted crude extracts and external- or matrix-matched calibration. Analytical results are corrected for the method bias, which origins from losses due to extraction recovery (RE) and signal suppression/enhancement (SSE). In everyday practice RE and SSE are evaluated based on replicate analysis of a single lot of a matrix. We hypothesized that i) RE and SSE of an analyte may vary in different lots of the same matrix (lot-to-lot variation), and ii) that the measurement uncertainty increases due to the lot-to-lot variation.
In this work, the measurement uncertainty of 70 mycotoxins in maize and figs was calculated by evaluating the intermediate precision and the uncertainty associated with the method bias. The contribution of the lot-to-lot variation to the method bias was evaluated by calculating RE and SSE values of 7 different lots of the same matrix.
In both matrices, the lot-to-lot variation contributed to the measurement uncertainty for the majority of the analytes. The dominating cause of the lot-to-lot variation were differences in RE for figs and SSE for maize.
Our findings highlight the need to consider the influence of the lot-to-lot variation on the performance of LC-MS/MS based mycotoxin determination during method validation and the evaluation of the measurement uncertainty.

[1] Malachova et al., J Chromatogr. A. 2014, 1362, 145-156.

Keywords: *relative matrix effect, LC-MS, matrix-matched calibration, mycotoxins, uncertainty budget*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012.*

K49

IDENTIFICATION AND DETERMINATION OF DEOXYNIVALENOL (DON) AND DEEPOXY-DEOXYNIVALENOL (DOM-1) IN PIG COLOSTRUM AND SERUM USING LC IN COMBINATION WITH HIGH RESOLUTION MS/MS; EXPERIMENTAL MODELING OF THE TRANSFER OF MYCOTOXIN DEOXYNIVALENOL FROM SOWS TO PIGLETS

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Deoxynivalenol (DON) is one of the most common mycotoxins produced by field fungi (especially Fusarium). Contamination of livestock feed is a significant risk factor, especially for pigs that are highly susceptible to the toxic effects of deoxynivalenol and its metabolite deep-deoxynivalenol (DOM-1). In this study a validated ultra - performance liquid chromatography (U-HPLC) combined with HR-Orbitrap-MS analysis method is described for the identification and quantitative determination of the mycotoxin compounds (DON, DOM-1) in pig colostrum (milk) and plasma. Detection of analytes was achieved on the tandem hybrid mass spectrometer Q Exactive with a H-ESI+. For identification (confirmation) analysis, the mass spectrometer worked in the full scan mode with resolving power (PR)=140,000 (FWHM) and for quantification analysis, worked in the Parallel reaction monitoring mode (PRM). The method has been fully validated according to the requirements of Commission Decision 2002/657/EC for confirmatory analyses, plus the addition of mass accuracy (MA) parametr. For identification (confirmation) of the presence of these analytes in pig colostrum and serum, matching of the retention time with mass accuracy for precursor ion from MS and product ions from MS/MS was used. The method allowed us to detect very low amount of DON (limit of detection 0.58 ng/ mL) in a blood, colostrum and also in different types of a tissue. The method has been successfully evaluated using incurred samples of pig colostrum and serum. Our data showed that transplacental transfer of DON occurred and seems to have much higher negative effect on piglets than transfer via colostrum. DON persists in piglet's blood up to ten weeks which represents potential healthy risk for piglets. On the other hand, long term persistence of DON does not represent toxicological risk from food safety point of a view based on the negative results from tissue samples, including muscle.

Keywords: *deoxynivalenol, pig colostrum serum, mass spectrometry with high resolution, mycotoxins*

Acknowledgement: *This project was financially supported by the Czech Ministry of Education, Youth and Sports (COST CZ LD15055).*

NOVEL FOODS & SUPPLEMENTS

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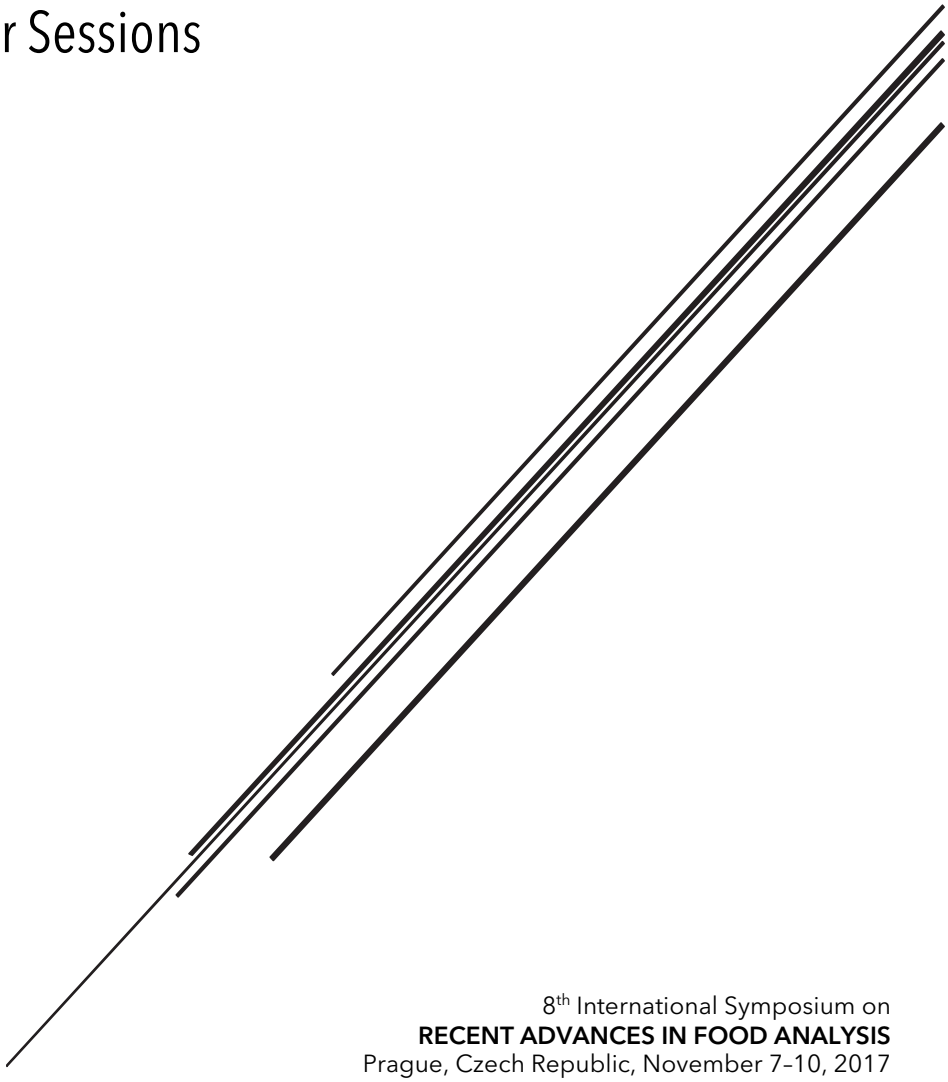

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Prague, Czech Republic, November 7–10, 2017

M1
MEDITERRANEAN MACROALGAE AS POTENTIAL FOOD SUPPLEMENTS: A FIRST MONITORING STUDY IN ELBA ISLAND (ITALY)

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INTRODUCTION: In European countries, there is a notable interest in marine macro algae as alternative food and feed components, due to the increasing demand of sustainable protein source of non-animal origin. Seaweeds are already part of the diet in many Asian countries since a long time and in Western countries, their beneficial properties are now intriguing new markets. In particular, in human nutrition the use of macro algae as food supplements is recommended to increase the contents of essential trace elements [1,2]. In fact, seaweeds contain higher concentrations of trace elements than plants [3] and their metals content is depending to macro algae families, genera and species, as well to geographical provenience [4].
MATERIALS AND METHOD: three seaweeds species belonging to Chlorophyta (green algae, *Codium bursa*), Ochrophyta (brown algae, *Dictyota dichotoma*) and Rodophyta (red algae, *Laurencia obtusa*) were collected in Elba Island (LI), NW Mediterranean Sea, the most populated island of the Tuscan archipelago. Seaweeds were rinsed with distilled water, freeze-dried and homogenized. Samples of approximately 1-1.5 g were subjected to trace element determination. The concentrations of nonessential trace elements (aluminum, antimony, arsenic, beryllium, cadmium, mercury, lead, tin, thallium and vanadium) and essential trace elements (cobalt, chromium, copper, iron, manganese, molybdenum, nickel, selenium and zinc) was performed using an ICP-MS instrumentation, after microwave digestion with concentrated nitric acid and hydrogen peroxide. Quantification limit (LOQ) was 0.010 mg Kg^{-1}.
RESULTS: Total metals concentration was found in the order green (11529 $mgKg^{-1}$ dry weight) > brown (8818 $mgKg^{-1}$) >red (5081 $mgKg^{-1}$) macro algae. The highest levels for nonessential elements were reached for Al (range 1663-2895 $mgKg^{-1}$) and V (range 12-31 $mgKg^{-1}$), while for the essential elements the highest concentrations were found in Fe (3189-8732 $mgKg^{-1}$) and Mn (91-250 $mgKg^{-1}$). Regarding the metals for which EU Regulations set maximum limits in food supplements (3 $mgKg^{-1}$ Cd and Pb; 0.1 $mgKg^{-1}$ Hg), in the green macro algae *Codium bursa* and in the brown macroalgae *Dictyota dichotoma* the limit for Pb was exceeded (4.8 $mgKg^{-1}$ and 8.0 $mgKg^{-1}$).
CONCLUSIONS: Lead is the element that could constitute the major limitation in the use of the examined algae for food supplements, in particular in green and brown seaweeds, because of the exceeding of maximum allowed level. The macro algae species intended to be used for food supplements should be carefully identified in order to prevent potentially toxic effects for consumers.
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Keywords: *seaweeds, food supplements, Mediterranean Sea*

Acknowledgement: *This work was founded by the Italian Ministry of Health (grant nr. IZS PLV 14/14 RC).*

M2
POTENTIAL USE OF CAROTENOGENIC YEASTS AND MICROALGAE FOR PRODUCTION OF HIGH VALUE PRODUCTS

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Red yeast species and algae have high industrial potential due to accumulation of lipids and carotenoid pigments. They are also increasingly playing a role in nutraceuticals and functional foods. Red yeasts and microalgae are able to enhance the nutritional value of conventional food and feed preparation and hence positively affect human and animal health due to their original chemical composition, sometimes more efficiently than traditional crops. Commercial production of enriched microalgae biomass for human nutrition is already a reality. Numerous form of tablets, powders or liquids as nutritional suplements can be found in the market.
Carotenoids are natural pigments occurring in plants and many microorganisms. They represent the most common group of antioxidants with significant biological effect. The best-known examples are astaxanthin and β-carotene, which are used as colouring agents and for health-promoting purposes. Many species of green algae and carotenogenic yeasts are able to produce valuable metabolites for different uses; examples are antioxidants, polyunsaturated fatty acids (linolenic acid, docosahexaenoic acid, eicosahexaenoic acid), vitamins, anticancer and antiviral drugs, algal extracts for use in cosmetics etc.
In many cases, these substances are secondary metabolites that are produced when the red yeasts and algae are exposed to stress conditions linked to nutrient starvation (limitation), high light intensity, temperature, salinity and pH. However, there is not complete information about optimization of the production of individual products, or the effects of stress conditions on their production.
This work deals with finding a cost-effective method for biotechnological production of selected metabolites. Selected strains of red yeasts (genera *Rhodotorula*, *Cystofilobasium* and *Sporobolomyces*) and microalgae (genus *Chlorella, Chlamydomonas* and *Desmodesmus*) were enrolled in a comparative study. The cells were subjected to several types of exogenous stress. Simultaneously, the strains were tested for ability to utilize selected low-cost waste substrates to increase the production of microbial biomass enriched in specific metabolites.

Keywords: *carotenogenic yeasts, microalgae, β-carotene, polyunsaturated fatty acids*

Acknowledgement: *This work was supported by project Nr. LO1211 „Materials Research Centre – Sustainability and Development" of the Czech Ministry of Education, Youth and Sports.*

M3
INFLUENCE OF FOOD MATRICES ON PROBIOTIC BACTERIA VIABILITY

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Probiotics are defined as live microorganisms which when administered in adequate amount beneficially affect the host by improving its intestinal balance.
Presented work was focused on study of the environment effects on the viability and growth of probiotic bacteria. For this study *Lactobacillus acidophilus, Bifidobacterium breve* and commercially complex of probiotics were chosen. Probiotics were incubated in the environment of selected types of model and real foods and beverages. For simulation of human digestion, probiotics were afterwards incubated in artifical gastrointestinal juices.
Additionally, the potential influence of encapsulation and lyophilization on viability of probiotic cells was observed. Probiotics viability was monitored by flow cytometry, fluorescence microscopy and by cultivation method during long-term storage.
According to preliminary results, it is recommended to consume probiotic bacteria right before or during meals. Moreover, the process of encapsulation can have a significant effect on viability of probiotic cells.

Acknowledgement: *This work was also supported by the project "Materials Research Centre at FCH BUT–Sustainability and Development" no. LO1211 of the Ministry of Education, Youth and Sports of the Czech Republic.*

M4
EFFECT OF NATURAL COMPOUNDS FROM HOP (HUMULUS LUPULUS L.) ON THE QUALITY OF SOFT SALAMI

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Meat products can be spoiled by two main causes: oxidative rancidity and microbial growth. Both processes could be slowed down by the addition of food preservatives. Traditional chemical food additives play an important role in meat production; some of them are even irreplaceable. However, there is discussion about nutritional impact and potential harmful effect of chemical preservatives on human health. Therefore, demand for natural additives, obtained especially from plants, has notably increased in recent years. A number of studies have shown antimicrobial and antioxidant effects of spices and herbs, such as rosemary, thyme, basil, garlic, ginger, clove, pepper etc. The use of natural compounds may allow developing novel meat products with improved shelf-life, increased oxidative stability, enhanced nutritional and health benefits.
In this study, we examined the influence of natural compounds from hop (*Humulus lupulus* L.) on shelf-life and on the key technological and organoleptic properties of soft salami. Laboratory and industrial samples of meat products were tested using two different hop products in different concentrations. The effect of natural compounds obtained from hop on shelf-life was established through microbiological analysis, which was carried out every few days for three weeks. The following technological properties were measured: pH, color (reflective spectrophotometry), lipid oxidation (TBARS) and texture (Warner-Bratzler shear force test). The samples were also subjected to sensory analysis. The technological and organoleptic properties of the samples with hop products were comparable to the standard sample, but with the additional benefits of reducing the content of lipid oxidation products. Our results suggest that hop products could be used as a source of natural antioxidants for soft salami.

Keywords: *natural compounds, hop, meat products, soft salami*

Acknowledgement: *Financial support from specific university research (MSMT No 20-SVV/2017)*

M5
VOLATILE METABOLOMICS AND BIOACTIVE COMPOUNDS BASED STRATEGIES DIFFERENTIATION UPON NOVEL OHMIC HEATED WHEY ACEROLA-FLAVOURED BEVERAGES

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Conventional pasteurization (CP) was mainly used to dairy products conservation over the past century, even though its sensorial, nutritional and bioactivity depleting effect. Due to these implications of CP, novel technologies, as Ohmic Heating (OH), have been investigated in order to deliver higher nutritional contents, sensorial acceptance, and a complete level of sterilization for microbiological safety.
The aim of this work was to investigate volatile metabolomic and bioactivity differentiation between whey acerola-flavoured beverage (WAFB) ohmic heated treated and pasteurized. For whey beverages ohmic heating processing two different systems were used. The first one at a 25V constant voltage and three distinct frequencies (10, 100 and 1000Hz) were applied. In the second system, a 60Hz frequency and three different voltages (45, 60 and 80V) were applied. A pasteurized WAFB (QC) was made at 64°C for 30min as a control. For bioactivity assessment 1g of sample were weighted in beakers, then 30mL of aqueous ethanolic solution (50:50, *v/v*) were added, and shake at 200rpm in orbital shaker. Finally, the extract was vacuum filtered and analyzed by DPPH, FRAP and Total Phenolic Content (TPC) assays.
For volatile organic compounds (VOCs) analysis around 3 g of WAFBs were weighted in 20 mL headspace vials then added 3 mL NaCl saturated solution. VOCs extraction was performed by SPME for 30 min at 40°C. VOCs were analyzed by a 7890A-5975C GC-MS equipped with a CP-Wax 52cb 60 m x 0.25 mm x 0.25µm column, in splitless mode. Ion source operated at 70eV. Single quadrupole operated at 40-400 *m/z* mass range.
VOCs were identified in deconvolution mode, with S/N ratio above 10, left Δ *m/z* =0,3 AMU and right Δ *m/z* =0.7 AMU, and hints obtained from NIST 11 Library. Increasing voltage (45-80 V) and reduction in frequency (1000-10 Hz) resulted in higher degradations in antioxidant capacity, except for DPPH results. Results ranged from 45.66–63.08 µg Acid Gallic/g for TPC; 196.27–248.15 µg Trolox Eq/g for FRAP; 8.30–8.88 µg Trolox Eq/g for DPPH. QC (61,54±1.30) showed less TPC then WAFB (63,08 ± 0.99) at 1000Hz and one of the lowest DPPH results (8,48 ±0.18).
Esters were the most abundant compounds in both OH and CP, where compound numbers ranged from 9 (100 Hz - 25 V) to 11 (1000 Hz - 25 V). Six Maillard Reaction Products (MRPs) were detected: Furfural, 2-Furanmethanol, 5-Hydroxymethylfurfural, Pyranone, 2(3H)-Furanone dihydro-4-hydroxy, 2H-Pyran-2.6(3H)-dione, where the best condition was in OH 100 Hz – 25 V, with 0 MRPs detected, and the worst condition was in the QC, with all six MRPs detected.
Hence, WAFBs ohmic heated presented a higher antioxidant/bioactivity than conventional pasteurization with none or less off-flavors in the final product. Overall OH could be a potential alternative to whey beverages pasteurization, since it preserves the nutritional, functional and sensory quality of the product.

Keywords: *VOCs, bioactive compounds, GC-MS, ohmic heating*

Acknowledgement: *This work was supported by Brazilian National Council for Scientific and Technological Development (CNPq).*

M6
FAST AND RELIABLE ANALYSIS OF ISOFLAVONES IN DIETARY SUPPLEMENTS

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Isoflavones are found primarily in plants of soy (*Glycine max*), red clover (*Trifolium pretense*), and Kudzu (*Pueraria lobata*). 12 major isoflavones found in these plants are daidzein, glycitein, genistein and their respective glucoside and malonyl- and acetyl- glucoside derivatives. The structures of 12 isoflavones and an internal standard (apigenin) are shown in Figure 1. These hormone-like compounds are often used in remedies to reduce menopausal and post-menopausal symptoms.
Standard methods for isoflavones in dietary supplements have been established by standard organizations such as USP[(1)] and AOAC[(2)]. These methods use reversed-phase LC with C_{18} columns and ultraviolet and visible light (UV-Vis) spectroscopy for separation and quantitation. Because of the close structural similarity of this group of compounds, the chromatographic run times of these methods are over 70 minutes long. It is highly desired to develop a fast isoflavone analysis method.
This study demonstrates the method transfer of the USP method on an ACQUITY® Arc™ system. The analysis time, including column wash and equilibration, is only 18 minutes with this fast method. Waters® QDa® mass detector was used in this study. The benefits of mass detection in peak identification and method optimization are also highlighted.

Keywords: *isoflavones, dietary supplements*

M7
ENTOFÔR PROJECT - FROM WASTE TO RESOURCE

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Trends towards 2050 predict a steady population increase to 9 billion people, forcing an increased food/feed output from available agro-ecosystems resulting in an even greater pressure on the environment. Scarcities of agricultural land, water, forest, fishery and biodiversity resources, as well as nutrients and non-renewable energy are foreseen. The increasing demand for food and feed proteins, forces us to produce and use food more efficiently. Finding innovative ways to increase food and feed production is important, but equally important is to avoid wasting valuable nutrients.
The project ENTOFÔR answers to this statement by increasing value creation from bio-based waste. This will be done by recycling, or rather upcycling bio-based waste by feeding it to insects which will turn it into protein source for feed industry. This new waste-based insect industry converts the traditional view of bio-based '*waste*' to be considered as a '*resource*'. However, converting bio-based waste in this way also creates barriers for insect producing companies, feed manufacturers and regulatory bodies. To suggest how this novel industry may overcome these barriers, a consortium of researchers from multiple disciplines (economy, social sciences, entomology, animal nutrition and feed safety) and the industry (bio-based waste- and insect industry) was built.
The specific objectives of the ENTOFÔR project is to deliver knowledge and tools that policy makers, companies and other stakeholders need to direct and develop a waste-fed insect industry for feed production in Norway by: (1) identifying attractive Norwegian organic waste streams that may be converted by insects, (2) evaluating and establishing insect species cultures and methods that are relevant to convert the identified waste streams into feed resources, (3) optimising of insect protein and lipid for feed, (4) developing proteomics based methods for detection and tracing of non-legal waste, (5) mapping pesticides and mycotoxins in waste-fed insects.

Keywords: *recycling, insects, valorisation, protein, aquafeed*

Acknowledgement: *This project is supported by the Norwegian Research Council.*

M8
DEVELOPMENT OF A REAL-TIME PCR TARGET FOR THE DETECTION OF HERMETIA ILLUCENS IN FEED

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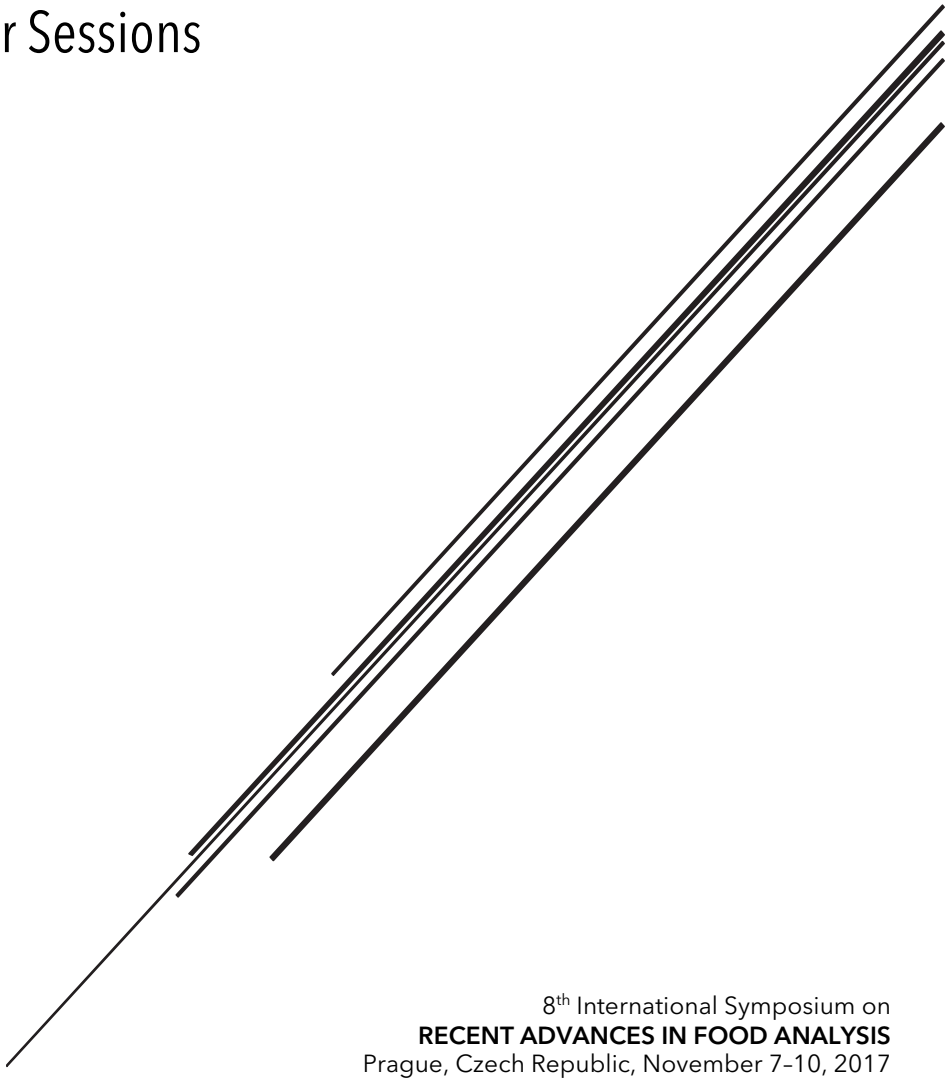
Insects are rich in proteins and could be an alternative source of macronutrients to feed animals and humans. Numerous companies have started the production of insects for feed purposes. In Europe, the processed animal proteins obtained from seven insect species are authorized since 1st July 2017 for aquaculture by EU regulation 2017/893. Methods of authentication are required to check the conformity of the products. In this study, we propose a real-time PCR method for the specific detection of the black soldier fly (*Hermetia illucens* L), one of the most widely used insects for feed production. The developed PCR assays amplify a fragment of 67 bp based on the mitochondrial COX3 gene coding for the subunit 3 of the cytochrome C oxidase. The qualitative method was tested according to several performance criteria. The specificity was tested against 50 insect species. The specificity was also checked against plant species and other animal species as crustacean, mammals and birds. The sensitivity, efficiency and robustness of the PCR test were successfully tested. The applicability of the test was proved through the analysis of real-life processed samples (industrial meals) of *H. illucens*.

OMICS INCLUDING FOODOMICS

Poster Sessions



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N1
NMR-BASED METABOLOMICS TO IMPROVE OLIVE TREE PRODUCTIVITY: UNRAVELLING METABOLIC BEHAVIOR OF FRUITLET DROP

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Olea europaea is one of the most important and widespread fruit trees in the Mediterranean basin. About 73% of the global olive oil production comes from European Union countries and 97% comes from Spain, Italy, and Greece. Olives are a cash crop of great economic importance even for the great adaptability to dry spells and drought. However, olive trees suffer several production problems, especially in challenging conditions. Generally, olives have a very low fruit-set and this is also due to the high levels of fertilized fruitlet drop. Another important problem affecting yield and productivity, is the climate change. Each crop and even each variety has different climatic and environmental requirements for normal growth (e.g., light, nutrients, soil, temperature, and water availability). Climate change mainly influences these variables. Climate warming will affect olive yield and quality across the Mediterranean basin and at local scale and profitability of small olive farms will decrease. Irrigation is not an easy task in several hilly areas and it may negatively affect water supplies, leading to water shortages for other purposes. The low yield (and the high costs of the grove management) makes olive growing a less profitable cultivation if compared with other crops with the risk of land abandonment. New and innovative solutions to improve olive tree productivity must be found. As above stated, olives are characterized by extremely high levels of post-fertilization/early growth fruitlet abscission. Furthermore, no specific information is available to better understand the molecular mechanism and the metabolic processes inducing fruitlet abscission. The elucidation of these phenomena represents a key step towards the improvement of olive tree productivity. This study focuses on the metabolic basis of mechanisms involved in fruitlet shedding using Nuclear Magnetic Resonance (NMR)-based metabolomics (600 and 900MHz Bruker spectrometers). Olive fruitlet samples, collected at different time points and categorized as "fast-"and "slow-growing", and as "dropped" according to their size and/or their tendency to drop, have been analyzed. The PCA analysis performed on one-dimensional spectra, proved to be effective in discriminating olive groups according to the developmental stage. The supervised approach built on the OPLS model is highly effective in discriminating the "not dropped" fruitlets from the "dropped" ones, meaning that the two groups have distinct profiles. Moreover, several water-soluble metabolites differ among groups: higher choline levels and lower levels of gallic acid, caffeic acid and 3,4-dihydroxyphenylglycol in "dropped" fruitlets suggest, according to literature, an impairment of stress response mechanisms. Instead, lower arabinose levels can be ascribed to an incomplete development in "dropped" fruitlets.

Keywords: *foodomics, NMR, climate change, olives, fruitlet abscission characterization*

Acknowledgement: *This study was conducted in collaboration with Prof. Pietro Tonutti, PhD Stefano Brizzolara and Athanasia-Maria Dourou from Scuola Superiore Sant'Anna (Pisa, Italy).*

N2
VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF BIOACTIVE COMPOUNDS IN ORGANIC TOMATOES APPLICABLE IN ROUTINE PRACTICE

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Consumer demand for organically grown food has become higher than ever in recent years, with its market share undergoing almost a three-fold increase in under a decade, from U.S. $22.1 bn (2002) to U.S. $62.8 bn (2011). European countries comprise almost half of the global market at U.S. $29 bn, Germany having the largest market for organic products. Furthermore, most of the production in southern European countries (Greece, Spain and Portugal) is exported for sale at foreign markets, in northern-western Europe [1]. The risk for counterfeit is high, since consumers are willing to pay higher prices for organically grown food, from which the need for a means of verifying the authenticity of organic food products arises. Tomatoes (*Solanum lycopersicum*) are a widely consumed fruit in Europe [2], and were the chosen food to develop a chemometric means to fight against counterfeit.
From an analytical point of view, the authentication of organic food products is a challenging issue. The determination of bioactive compounds to distinguish between organic and conventional tomatoes can be a promising strategy. The first step in order to look for these natural food compounds (NFCs) is the method validation. Thus, the objective of this study was to validate a simple, rapid and sensitive analytical approach for the detection and quantification of bioactive compounds in organically and conventionally grown tomatoes under controlled agronomic conditions, and in a climatic region of leading EU production such as the Mediterranean. To this end, an easy extraction method combined with a liquid chromatography high resolution accurate mass spectrometry (HRAMS) system was the procedure validated in this study. The more demanding requirements regarding mass spectrometric confirmation currently set by EU regulations (SANTE/11945/2015) were taken into account [3].The efficiency of the extraction solvent was determined by comparison of the detected bioactive compounds between the methanolic and the acetonitrilic extracts, after which methanol was chosen as the most suitable extraction solvent. The validated method will be employed for the study of these tentative biomarkers and the research of their potential use as a means to fight counterfeit in organic food products. *References*

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Keywords: *organic food, natural food components, method validation, HRAMS*

N3
COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH TOF-MS, A POWERFUL TOOL FOR ANALYSIS OF THE VOLATOMES OF GRAPES AND WINES

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Comprehensive two-dimensional gas chromatography (GCxGC) has emerged as a powerful analytical technique for unraveling the volatile composition of complex matrices. This work will present three applications of GCxGC Tof-MS to the oenological field, aimed to identify novel biomarkers to be used in the quality control process of the wine industry.
Comprehensive mapping of volatile compounds was conducted in a large sample of 70 sparkling wines, produced by 48 different wineries across 6 vintages and representative of the two main production areas for premium Italian sparkling wines (Franciacorta (FC) and Trentodoc (TN)), using HS-SPME followed by GCxGC-Tof-MS and multivariate analysis. Selection and identification of 196 putative biomarkers allowed clear separation of sparkling wines from FC and TN.
A spatial investigation of Shiraz wines fermented in triplicates from grapes collected from climatically diverse (warm/hot versus cold/temperate) regions of Australia (New South Wales) was made using HS-SPME followed by GCxGC-Tof-MS. Wine volatile profiles from warm/hot and cool/temperate climate could be distinguished according to the first two principal components. Wines from cool/temperate climate were characterised by higher levels of several terpenes such as alpha terpineol, linalool oxide, citronellol acetate, 1-*p*-menthen-9-al, *cis*-rose oxide, ho-trienol) and sesquiterpenes, whereas trend for norisoprenoids was less consistent. Higher TDN levels in wines from warm/hot climate were observed.
Volatile composition of wines from four grape cultivars was investigated with GCxGC Tof-MS in association with multivariate analyses. Eighteen samples of Müller Thurgau, 48 samples of Pinot Gris, 36 samples of Chardonnay and 18 samples of Gewürztraminer were analyzed. A clear varietal differentiation according to the wine volatiles was affirmed by PCA and potential cultivar-specific biomarkers were identified.

Keywords: *wine, aroma compounds, GCxGC ToF-MS*

N4
IDENTIFICATION OF URINARY BIOMARKERS FOR TOMATO INTAKE BY HPLC-HRMS BASED METABOLOMICS

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Food and nutrition substantially contribute to the support and improvement of human health as well as to the prevention of diseases. Consequently, the ability to conclusively determine the relation between consumption of specific foods and their biological effects is crucial. The major challenge in the latter is the objective and quantitative assessment of the human dietary status. Data collection tools like food frequency questionnaires or 24-hour dietary recalls are conventionally applied to collecting dietary intake data. However, these methods are prone to error due to the subjective nature of self-reporting, whereas the use of dietary biomarkers represents a promising alternative. Dietary biomarkers are food constituents or metabolites thereof quantifiable in biofluids after consumption of the respective food items. Therefore, they allow an objective and accurate measurement of the food intake taking the nutrients bioavailability as well as metabolism into account. The number of (valid) dietary biomarkers is currently very limited, so further research in terms of identification as well as validation of dietary biomarkers is mandatory.
The objective of our study is the identification and subsequent validation of novel urinary biomarkers for the consumption of tomatoes, one of the most frequently consumed 'vegetables' worldwide with several beneficial health effects. For that purpose, we conducted an acute intervention study and compared the urine metabolome, i.e. all the small molecules or metabolites present in a given biological sample, of a case and control group after consumption of tomato juice based on HPLC-Orbitrap-HRMS data for different points in time after consumption. The collected mass spectrometry data were subjected to multi-step data processing with MZmine 2, followed by application of univariate as well as multivariate statistics using MetaboAnalyst 3.0 to identify significant differences in the metabolome of the case group in comparison to the control group.
In the course of our study, we identified several features, i.e. exact masses at given retention times, with significantly higher intensities in the case group's metabolome at the investigated time points compared to the control group, thus representing potential dietary biomarkers. Several of them were found to be glucuronidated or sulfated compounds identified by characteristic mass spectrometric fragments. For structure elucidation of features representing potential dietary biomarkers, their exact masses and corresponding isotopic patterns were used for molecular formula prediction and checked against several databases. Additionally, fragment spectra were recorded for further characterizing and assisting in structure identification.

Keywords: *dietary biomarkers, metabolomics, HPLC-HRMS, tomatoes*

N5
WHEN METABOLOMICS GETS ISO17025 ACCREDITED AND READY FOR OFFICIAL CONTROLS

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Within the particular context of controlling chemical residues in food, an alternative to targeted approaches has emerged; it consists in the characterization of physiological perturbations induced upon exposure of animals to a given chemical substance/class of substances to highlight suitable biomarkers addressing safety and/or regulatory issues. Metabolomics in particular has been investigated in the hope of identifying such biomarkers, and a range of studies have from that time forward demonstrated the efficiency of the strategy.
Until very recently, steps toward official or commercial implementation of corresponding tools had however still to be taken. In particular, the lack of guidelines and criteria to validate such methods that do not target specific chemical species *per se*, constituted a bottleneck.
In the present work, a metabolomics model dedicated to the detection of b-agonists administration in bovine has been developed and fully validated. Validation criteria (selectivity, robustness, stability, suspicion threshold, false positive and false negative rates) have been proposed in agreement with EU expectations (Dec 2002/657), enabling demonstrating performances complying screening requirements.
Although some of the biomarkers involved in the prediction model remain still un-elucidated, the corresponding LC-HRMS method has recently been ISO17025 accredited, allowing for the very first official implementation of a metabolomics based strategy within National Monitoring Plans.

N6
DETECTION OF HONEY ADULTERATION FOLLOWING BEE FEEDING WITH BEET SUGAR SYRUPS: A PRELIMINARY STUDY USING A METABOLOMIC APPROACH

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Honey is a natural product with an excellent nutritional value and healing properties. The limited availability and high price of honey contribute to encourage the illegal practice of adulterating honey. The most frequently used methods for honey adulteration are the direct addition of sugar syrups and the overfeeding of bees with excessive amounts of syrup during the main honey-producing season. As in other food products, such as wine or fruit juices, honey adulteration has evolved over time from the simple addition of sugar and water, to the use of specially produced syrups for which the chemical composition reproduces the sugar composition and ratios of honey. There is therefore a need to develop more sophisticated methods to detect honey adulteration. The present study aimed at identifying potential biomarkers to detect honey adulteration using an untargeted metabolomic approach. Metabolomics is aimed at studying low molecular weight (<1 kDa) molecules using bioanalytical and bioinformatic tools. The fingerprints of six surely "non adulterated" honey of different botanical origin [multiflower, acacia (*Robinia pseudoacacia L.*), dandelion, lime and alfalfa (*Medicago sativa*) honey] and five different industrial beet syrups have been investigated by using liquid chromatography coupled to hybrid high resolution mass spectrometry (LC-HRMS). The complex statistical elaboration of the high number of signals obtained has been performed by Thermo SIEVE™ application to assess the differences between honey and syrup samples and to identify significant markers, which are present exclusively or more significantly in syrups. Metabolomic data analysis resulted in nine potential markers for electrospray ionization (ESI) in positive polarity and eight for ESI in negative polarity. Accurate mass of the selected ions have been obtained by analyzing the syrup mixture in high-resolution capture mode (140000 FWHM, Full Scan), and an inclusion list has been created to obtain MS/MS spectra. Honey from overfeeding was produced by administering a total of 7.5 kg of beet sugar syrup in a week. It resulted strongly positive for all selected potential markers. Fortified honey was obtained by adding "non adulterated" honey with 5, 10, 25, 50 and 75% of a syrup mixture. Gradually decreasing the percentage of added syrup, there is a reduction in the significant differences between test and control group. We have so far detected three ions which appear to be promising selective markers for beet syrup. In particular, one ion resulted to be the most promising marker given its exclusive presence in beet syrup and not in honey. The method is sensitive to this analyte, detectable down to 5% adulteration level. The work is still going on testing other honey and syrup samples, and trying to identify the potential markers so far detected.

Keywords: *metabolomics, honey, food fraud, bee feeding, LC-HRMS*

Acknowledgement: *We acknowledge the Council of Veneto region for the financial support to the Project "SYRUP_HONEY_16: study on the presence of exogenous sugars in honey following artificial bee-feeding" (Decree n. 1242 of 1 August 2016).*

N7
POTENTIAL OF LC-ESI/APCI-MS AND GC-APCI-MS IN FOOD METABOLOMICS: CHARACTERIZATION OF NINE DIFFERENT MATRICES (OILS AND TISSUES) DERIVED FROM THE OLIVE TREE

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Over the last decades, virgin olive oil consumption has been associated to a lower incidence of some chronic diseases such as cancer, diabetes or coronary diseases, largely due to the non-glyceridic minor compounds found in it. Some of these phytochemicals with healthy properties are secondary metabolites of plants, which can also be found in other vegetal tissues derived from the olive tree. Currently, the transformation of the olive fruit and the valorization of by-products are considered as parts of the same integral cycle of use, so the deep characterization of the main parts of the olive fruit and tree is a key step when trying to find natural sources of bioactive compounds.
Herewith, we present the results of the study of nine different samples coming from Picudo *cv*. olive trees, which can help to understand the biosynthesis and distribution of these compounds on the studied matrices (lyophilized olive pulp, dehydrated and defatted olive pulp, olive seed, fruit skin, leaves and wood from the branches, as well as virgin olive oil, olive oil obtained from pitted and dehydrated fruits and oil obtained from the seed contained inside the pit).
Samples were prepared using a very unselective liquid/solid-liquid extraction protocol pursuing the extraction of as many compounds as possible. Afterwards, the extracts were analyzed by LC and GC coupled to a QTOF detector (Bruker) by means of ESI and APCI interfaces in the case of LC and an APCI source in GC.
Chromatographic and MS conditions were optimized to facilitate the determination of analytes in a very wide range of polarity/volatility within a single run. In LC, the analytes were eluted in a C18 column (2.1x 100 mm, 1.8 µm), with acidified water and acetonitrile as mobile phases at 40°C. The same extracts (after a further silylation step) were analyzed in GC (BR-5 column) with a T gradient from 150 to 320°C (ramped at 4°C/min). Spectra in both positive and negative polarities were acquired and the collected data was processed with MetaboScape® 3.0 and the T-ReX 3D algorithm for time-aligned, region-complete feature extraction (Bruker).
The analysis of more than 40 commercially available pure standards, relative retention times and MS data, together with information found in databases and published reports were carefully studied in an attempt to assign a feasible identity to most of the detected chromatographic peaks. The identified metabolites belong to different chemical classes: phenolic compounds (phenolic acids and alcohols, lignans, flavonoids, secoiridoids and some of their glycosides), triterpenic acids and dialcohols, tocopherols, sterols and free fatty acids.
The use of LC and GC coupled to a high resolution MS (through different ionization sources) and annotation strategies within MetaboScape (based on retention times, accurate masses, isotopic patterns and MS/MS spectra) has allowed the identification of around 100 compounds in the profiles, showing a great complementarity.

Keywords: *olive tree, bioactive compounds, food metabolomics, mass spectrometry, compounds identification*

Acknowledgement: *L O-G wants to thank the financial support from the Spanish Government (FPU13/06438 and FPU short stay grant).*

N8
THE ADVANTAGES OF USING MS/MS ALL WITH SWATH® ACQUISITION FOR CONFIDENT COMPOUND IDENTIFICATION IN NON TARGET SCREENING ANALYSES

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The increased performance of high resolution mass spectrometry instrumentation has allowed chemists to have an unprecedented ability to identify completely unknown compounds in complex matrices. In earlier instrumentation, this has been accomplished with a data independent acquisition. In these types of acquisitions, all of the ions from a wide mass range, 100-1000 for example, are allowed into the fragmentation chamber and the resulting fragments are analyzed. In an effort to generate MS/MS spectra that are library searchable, different workers have developed schemes for de-convoluting the resulting spectra based on differences in retention times. Unfortunately, in the case where compounds completely co-elute it is impossible to generate clean spectra. SCIEX has developed the MS/MS ALL with SWATH® acquisition to provide better spectra from data independent acquisitions. In this presentation SWATH® will be described and the advantage of such an approach will be shown. Data from actual matrices will be presented along with library searching results to demonstrate the improved quality of mass spectra using MS/MS ALL with SWATH® acquisition.

Keywords: *SWATH, library, unknown screening, LC-MS/MS, HR-MS*

N9
A NON-TARGETED SMALL SCALE METABOLOMIC STUDY OF RETAIL POMEGRANATE JUICE PRODUCTS TO INVESTIGATE THE NUTRITIONAL AND QUALITY CHARACTERISTICS USING A NOVEL DATA INDEPENDENT ACQUISITION MODE AND ION-MOBILITY ON A QTOF MS INSTRUMENT

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Comprehensive identification of the phytoactive compounds is a critical starting point for assessing the biological and technological properties in food matrices. Due to the complexity of plant secondary metabolism the full characterisation of phytochemicals in fruits and vegetables is recognised as a significant analytical challenge and requires sensitive and accurate techniques to be employed. Pomegranate fruit (*Punica granatum* L.) is commonly reported as a rich dietary source of phenolic compounds with regular consumption being linked to a wide range of associated health benefits[1]. Phenolic compounds are also known to play an important role in the quality and sensorial performance of fruit juice products and as such of value to the food industry.
The diverse array of different (poly)phenolic structures including flavonoids, phenolic acids and hydrolysable tannins can make the accurate screening of their profile difficult. High resolution chromatographic techniques, coupled with Quadrupole Time of Flight (UPLC-QToF) MS detection can provide the information to achieve this. However, the resulting data output often consists of retention time, precursor ion exact mass, fragment ion(s) exact mass, isotopic profile and intensity data for every component in each sample. In order to rationalise the results and identify compounds of significance to the food industry, multivariate statistical approaches including principal components analysis (PCA) and orthogonal partial least squares discriminate analysis (OPLS-DA) are commonly used approaches to elucidate population variations and interpret these complicated data sets.
In this study, we report the potential of a new data independent acquisition (DIA) mode on a QTof instrument in combination with a scanning quadrupole mass filter and ultra fast detection system. This methodology alongside ion mobility enabled QTof-MS (IM-QTof-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step. The resulting information was further subject to database searching which indicates the presence of several significant polyphenolic compounds and processing additives in a selection of commercially available processed juice products in the U.K.

Keywords: *metabolomics, LC-MS/MS, pomegranate, nutrition, food quality*

Acknowledgement: *Mena P., et al "Rapid and Comprehensive Evaluation of (poly)phenolic compounds in Pomegranate (Punica granatum L.) Juice by UHPLC-MS. Molecules 2012, 17, 14821-14840.*

N10
MSCOMPARE: AN UNTARGETED GC/MS METABOLOMICS PLATFORM FOR QUALITY CONTROL, PRECISE DECONVOLUTION AND DATA ANALYSIS

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A GC/MS workflow for Metabolomics includes a number of distinct steps:
Experimental Design, Sampling, Sample Preparation, Data Analysis, Identification and Data Interpretation. The MsCompare platform includes all tools to properly control each step in this workflow.
One of the key issues in this field is the precise and sensitive detection of all components present in a series of samples. GC/MS deconvolution remains by far the most difficult step for low level components, especially when highly similar co-eluting or nearly co-eluting compounds are present. In these cases, precise GC/MS Peak Detection and Deconvolution is necessary with minimal user interference. Proper deconvolution also allows for correct identification of all components.
Another problematic area in GC/MS Metabolomics studies might be the proper alignment of chromatograms before the actual data processing starts. Depending on the application, we often see individual components in GC/MS having bad peaks shapes or bad reproducibility regarding retention times. MsCompare contains a number of alignment algorithms to correct for this behavior. Data analysis in MsCompare comprises both Univariate and Multivariate analysis methods like PCA, PLS-DA, Clustering etc. However, it will be shown that for many cases, due to the high selectivity of GC/MS, univariate analysis methods are adequate in solving the main questions.

Examples from a number of different studies (small and large) will be given, showing an overview of the workflow and implemented tools.

Keywords: *metabolomics, GC/MS, data analysis, comparative analysis, multivariate statistics*

Acknowledgement: *C.C.M. van der Kroft, University of Utrecht*

N11
NEW U-HPLC–HRMS METHOD OF METABOLOMIC FINGERPRINTING FOR DIFFERENTIATION OF CANNABIS SATIVA VARIETIES

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Cannabis sativa plants are known to produce a broad spectrum of biologically active secondary metabolites. So far, more than 500 natural compounds belonging to terpenes, aldehydes, ketones, other hydrocarbons, nitrogenous substances, phenols, flavonoids, fatty acids and others have been discovered. Approximately 150 of these substances are specific to *Cannabis* plants and are known as phytocannabinoids. Based on the hypothesis that representation of such metabolites is characteristic for particular *Cannabis* varieties, we aimed to develop a fingerprinting method able to differentiate between various *Cannabis* plant species. Within the presented study, optimization of non-target screening using separation and detection by ultra-performance liquid chromatography coupled with high resolution tandem mass spectrometry (U-HPLC–HRMS) with quadrupole-time-of-flight mass analyzers was realized. The effort was devoted mainly to optimization of the extraction step, which is critical for non-discriminatory isolation of a wide range of present bioactive substances. As the most suitable extraction solvents, a mixture of ethanol:water (80:20, *v/v*) for isolation of polar substances, and a mixture of dichloromethane:methanol (50:50, *v/v*) for extraction of less polar part of the metabolome, have been shown. Further, the U-HPLC conditions were also optimized in order to obtain effective separation of metabolites potentially influencing the study results. Two different columns (BEH C18, 100 x 2.1 mm, 1.7 µm and HSS T3, 100 x 2.1 mm, 1.8 µm), mobile phases and gradients were tested for analysis of both extracts. The optimized method was further applied for analysis of five varieties of *Cannabis sativa* plants grown under different cultivation conditions (128 samples in total). The acquired data were processed by utilizing the methods of multivariate analysis (principal component analysis, PCA, together with partial least square discriminant analysis, PLS-DA), and the obtained models were validated. The best classification was obtained for following *Cannabis* variety groups: (i) Santhica, (ii) Finola + Bialobrzeskie, and (iii) Carmagnola + Uniko B, with 100% recognition ability (R2) and 98% prediction ability (Q2). In a separate model for differentiation of Finola and Bialobrzeskie varieties, both R2 and Q2 were 100%. R2 and Q2 for model separating Carmagnola and Uniko B were 100 and 94%, respectively. The markers responsible for species classification belonged mostly to the cannabinoids group.

Keywords: *Cannabis sativa, U-HPLC–HRMS, metabolomics, multivariate analysis*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015).*

N12
TARGET AND NON-TARGET ANALYSIS OF (NEURO)PEPTIDES IN HONEY BEES TO PROVIDE INSIGHTS INTO THE EFFECTS OF PESTICIDE EXPOSURE

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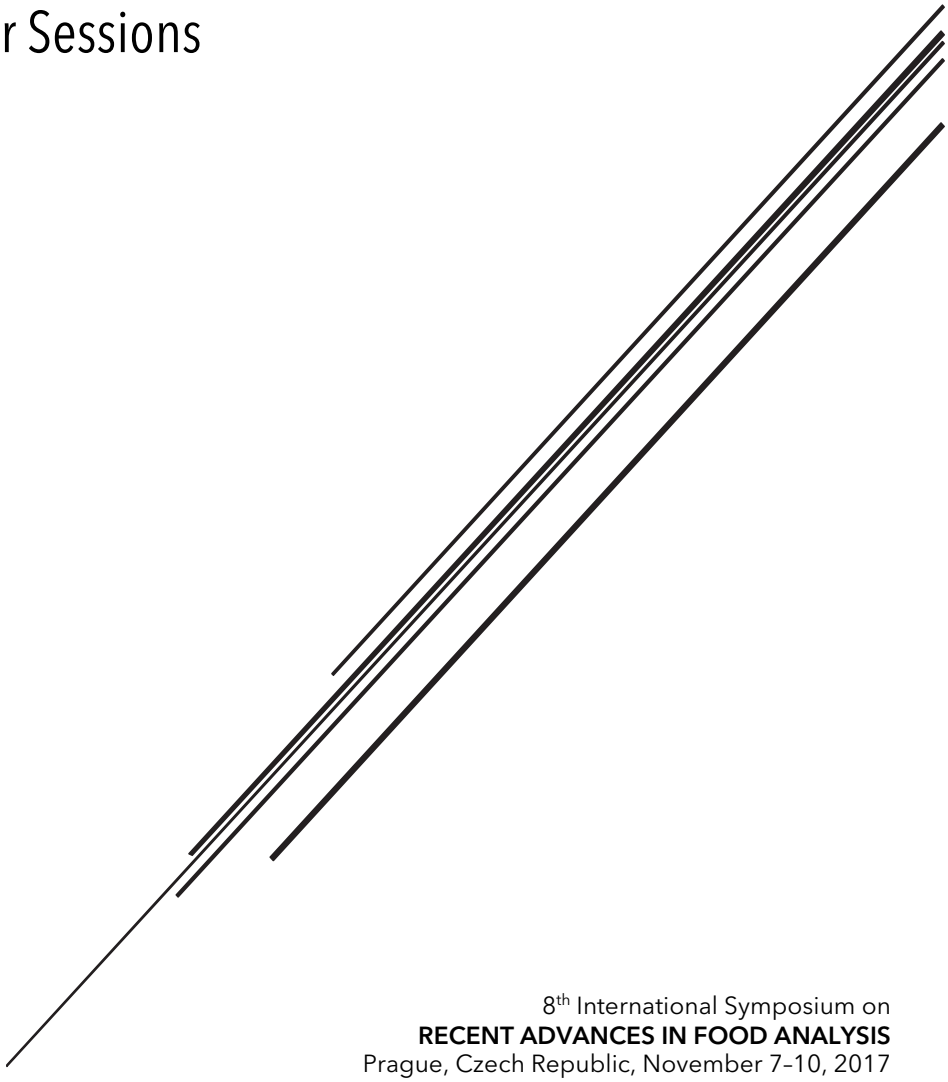
The European honey bee, *Apis mellifera*, is an important pollinator of agricultural crops. During last years, declines in bee colonies are being registered as much in Europe as in other parts of the world. Pesticides that target cholinergic neurotransmission and their extensive use, has been correlated to the decline of bees and other pollinators. It has been shown that pesticides and other environmental contaminants act as neuroendocrine disruptors capable of acting as agonist/antagonist or modulators of the metabolism of neuropeptides. Neuropeptides are vital signaling molecules, playing a critical role in most major physiological and behavioral processes in animal species such as reproduction, feeding, growth, dealing with stress, circadian rhythms and social interaction. Even though the mode of action of neuropeptides in insects has been vigorously studied, the physiological and behavioral functions of most neuropeptides remain largely unknown. In this study, we present an analytical workflow to evaluate pesticide effects on honey bees through the analysis of (neuro)peptides. The workflow consists of a rapid and simple extraction method, liquid chromatography with triple quadrupole, high resolution mass spectrometry, multivariate analysis and automatic identification of discriminating peptides using specific software and protein sequence databases. The analytical method was applied to the analysis of target and non-target (neuro)peptides in honey bees with low and high exposure to a wide range of pesticides in field conditions. Our findings show that the detection frequency of most target neuropeptides decreases significantly in honey bees exposed to high concentration of pesticides (pesticide concentrations ≥ 500 µg kg-1) in comparison with the honey bees with low content of pesticides (pesticide concentrations ≤ 20 µg kg-1). Moreover, the principal component in non-target analysis shows a clear distinction between peptide content in honey bee exposed to high level of pesticides and honey bees with a low level of exposure. In addition, the use of high resolution mass spectrometry has allowed the identification of 25 non-redundant peptides responsible of discrimination between the two groups, derived from 18 precursor proteins.

Keywords: *Neuropeptides, Pesticides, Field conditions, Honey bees, High Resolution Mass Spectrometry*

Acknowledgement: *The authors acknowledge funding support from the National Plan for Scientific and Technical Research and Innovation 2013–2016 and the National Institute for Agricultural and Food Research and Technology–INIA, Ref. Project RTA2013-00042-C10-01. Dr María José Gómez Ramos acknowledges funding obtained from the Spanish Government for a Ramón y Cajal Research Fellowship (RYC-2015-17959). The authors also would like to thank Zac Anderson and Dan Maloney from Bioinformatics Solutions Inc. for providing technical support of PEAKS Studio software.*

PROCESSING CONTAMINANTS

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

PROCESSING CONTAMINANTS

O1
POLYCYCLIC AROMATIC HYDROCARBONS SURVEY IN TEAS USING QUECHERS SAMPLE PREPARATION AND HPLC-FLD

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Polycyclic aromatic hydrocarbons (PAHs) are considered food processing contaminants and are formed, due to incomplete combustion of organic materials. Some PAHs are considered to be carcinogenic and genotoxic. Tea is one of the oldest beverages in the world, however due to its drying process stage it may be contaminated by PAHs. The aim of the present study was to validate an analytical method involving QuEChERS for determination of four PAHs (PAH4) in teas and teas infusions and evaluate the contamination levels in 10 different types of teas from Brazil. Analytical method involved a modified QuEChERS sample preparation using ethyl acetate for extraction and clean-up with PSA and silica gel and determination by HPLC-FLD. Recoveries varied from 54 to 99%, with RSDs from 1 to 21% (repeatability) and 4 to 9% (reproducibility). LODs and LOQs were from 0.2 to 0.3 µg/kg (leaves), and 0.03 to 0.05 µg/L (infusion); and 0.1 µg/L (infusion) to 0.5 µg/kg (leaves), respectively. Mate tea presented the highest PAH levels, with PAH4 varying from 193.60 to 1794.84 µg/kg; followed by black tea (1.81 to 186.35 µg/kg), white tea (23.80 to 119.05 µg/kg) and green tea 3.06 to 91.76 µg/kg. Teas with lowest PAH4 content were: strawberry (0.74-5.07 µg/kg), lemongrass (not detected-9.53 µg/kg), peppermint (2.91-9.59 µg/kg) and boldo (2.82-9.63 µg/kg). A large variation in PAHs levels between different brands and between different batches of a same brand was noticed. As for tea infusions, only trace levels of PAHs were detected (below LOQ), so apparently it would not affect PAHs intake by Brazilian population.

Keywords: *polycyclic aromatic hydrocarbons, QuEChERS, tea*

Acknowledgement: *This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) under Grant 2012/19142-7. R.M.R. (Instituto de Química, UNICAMP, Campinas, SP, Brazil) thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (444146/2014-8 and PIBIC/CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo - Fapesp (2011/10966-4).*

O2
QUANTITATIVE DETERMINATION OF ACRYLAMIDE IN FOOD USING 1D- AND 2D-LC-ESI-MS/MS

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Since 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, Eurofins WEJ Contaminants has been offered the analysis of this substance for customers from the very beginning [1]. 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 [2]. 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 [3]. In 2004, Eurofins WEJ Contaminants had published the development of two different sample preparation methods for the extraction of acrylamide from various food stuffs [4]. Since this time the apparatus-assisted analysis (UHPLC, MS) has developed strongly. Due to improved device performance to 2D-LC approaches the laboratory-side sample processing has now been reduced to a minimum. With the aid of these new opportunities a simple and uniform sample preparation procedure for various food stuffs like French fries, potato crisps and cookies, but also "difficult matrices" like roasted and instant coffee, molasses as well as cacao and yeast, could be successfully developed and validated. While a classical 1D-LC-ESI-MS/MS method is sufficient for the first-mentioned matrices, the difficult matrices can be measured now using the described 2D-approach. For this powerful 2D method, two polar modified stationary RP-phases were used with orthogonal selectivity. After initial separation on the first column a "heart cut" at the retention time of acrylamide is made. The small acrylamide-containing volume of this heart cut is then further separated over a second LC column with different selectivity. With this technique, a minimum of ionic suppression and thus a lower limit of quantification (5 instead of 30 µg/kg) could be achieved.

[1] Tareke, E. et al.: Analysis of Acrylamide, a Carcinogen Formed in Heated Foodstuffs; J Agric Food Chem, 50 (17), 2002.
[2] European Commission; Commission Recommendation on investigations into the levels of acrylamide in food, (2013/647/EU), vom 08.11.2013.
[3] Statement No. 043/2011 Federal Institute for risk assessment (BfR), 29.06.2011.
[4] Hoenicke, K. et al.: Analysis of acrylamide in different foodstuffs using liquid chromatography– tandem mass spectrometry and gas chromatography–tandem mass spectrometry; Anal Chim Acta, 520 (1-2), 2004.

Keywords: *acrylamide*

O3
DIFFERENT STRATEGIES FOR INDIRECT DETERMINATION OF CHLOROPROPANOL ESTERS BY GC-MS IN PROCESSED FOOD

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During the processing of foods at high temperatures, potentially toxic compounds, such as fatty acid diesters of 3-monochloropropane-1,2-diol (3-MCPD) are generated. This contaminant decreases the nutritional value of the food, having a negative impact on the health of consumers and are a constant concern of the FDA and EFSA. The presence of esters is considered a possible source of chloropropanols that can be released into the gastrointestinal tract during digestion by lipase catalyzed hydrolysis reaction. They have become important with their significant appearance in foods, particularly refined vegetable oils, in recent years.
A requirement of international and national authorities involved in food safety and the prevention of risks to human health is the development and validation of sensitive, accurate and economical analytical methods that can determine the contaminants of food processing in different food matrices.
The availability of direct and indirect methods is broad but also confusing and tedious and requires study to advance in the improvement of these methodologies. Indirect methods are based on GC-MS determination of free 3-MCPD and requires a hydrolysis step in which the 3-MCPD esters generate the free form. In addition to hydrolysis the 3-MCPD requires a derivatization reaction, due to its high polarity and low volatility. The hydrolysis may be carried out in basic medium using CH_3ONa or in acid medium with sulfuric acid and using enzymes which simulate human digestion.
The aim of this study is to develop and optimize the factors affecting the sample preparation for indirect determination of 3-MCPD diesters, and particularly to compare the obtained results from different hydrolisis approaches combining with ultrasound assisted-dispersive liquid-liquid microextraction (DLLME)[1]. UA-DLLME involved derivatization with HFBI and purification in one step.

[1] P. Gonzalez-Siso, R.A. Lorenzo, M. Regenjo, P. Fernández, A.M. Carro J. Sep. Sci. 2015, 38, 3428–3434.

Keywords: *3-MCPD diesters, indirect method, hydrolysis, DLLME*

Acknowledgement: *This research was supported by the Project AGL2014-53647-R Plan Estatal de Investigación Científica y Técnica y de Innovación 2013–2016 and FEDER.*

O4
VALIDATION OF A SALTING-OUT ASSISTED LIQUID-LIQUID EXTRACTION METHOD FOR ANALYSIS OF 3-MCPD DIESTERS

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Food processing induces the formation of undesirable compounds such as 3-MCPD. Fatty acid esters of 3-MCPD are food process contaminants that can be found in refined oils. Since fatty acid esters could release non-bound 3-MCPD, compound with proved genotoxic and carcinogenic activity, these compounds have been classified as "possibly carcinogenic for human" (Category 2B) and a TDI of 0.8 µg/kg have been stablished [1,2].
The salting-out has been applied in sample preparation processes to application of the salting-out process is in the separation of water-miscible organic solvents inducing the formation of a biphasic system [3]. The fat matrix of the samples conditions the type of solvent used and requires the purification of the extracts obtained. Here, a sample preparation procedure based on salting-out assisted liquid-liquid extraction (SALLE) with two dispersive solid phase extraction (d-SPE) clean-up steps is presented. The main challenge has been achieved a selectivity enough for simultaneous isolation of seven 3-MCPD fatty acid diesters from oil and oil-based foodstuff. Several parameters, such as organic solvents mixtures, ammonium salts, and sorbents such as PSA, Strong Anion Xchange Silica (Si-SAX), and Z-Sep$^+$ were tested and optimal conditions were obtained using experimental designs. The sample preparation procedure followed by HPLC-MS/MS with HESI-II source in positive mode, was validated by FDA guidelines [4].

[1] IARC, I. A. (2013). 3-monochloro-1,2-propanediol. Lion, France: IARC Monographs-101.
[2] EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2016. Scientific opinion on the risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. EFSA Journal 14 (2016) 4426.
[3] Inês Maria Valente, Luís Moreira Goncalves, José António Rodrigues. J. Chromatogr. A, 1308 (2013) 58– 62.
[4] US Department of Health and Human Services. (2001). FDA Guidance for Industry, Bioanalytical Method Validation. http://www.fda.gov/cder/guidance/index.htm. Available, July 27, 2017.

Keywords: *diesters chloropropanols, salle, d-SPE, edible oils*

Acknowledgement: *The authors would like to thank the Spanish Ministry of Science and Innovation (Project AGL-2014-53647-R) and FEDER, for their financial support.*

O5
OCURRENCE OF 3-MCPD DIESTERS IN EDIBLE OILS AVAILABLE ON SPANISH MARKET

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Monochloropropanediol (MCPD) and fatty acid of MCPD esters are heat-induced food contaminants formed mostly during processing of oils and foods with high fat content. 3-Monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant classified in the group 2B as a possible human carcinogen by the International Agency for Research on Cancer (IARC), for which recently the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) of 0.8 µg/kg body weight [1].
In this study, a Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) method for the direct determination of seven 3-monochloropropanediol diesters (MCPDEs) is developed and validated. The analytes extraction is based on a partitioned liquid-liquid extraction (PLLE) and dispersive solid phase extraction (d-SPE) clean-up. The method showed excellent linearity (R^2>0.9960) for target compounds. The recoveries were over the range of 71-123% with a relative standard deviation (RSD) lower than 13 %. Based on the established method, the levels of MCPD diesters found in a variety of edible oils and margarines were evaluated and reported. A total of eighty-three samples of edible oils and twelve samples of margarines were evaluated, analyzing different oils categories: Olive, soy and sesame, avocado, coconut and palm, sunflower and corn, seed, other oils and margarines. The six samples of extra virgin olive oil (not refined) did not contain detectable levels of MCPDEs. Two different brands of sesame oil showed the highest concentrations of 1,2-dioleoyl-3MCPD (OLOL) and 1-oleoyl-2-steareoyl-3MCPD (OLST), which were of the order of 7.3 and 7.1 µg/g, respectively. Also a sample of olive oil from a canned tuna showed high concentrations of OLOL, OLST and 1,2-dilinoleoyl-3MCPD (LILI). The levels of 3-MCPD from diesters in margarines ranged between 0.27 and 2.50 µg/g.

[1] European Food Safety Authority (EFSA). (2016). Risk for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. EFSA Journal, 15(5), 4426.

Keywords: *3-MCPD diesters, oils, margarines, exposure, food contaminants*

Acknowledgement: *The authors would like to thank the Spanish Ministry of Science and Innovation (Project AGL-2014-53647-R) and FEDER, for their financial support.*

O6
AUTOMATED DETERMINATION OF 3-MCPD ESTERS AND GLYCIDYL ESTERS IN FOODSTUFFS WITH GC-MS/MS

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3-monochloropropanediol fatty acid esters (3-MCPD esters) and glycidyl fatty acid esters as well as free MCPD belong to the process contaminants. Free compounds are formed during the manufacturing process of fatty and salt-containing foodstuffs at high temperatures (for example soy sauce, toast, pastry and smoked foods). The esterbound compounds are formed during the deodorization step of fat and oil refining. Due to their formation during the processing of foodstuffs and their toxicological properties, these compounds are referred to as "foodborne toxicants". A permanently increased uptake of 3-MCPD is toxic for renal and testicles. In addition, glycidol is described as genotoxic. Free 3-MCPD and glycidol are classified by the IARC (International Agency for Research on Cancer) to be "potentially carcinogenic to humans". In 2016, the EFSA (European Food Safety Authority) found that 3-MCPD fatty acid esters in the human organism are almost completely split into free 3-MCPD by digestion enzymes. Hence, the toxicological considerations refer exclusively to the free compounds. Due to the toxicological properties of free and bound 3-MCPD, the EFSA 2016 caused the TDI (Tolerable Daily Intake) to be reduced from 2 µg to 0.8 µg per kilogram of body weight. The JECFA (Joint FAO / WHO Expert Committee on Food Additives) proposed a contradictory risk assessment of 3-MCPD in 2016 the same year. According to the JECFA, the group TDI (sum of free and bound 3-MCPD) has to be raised up to 4 µg per kilogram body weight. A final decision on the TDI is still pending. Regarding the maximum levels in foodstuffs and food additives, limit values for free 3-MCPD have been determined for soy sauce and hydrolyzed plant protein in Regulation (EC) No 1881/2006. In addition, Regulation (EU) No 231/2012 specifies a limit value for glycerol as an additive. In general, the ALARA principle (as low as reasonably achievable) applies.
This work describes the fully automated determination of 3-MCPD esters and glycidyl esters in fats and oils as well as in fatty foods. The analysis is based on the ISO 18363-1 (AOCS Cd 29c-13, DGF C-VI 18 (10)). The automation enables the complete sample preparation of fats and oils. Only the fat extraction of the fatty foods must be carried out manually. The sample preparation, from the dissolution of the sample to the derivatization, is automated by means of a PAL system. The combination of the PAL system with a GC-MS/MS enables gas chromatographic separation by overlapping detection immediately after sample preparation. In addition, a maximum sample throughput is achieved. While one sample is analyzed with GC-MS/MS, a new sample is already prepared. Automated determination is characterized by high precision, correctness and robustness, thus offering a reliable and fast process.

Keywords: *3-MCPD esters, glycidyl esters, foodborne toxicants, processing contaminants*

O7
DETERMINATION OF 3-MCPD, 2-MCPD AND GLYCIDYL FATTY ACID ESTERS IN OLIVE OIL AND WAFFLE USING GC-MS/MS METHOD

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Fatty acid esters of 3-monochloropropanediol (3-MCPDEs), of 2-monochloropropanediol (2-MCPDEs) and of glycidol (GEs) are formed during the refining of edible fats and oils. A selective analytical method for the determination and quantification of 3-MCPD, 2-MCPD and glycidyl fatty acid esters in olive oil and waffle was developed. The indirect gas chromatography-tandem mass spectrometry (GC-MS/MS) method is based on the Standard Operating Procedure (SOP) by EC-JRC-IRMM and it is suitable for olive oil and waffle products.
Due to the different sample matrices the waffle samples were first freeze-dried and extracted using the pressurised liquid extraction (PLE), whereas the olive oil samples were used as such. Isotope labelled standards were added and glycidyl esters were converted into 3-monobromopropanediol (3-MBPD) monoesters in a sodium bromide solution. Thereafter, the ester-bound analytes were transesterified and defatted and the released free MCPD and MBPD were extracted and derivatised with phenylboronic acid (PBA) before the GC-MS/MS analysis.
The validation was carried out in two concentration levels (470 µg/kg and 941 µg/kg) during three different days. Each day three replicate samples per level were prepared. The advantages of GC-MS/MS compared to other detection methods are noteworthy in terms of sensitivity, selectivity and interpretation. The calibration curves showed a good linearity for all analytes within the concentration range. Specificity and selectivity were evaluated based on blank samples (n=12) and no interfering peaks were detected in chromatograms. Repeatability, internal reproducibility and recovery were fit for purpose. Measurement uncertainty was calculated based on the validation results.
According to Commission Recommendation 2014/661/EU the limit of quantification (LOQ) should not be higher than 100 µg/kg for the analysis of MCPD and glycidyl bound to fatty acid esters in edible oils and fats. For other foods which include more than 10% fat, the LOQ should not be higher than the fat content of the food. The calculated LOQs were below the set level. The trueness was evaluated utilising the proficiency test material by EU-RL.
The development of this method is a topical task since there is only limited information available on the determination of MCPD esters and GEs in processed food.

O8
TRENDS IN ACRYLAMIDE CONTENT IN SELECTED FRENCH FRIES, POTATO CHIPS AND CEREALS ON THE CANADIAN MARKET

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Concentrations of acrylamide were measured in four brands/products of potato chips, three breakfast cereals and French fries from three national fast food chains. The same brands/products and outlet locations were sampled in the past (2004-2010), allowing for a temporal comparison. All of the products were purchased in a single city, Ottawa, Ontario, Canada. Samples were analyzed by an isotope dilution ($^{13}C_3$) acrylamide method. They were extracted with water, partitioned with dichloromethane, filtered through a 5kDa centrifuge filter, cleaned-up on HLB Oasis polymeric and Accucat mixed mode anion and cation exchange SPE columns and analysed by LC-MS/MS.
Acrylamide concentrations in potato chips varied from 152 to 1010 ng/g, in cereals from 24 to 426 ng/g and in French fries from 67 to 842 ng/g. Wide variations were observed between brands, between lots of the same brand, between different national chains and in some instances between different outlets belonging to the same fast food store chain. The trends over 10 years indicate that there may have been some active mitigation efforts for certain potato chip products on the Canadian market. Amongst three cereals monitored, rice cereal variety also showed a trend towards lower acrylamide concentrations over time.
The exact trends in acrylamide concentration in French fries from fast food outlets are more difficult to assess due to the limited number of data points. However, it seems that over the last 5 years, at least one chain may have implemented substantial mitigation measures. The other inference which could be obtained from the data is a large degree of variability in producing fries with consistent acrylamide concentration between different outlets belonging to the same store chain.

PROCESSING CONTAMINANTS

O9
3-MCPD, 2-MCPD AND GLYCIDYL ESTERS IN EXTRA VIRGIN OLIVE OIL

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Esters of 3-monochloropropane-1,2-diol (3-MCPDE), 2-monochloropropane-1,3-diol (2-MCPDE) and glycidyl (GE) have received considerable attention due to their wide occurrence in food and the fact that they are potentially toxic contaminants. The International Agency for Research on Cancer (IARC) classified the 3-MCPD as a possible human carcinogen and glycidol as a probable human carcinogen. These esters are formed mainly during the processing of vegetable oils under high temperatures in the refining process. Since processing of extra virgin oil does not require the use of high temperatures, significant amounts of 3-MCPDE, 2-MCPDE and GE are not expected, unless the product is adulterated with the addition of a refined vegetable oil. Therefore, levels of 3-MCPDE, 2-MCPDE and GE were determined in extra virgin oils available on the Brazilian market (n=25) using the official AOCS method (Cd 29a-13). The method is based on the conversion of GE to 3-monobromo-1,2-propanediol esters (3-MBPDE). Then, 3-MBPDE, 3-MCPDE and 2-MCPDE are converted in their free forms by a transesterification reaction, compounds are later derivatized and analyzed by gas chromatography-mass spectrometry. The method performance verification was done according to the guidelines of the Brazilian National Institute of Metrology, Quality and Technology (INMETRO). For analytical quality control, a certified reference material of vegetable oil was used. The method presented good linearity for 3-MCPDE (0 – 8.8 mg/kg; r^2=0.993), 2-MCPDE (0 – 9.1 mg/kg; r^2=0.996) and GE (0 – 20.9 mg/kg; r^2=0.998). Limits of detection and quantitation were respectively 0.01 and 0.03 mg/kg (3-MCPDE) and 0.02 and 0.08 mg/kg (2-MCPDE e GE). Recoveries and precision were 91.8% and 4.4% for 3-MCPDE, 101.4% and 2.7% for 2-MCPDE, 104.4% and 6.3% for GE. Concentrations of analyzed samples ranged from "not detected" to 1.16 mg/kg, "not detected" to 0.58 mg/kg and "not detected" to 1.98 mg/kg for 3-MCPDE, 2-MCPDE and GE, respectively. The contaminants were detected in 14 samples out of 25 samples of extra virgin olive oils evaluated. Therefore, the presence of these contaminants may be an indicative of adulteration in olive oils commercialized as extra virgin, which can contribute as a risk to consumer health in view of the toxicity associated with 3-MCPDE, 2-MCPDE and GE.

Keywords: *3-MCPD esters, 2-MCPD esters, glycidyl esters, extra virgin olive oil*

O10
PROFILE OF BIOGENIC AMINES IN SERBIAN FERMENTED MEAT PRODUCTS

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Biogenic amines (BAs) arise as a consequence of microbial activity in food. Fermented foods, including meat products, are of particular concern regarding formation of BAs, which are often used as chemical indicators of products´ hygienic quality. Regarding biological activity of BAs, histamine and tyramine are considered as the most toxic and food safety relevant due to their effects on cardiovascular and neurological systems. Based on limited information, no adverse health effects were observed in healthy individuals after exposure to 50 mg of histamine or 600 mg of tyramine in one or daily dose. For other BAs, available information was not sufficient for hazard characterization.

In this work, a group of 50 samples of fermented meat products representing market in the City of Novi Sad (Serbia) at the beginning of 2017 was analysed with respect to the occurrence of tryptamine (TRY), phenylethylamine (PEA), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), spermidine (SPD), and spermine (SPM), using a method based on perchloric acid exctraction, derivatization with dansyl chloride and HPLC separation with UV detection.

All eight BAs were present in all analysed fermented meat products, except PEA (90%) and TRY (20%). TYR was the most abundant BA in half of the samples, corresponding to about 50% of total BAs in one fifth of the samples. It was followed by PUT - dominant BA in 20% of the samples, amounted for more than 30% of total BAs in one fifth of the samples. TYR amount ranged from 0.5-412.3 mg/kg, PUT 1.0-273.0 mg/kg, CAD from 0.2-409.4 mg/kg. Considering the mean levels of individual BAs, the major BA was TYR, followed by PUT and CAD: 73.0, 55.8 and 40.6 mg/kg, respectively. HIS was detected in all the samples, in the range from 1.4-118.8 mg/kg, with mean at 15.6 mg/kg. SPM and SPD showed the narrowest concentration ranges: 0.2-43.7 and 0.2-58.8 mg/kg, respectively. Total BA content ranged from 37.3-1185.9 mg/kg, with mean at 238.2 mg/kg. Comparison of two groups of the samples, formed according to the production type – family manufactures (19 samples, sold on green markets), and industrial production (31), has not revealed significant differences regarding mean content of total or individual BAs.

Daily portion of 50 g of three fermented sausages with the highest HIS levels would cause HIS intake of 11.9, 7.7 and 5.2% of HIS threshold dose. However, mere presence of HIS could pose risk for individuals with histamine intolerance. Regarding TYR, threshold dose of 600 mg/day was far above the intakes that could be expected through consumption of analysed products – 50 g of the one with the highest TYR level would reach 3.4%. If consumed by a person taking MAOI drugs, TYR quantity found in the most contaminated sausage would reach 41% of threshold level of 50 mg/day – not to be neglected taking into account possible BAs contribution from other food sources.

Keywords: *biogenic amines, fermented meat*

O11
DETERMINATION OF ACRYLAMIDE IN TRADITIONAL, INDUSTRIAL AND SEMI-INDUSTRIAL BREADS USING A NOVEL EXTRACTION AND CLEAN UP METHOD BY LC-MS/MS AND ESTIMATION OF ITS DIETARY INTAKE

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Acrylamide is a chemical compound that has been classified by the International Agency for Research on Cancer (IARC) as Group 2A 'probably carcinogenic for humans'. Since bread is one of the primary foods of the Iranian people, the determination of acrylamide content in bread might be considered important. A very sensitive, rapid, easy, cheap and safe extraction and clean up method was used for determination of Acrylamide in 86 bread samples using LC-MS/MS. In order to overcome on matrix effect for drawing of the calibration curve, spiked samples were used in different levels. Sample preparation includes extraction of Acrylamide into methanol followed by cleanup with dispersive solid phase extraction. The limit of detection and limit of quantification were 0.3 ng/g and 1 ng/g, respectively. The values for recoveries and RSD were calculated as 96.01-105.34% and <10.8% respectively. About 94 % of all samples were contaminated with Acrylamide at the amount higher than LOQ (1 ng/g). The average concentration of Acrylamide in Sangak bread samples (sum of traditional and semi-industrial Sangak bread samples) of Shiraz and Tehran (traditional Sangak bread samples) was 45.95 and 21.52 ng/g, respectively. Average Acrylamide concentration in industrial bread samples was 8.02 ng/g.
The result showed that industrial bread samples had lower contamination with Acrylamide than traditional bread samples. Consideration the high consumption bread of Iran from one hand and contamination of Sangak bread with Acrylamide of the other hand, a comprehensive survey concerning Acrylamide contamination in traditional bread is necessary.

O12
ANTHRAQUINONE IN SMOKED MEAT PRODUCTS

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Anthraquinone belongs to the group of oxidized polycyclic aromatic hydrocarbons. In the past it was used for the production of cellulose fibers. In the year 2013 anthraquinone was removed from the list of recommendations for food packaging [1]. In the European Union anthraquinone was banned in 2014, and therefore a maximum residue level (MRL) had to be established for this substance. Furthermore, anthraquinone is formed by the incomplete combustion of organic material [2]. Anthraquinone acts as a carcinogen in the kidney and the liver of mice and rats. The International Agency for Research on Cancer (IARC) has classified this substance as a possible carcinogenic substance to humans (group 2B). Moreover, anthraquinone has direct mutagenic potential and is cytotoxic [3].
In the past, anthraquinone was not studied extensively in food. For this reason, no analytical method has existed for the determination of the contents of anthraquinone in fatty matrices until now. Based on a method for the analysis of polycyclic aromatic hydrocarbons (PAH) in smoked meat products [4], a new analytical method for the quantification of anthraquinone, anthracene, and, in addition, the PAH-4 (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[a]pyrene), has been developed. The extraction of the sausages was carried out using accelerated solvent extraction with n-hexane. The extract was cleaned up by solid phase extraction (SPE). The qualification and the quantification were carried out by gas chromatography coupled with high resolution mass spectrometry.
The analyzed Frankfurter-type sausages (N=7) showed anthraquinone contents between 0.8 and 3.6 µg/kg and, consequently, contents below the MRL of 10 µg/kg [5]. Samples purchased from butcheries (N=4) had slightly higher anthraquinone contents than samples from local supermarkets (N=3). Furthermore, in a first study with smouldering smoke it could be shown that the PAH contents and the content of anthraquinone increase with the duration of the smoke as expected.

[1] BfR opinion No. 005/2013, 12 February 2013; http://www.bfr.bund.de/cm/349/bfr-removes-anthraquinone-from-its-list-of-recommandations-for-food-packaging.pdf
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[5] Commission Regulation (EU) No 1146/2014 of 23 October 2014, Official Journal of the European Union L308/3.

Keywords: *anthraquinone*

O13
NEW METHOD FOR THE EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) FROM EDIBLE OILS USING MOLECULARLY IMPRINTED POLYMERS (MIP)

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Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds with two or more fused aromatic rings. PAHs comprise the largest group of chemical compounds known to be cancer causing agents.
Human beings are exposed to PAHs mostly by intake of food. In addition, they have a relatively low solubility in water, but are highly lipophilic. So edible oils can be an important source of contamination by PAHs. Analysis of oily samples presents an analytical challenge due to the heavy matrix effects often encountered.
In 2011, European Union Commission Regulation No 835/2011 set a maximum limit in various foodstuffs, including edible oils. The limits for oils and fats (excluding cocoa butter and coconut oil) have been set to 2 ng/g of benzo[a]pyrene individually, and 10 ng/g of benzo[a]pyrene, benzo[b]fluoranthene, chrysene and benzo[a]anthracene combined.
This poster shows sample cleanup for the analysis of benzo[a]pyrene, benzo[b]fluoranthene, chrysene and benzo[a]anthracene in edible oil such as olive or colza prior to the analysis by HPLC - Fluorescence. The pretreatment method is based on Solid Phase Extraction (SPE) cartridges based on Molecularly Imprinted Polymer (MIP). Indeed, a MIP is a synthetic material with artificially generated three-dimensional network, able to specifically rebind a target molecule.
Thanks to MIP selectivity, perfect clean-up and good recoveries (>80) were obtained at concentration required by regulation.

Keywords: *polycyclic aromatic hydrocarbons (PAHs), molecularly imprinted polymers (MIP), edible oil, solid phase extraction*

O14
VALIDATION OF A NOVEL (NEW) EXTRACTION AND CLEAN UP METHOD FOR DETERMINATION OF TRACE LEVEL ACRYLAMIDE IN BREAD AND APPLICATION OF THIS METHOD ON REAL BREAD SAMPLES IN IRAN

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Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as 'probably carcinogenic for humans' and recognized by the European Union (EU) Scientific Committee on Food as a genotoxic carcinogen.
Complexity of food matrices and toxicity of this process contaminant require development of some analysis methods, sensitive and selective for proper separation, detection and quantification in food. Sample preparation is an essential step of vital part of such analytical procedure, irrespective of the chromatographic techniques used with the main purpose to purify and remove interfering substances.
New sample preparation procedure was optimized and a liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed for the quantitative analysis of acrylamide in bread. Method is based on sample extraction in methanol, purification with Carrez solutions and clean up with Primary Secondary Amine (PSA).
The developed method offers an efficient, inexpensive, easy sample preparation and very sensitive procedure for the determination of Acrylamide in bread. The method is advantageous in terms of total solvent utilization. The use of spiked calibration curves for constructing the calibration curve substantially reduced adverse matrix-related effects.
Good results were obtained with respect to repeatability (RSDs < 11%). The recoveries between 96% and 105.3%. Limit of Quantification was sensitive enough to detect AA at a concentration of 1 µg/kg.
The developed method was used for determination of Acrylamide in 25 Sangak bread samples collected from Shiraz. These results showed that approximately all Sangak samples were contaminated with Acrylamide. Therefore, a comprehensive survey for monitoring of Acrylamide in Sangakbread samples seems to be needed. This is the first report concerning contamination of Sangak bread samples with acrylamide in Iran.

O15
POLYCYCLIC AROMATIC HYDROCARBONS IN EDIBLE FATS AND OILS SOLD IN THE BRAZILIAN MARKET

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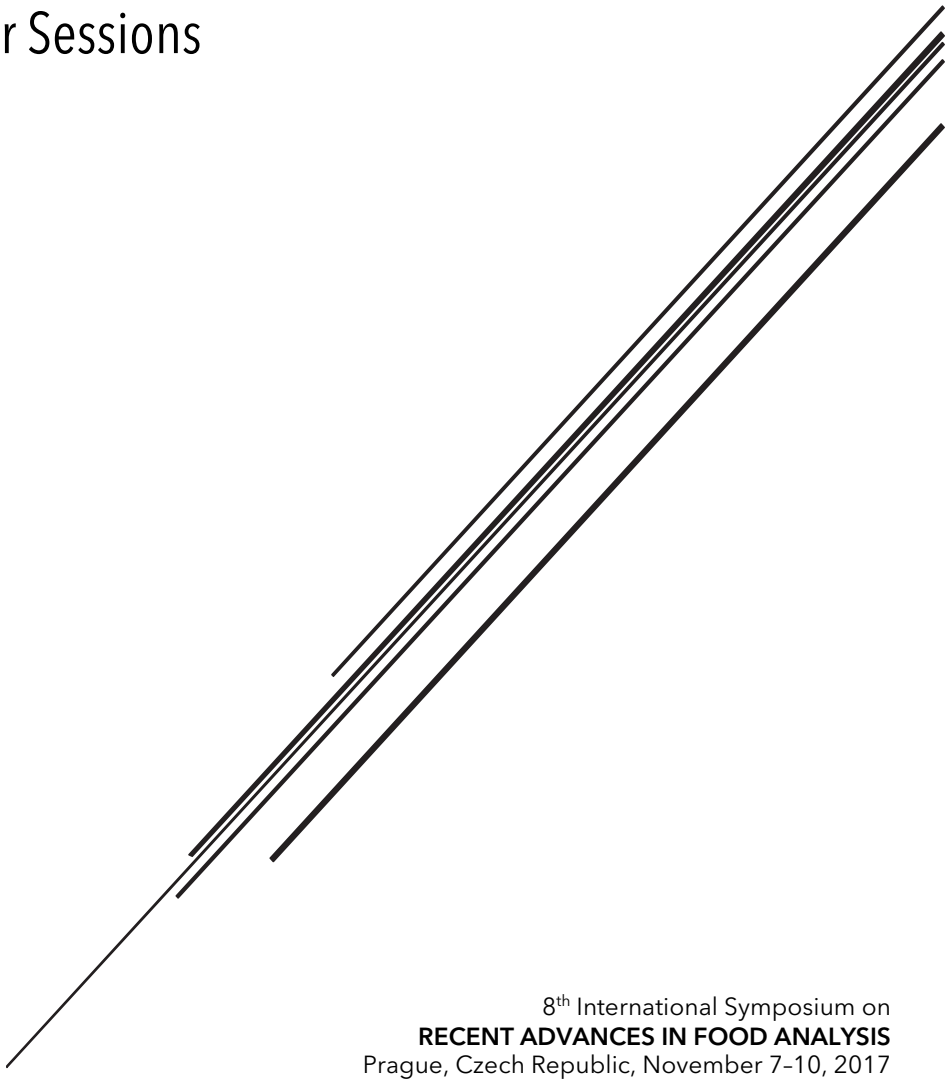
Polycyclic aromatic hydrocarbons (PAHs) are food and environmental contaminants that are formed due to incomplete combustion of organic matter; some are considered to be carcinogenic and genotoxic. Some studies have indicated the category of oils and fats as one of the main sources of PAHs intake in human diet. The presence of 13 PAHs was evaluated in different brands and batches of canola (n=23), sunflower (n=26), corn oils (n=21) and olive oils (n=70), oil blends (n=36) and margarine (n=12) available in the Brazilian market. Analytical method involved extraction with hexane and dimethylformamide-water, clean-up with SPE C18 cartridges and analysis by HPLC with fluorescence detection. Calibration curves were linear. Recoveries and RSDs were, respectively, in the range of 53-117% and 1-29% (margarine), 71-110% and 4-20% (oils), 62-115% and 2-20% (oil blends and olive oil). Limits of detection and quantification were from 0.02 to 0.30 µg/kg and 0.30 to 3.00 µg/kg. Linearity, accuracy, precision, LOD and LOQ were adequate and comply with the European Commission Regulation (EC 333/2007) performance criteria for benzo[*a*]pyrene analytical methods in foodstuffs. In all types of oils and fats PAHs were detected in at least 93% of the samples. Levels of summed 13 PAHs varied from not detected to 41.10 µg/kg for olive oils, 2.59 µg/kg to 85.30 µg/kg for oil blends, 3.21 µg/kg to 9.82 µg/kg for margarine, not detected to 31.70 µg/kg for canola oil, 0.65 to 17.88 µg/kg for sunflower and 2.61 to 38.23 for corn. A high variability in PAHs levels between brands and between different batches of the same brand was observed. Levels of benzo[*a*]pyrene were over the maximum limit established by European regulation (2.0 µg/kg) in 60 of the 188 samples (4 samples of olive oils, 18 oil blends, 2 margarines, 13 canola, 15 corn and 8 sunflower oils). As for PAH4 (sum of benzo[*a*]pyrene, chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene), 58 samples presented levels above permitted (10.0 µg/kg): 7 olive oils, 18 oil blends, 11 canola, 15 corn and 7 sunflower. Results show that vegetable oil is still a product subject to contamination by these potentially carcinogenic compounds, so any action to reduce and control their presence in this type of products should be encouraged. It is recommended that maximum limits for PAHs presence in edible fats and oils are set by Brazilian regulation in order to improve the safety of these products.

Keywords: *polycyclic aromatic hydrocarbons, vegetable oil, fat*

Acknowledgement: *Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (444146/2014-8 and PIBIC/CNPq) and Fundação de Amparo à Pesquisa do Estado de São Paulo - Fapesp (2011/10966-4).*

RESIDUES – PESTICIDES

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
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RESIDUES – PESTICIDES

P1
FOLLOW UP METRIBUZIN AND ITS METABOLISM IN LACTOBACILLUS CASEI 01 AND IN COCKTAIL TOMATO

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Metribuzin is a widely used herbicide approved for vegetables especially in tomatoes, peppers and potato cultures. It is well known, that metribuzin can be intensively metabolized in plants, but the human effect of metabolites is not well established. Because during plant and bacterial detoxification processes sometimes occur biologically accessible pesticide metabolites for animals and humans [1]. The aim of this study was to (i) to examine whether the well-known metabolites are present in fresh consumed tomato and (ii) whether metribuzin can be metabolized by a non-target organism, a *Lactobacillus* species (*L. casei* Shirota), a probiotic strain of the human gastro-intestinal tract. In this study, we aimed to develop a qualitative and quantitative method for measuring metribuzin and its metabolites by LC-QTRAP-MS and LC-TOF-MS. Furthermore, sample preparation method was developed for tomato as well as for *Lactobacillus casei 01* (nutrient broth, cells) which was to propagate in the presence of metribuzin.
In the first part of the experiment, metribuzin and its characteristic metabolites were investigated from cocktail tomato culture. To carry out this experiment a sample preparation method was developed and the sample solution was concentrate and introduce to LC-QTOF-MS and LC-MS/MS systems. Metribuzin was used in two recommended dosage: 2.5 and 5 L/100 ml, after waiting time (7 days) leaves, green tomatoes and ripe tomatoes were collected. With this study we wanted to simulate the household using situation and find the metribuzin metabolites and degradation products in the different parts of the plant. Deaminometribuzin (DA), diketometribuzin (DK) and deaminodiketometribuzin (DADK) [*2]* as well as metribuzin-glucoside was clearly detectable from the green parts of tomato at both doses. Ripe tomatoes also contained these metabolites, but metribuzin-glucoside was only detectable by the use of 5 L/100cm^2 metribuzin dose. This conjugated product of metribuzin could be determined semiquantatively with metribuzin-glucoside synthesized at Budapest University of Technology and Economics (10% efficiency), after further chromatographic enrichment and purification.
In the second part *Lactobacillus casei* cells were inoculated intro MRS broths containing D-glucose and metribuzin. It was incubated in 37°C during 48, 72 and 96 hours. The degradation products of metribuzin were examined and DA, DK, DADK and metribuzin-glucoside were identified in the *Lactobacilus casei* 01 cells after 96 h incubation [3].
The formation of metribuzin-*N*-glucoside by a lactic acid strain hosted in the human gastro-intestinal tract and tomato samples means a more hydrophilic compound compared to metribuzin, therefore the bioavailability and toxicity patterns of metribuzin might be reconsidered.

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Keywords: *cocktail tomato, metribuzin-N-glucoside, Lactobacilus casei 01*

P2
A ROBUST ANALYSIS OF GLYPHOSATE AND OTHER POLAR PESTICIDES IN FOOD AND FEED A CHALLENGE NO MORE

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The direct analyses of Glyphosate and other polar pesticides like Glufosinate, Fosetyl, Erhephon, chlorate and their metabolites with LCMS is well described in the EURL QUPPE-method. But the chromatographic separation is not robust and a lot of maintenance is required. For Glyphosate, AMPA and Glufosinate a known robust method is to derivative these compounds with FMOC. This method however is laborious and one still need a separate method for the other pesticides. At Nofalab a robust method is developed on a SCIEX 6500+ with a Shimadzu UHPLC. Basically, the chromatographic separation is done on a Polyvinyl alcohol with quaternary ammonium groups column at a pH of 9. This ionograchic condition puts the glyphosate in the ideal configuration allowing a stable separation whale the other anions are still well separated. The poster gives an overview of the method is shown as long-term stability, robustness and validation results.
The developed method meets the SANTE requirements regarding the Reproducibility (<20%) and recovery (80-110%). The LOD of the method is below 0.01 mg/kg.

Keywords: *glyphosate, food and feed, LC-MSMS*

Acknowledgement: *Sciex EMEA*

P3
FURTHER IMPROVEMENTS IN PESTICIDE RESIDUE ANALYSIS IN FOOD BY APPLYING GAS CHROMATOGRAPHY TRIPLE QUADRUPOLE MASS SPECTROMETRY (GC-TQ-MS/MS) TECHNOLOGY

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The permanent high demand of consumers for more sensitive and faster controls is driving to the development of improved analytical methodologies that increase the performances of sensitivity, robustness, and reduce the analyses time. This fact is promoting the development of upgraded GC or LC-MS platforms to arise such performances.
This work is focused on the evaluation of a new GC-TQ-MS/MS system, checking its capability in reducing runtime and decreasing the LOQs for pesticide residues in food matrices. The proposed GC-TQ-MS/MS method allowed reducing analysis time by a factor of 2 by modifying the oven temperature program. The optimum method (12.4 min runtime method) was selected based on peak shapes, resolution, and data points per peak. The 12.4 min fast GC runtime method was fully validated for 203 multiclass pesticides in 3 food matrices (tomato, apple, and orange), providing LOQs of 2 $\mu g.kg^{-1}$. Linearity, recoveries, limit of quantitation, sensitivity, matrix effects, intra-day and inter-day precisions, as well as uncertainty measurement fulfilled the requirements of the EU Quality Control Guidelines.
Good linearity was achieved for almost all pesticides in a range of 1 to 200 $\mu g.kg^{-1}$. Satisfactory recoveries results (between 70 and 120%) were obtained for97 % of the compounds. Matrix effect expressed as signal enhancement was resolved by performing matrix-matched calibration for quantitation purposes. Good reproducibility was achieved (RSDs below 10 %) for all analytes. Satisfactory sensitivity results were achieved by reaching a LOQ of 2 $\mu g.kg^{-1}$ for 97% of the compounds. Intra-day and inter-day precisions were below 20 % for all compounds. Overall measurement uncertainty values obtained were lower than 50% for all compounds.
The validated method has confirmed the proposed challenges with adequate robustness by its application to routine analyses for more than 70 real samples. The presented method will be of great benefit for pesticide control as it allows increasing samples throughput, improving the cost/time ratio. Despite the fact that MRLs are usually set in a range of 0.01-10 $mg.kg^{-1}$, detecting lower concentrations can be an important tool for monitoring of organic products, for monitoring the use of banned pesticides in agricultural practices, and for risk assessment.

Keywords: *fast GC-MS/MS analysis, low reporting limits, multiresidue method, method validation*

Acknowledgement: *The authors acknowledge funding support from the European Commission, DG SANTE (Grant decision SI2.726352).*

P4
TARGETED AND UNTARGETED SCREENING OF PESTICIDES AND OTHER CONTAMINANTS IN FOOD MATRICES USING A NOVEL HIGH RESOLUTION GC/Q-TOF

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Due to the large number of potential pesticide residues and metabolites present in food products, fast and reliable analytical methods for the screening, identification and quantification of pesticides in low levels and in a broad range of food matrices are needed. Criteria for the identification of analytes and confirmation of results are defined in the guidance document SANTE/11945/2015 and require the presence of two ions including a fragment for each compound with a good mass accuracy.
In this study, we demonstrate a novel GC/Q-TOF based workflow for targeted, suspect and unknown screening in two example matrices (grape and onion). The samples were analyzed by full scan GC/Q-TOF with a mid-column backflushing configuration and 20 min retention time locked (RTL) method.
The results demonstrate that the combination of high resolution accurate mass GC/Q-TOF and an accurate mass library serve as an effective and robust tool for qualitative and quantitative targeted screening of pesticides. A great majority of spiked pesticides were identified at all five spiking levels with good mass accuracy < 5 ppm. The novel data acquisition system enabled a linear response of those pesticides over a wide concentration range, yielding linear calibration curve fitting coefficient (R^2) of ≥ 0.99 in the matrix matched calibration from 5 to 100 ng/mL. The repeated experiments also yielded stable ratio of fragment ion abundance, with small variations observed. The mid-column backflushing method resulted in good instrument precision, with standard deviation (SD) of retention time ≤ 0.01 min for most identified pesticides obtained for >100 pesticides. The untargeted screening of those food matrices enabled the investigation of other contaminants in the matrices

Keywords: *GC–Q/TOF, high resolution, pesticide screening*

P5
TOTAL DETERMINATION OF RESIDUAL FLUTOLANIL AND ITS METABOLITES IN LIVESTOCK PRODUCTS AND SEAFOOD USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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We have developed a simple and sensitive LC-MS/MS analytical method for the determination of residual flutolanil and its principal metabolites, including α,α,α-trifluoro-3′-hydroxy-*o*-toluanilide (M-4) and its conjugates, in livestock and seafood products. Both flutolanil and its metabolites contain the 2-(trifluoromethyl)benzoic acid (2-TFMBA) moiety. In this method, flutolanil and M-4 are converted to 2-TFMBA by hydrolysis. The method comprises direct hydrolysis with sodium hydroxide at 200°C, acidification, partitioning into a mixture of ethyl acetate/*n*-hexane (1:9, *v/v*), clean-up using a strong anion exchange cartridge (InertSep SAX), and then quantification using LC-MS/MS. The optimal conditions for the complete hydrolysis of flutolanil to 2-TFMBA were an incubation time of 6 hours and a temperature of 200°C. The developed method was evaluated on seven types of food: bovine samples of muscle, fat, liver and milk, as well as eggs, eel and freshwater clams. Samples were spiked both at 0.01 mg/kg and at the Japanese Maximum Residue Limit (MRL) established for each food type. The validation results show excellent recoveries (88-109%) and precision (<15%) for flutolanil and M-4. The limit of quantification (S/N≥10) of the developed method is 0.01 mg/kg. The developed method will be useful for the regulatory monitoring of residual flutolanil and its metabolites in food products.

Keywords: *flutolanil, α,α,α-trifluoro-3′-hydroxy-o-toluanilide, 2-(trifluoromethyl)benzoic acid, LC-MS/MS*

Acknowledgement: *This research was supported by a grant from the Ministry of Health, Labour and Walfare of Japan.*

P6
VALIDATING HIGH RESOLUTION LC-MS/MS ANALYSIS OF PESTICIDES IN BABY FOODS FOR ROUTINE USE

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Infants and children are especially vulnerable to chemical exposures. They are exposed to pesticides relatively higher than adults because their diet is comprised of a higher percentage of fruits and vegetables and their consumption per kilogram/body weight is greater. Furthermore, children may also absorb pesticides more easily because their gastrointestinal tract is still developing.
Commission Directive 2006/125/EC establishes strict MRLs for a set of pesticides (0.003-0.008 mg/kg) in baby food. Bearing in mind the low concentration levels needed for successful monitoring of pesticide residues in infant foods, sensitive, reliable identification and quantification methods are required. In this study, the capabilities of QToF in non-target data independent mode were evaluated for that purpose. Validation of the method in 3 baby food matrices was conducted, and 20 real samples were analysed.
Citrate QuEChERS method without clean-up was used to extract the baby food samples. UHPLC (Exion LC) coupled to a QToF X500R was used for analyzing the samples in sequential window acquisition of all theoretical fragment ion spectra (SWATH acquisition mode). This is a non-targeted data independent mode which works in full scan (FS) and MS^2 simultaneously. The possibility to use a different number of mass windows when acquiring was tested. SWATH acquisition mode with 1, 5, 10, 15 and 20 mass windows was tested. After checking the exact mass accuracy and the ion ratio tolerance for those compounds in matrix, the method using 20 mass windows was selected for validation. Identification criteria used for all the experiments were: mass error in full scan below of 1 mDa, retention time within ±0.1 min, and ion ratio within ± 30% from the reference. For 93% of the compounds, mass error in FS mode were equal or lower than 1 mDa in matrix. The ion ratio was within ± 30% for 94% of the compounds at 0.003 mg/kg.
Full validation of the method was performed for 16 compounds following Directive 2006/125. Recoveries were tested at 2 concentration levels (0.003 and 0.006 mg/kg) in 3 baby food matrices (apple and pear, peach and banana, and fruit salad). In all cases, recoveries ranged from 77-120% with RSDs<13%. Linear range was tested from 0.003 to 0.20 mg/kg. Matrix effects were also checked. Limits of quantification of 0.003 mg/kg were obtained for all the compounds. Twenty real samples were analysed and also a sample from the EUPT-BF01.

Keywords: *high resolution mass spectrometry, baby food, SWATH, LC-QToF-MS/MS*

Acknowledgement: *The authors acknowledge funding support from the European Commission, DG SANTE (Grant decision SI2.726352).*

P7
MULTI-RESIDUE METHOD FOR THE ANALYSIS OF MORE THAN 600 PESTICIDE RESIDUES IN FOOD BY GC-MS/MS AND LC-MS/MS

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To ensure the food safety and compliance, Nestlé Quality Assurance Center Laboratories monitor pesticides in raw materials, processed food of animal & plant origin and finished products. Regarding the regulation, the strictest Maximum Residue Level (MRL) set by authorities is considered for Nestlé methods as a target value. To simplify, align and increase efficiency of pesticides methods across Nestlé Laboratories, new state of the art methods with a larger scope of pesticide/commodity combinations and fast sample preparation have to be provided. To do so, a multi-residue method for the analysis of more than 600 pesticides in cereals, fruits & vegetables and related processed ingredients have been developed and validated based on CEN 15662:2008 and SANTE N°11945/2015 documents.
The protocol was based on a single QuEChERS solvent-based extraction procedure and detection was performed using LC-MS/MS and GC-MS/MS. The LC-MS/MS workstream included four approaches: (a) a positive/negative mode quantification approach to cover 100 pesticides, (b) a positive mode "screening" approach, for the analysis of 352 pesticides (c) a positive mode quantification approach in case of positive findings during the "screening" approach and (d) a quantification approach for 3 polar pesticides: chlormequat, mepiquat and cyromazine. The GC-MS/MS workstream was used to determine 177 apolar pesticides. For both LC-MS/MS and GC-MS/MS quantification was based on 2-level standard addition to the final extract, except for the three polar pesticides where labelled isotopic dilution was used.

Keywords: *food safety and compliance, pesticide residues, QuEChERS extraction*

P8
APPLICATION OF SUPERCRITICAL FLUID CHROMATOGRAPHY FOR PESTICIDE ANALYSIS IN FRUITS AND VEGETABLES

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Supercritical fluid chromatography (SFC) uses carbon dioxide (CO_2) as a component of the mobile phase. The CO_2 when is put through specific conditions of temperature and pressure acts as a solvent. This type of mobile phase provides different separation than combination of water and an organic solvent. A fast analytical method supercritical fluid chromatography coupled to triple quadrupole mass spectrometry was validated to quantify 166 pesticides in three different matrices (tomato, orange and leek). A CO_2 gradient with MeOH as co-solvent was employed. Methanol was used also as make-up solvent (added after the chromatographic column). The duration of the method of analysis was 12 minutes eluting the last compound at the minute 6.8.
A rigorous validation procedure according to SANTE/11945/2015 Guidelines was performed to ensure high quality analytical measurements [1]. The reproducibility, limit of quantification (LOQ) and matrix effect for the 166 analytes in tomato, orange and leek were evaluated. More than 90% of pesticides were identified at 5ppb in all matrices. For 98% of the study pesticides in tomato, 93% in orange and 70% in leek, the suppression was lower than 20%, therefore, the majority of the analytes showed no significant matrix effects.
SFC is an alternative for conventional liquid chromatography applied for the analysis of pesticide residues (organic and aqueous phase). In conclusion it can be stated that the CO_2/MeOH mobile phase has advantages such as absence of water, less harm to the environment, reduced matrix effect and shorter analysis time.

[1] European Commission 2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11945/2015

Keywords: *supercritical fluid, SFC, method validation, pesticides*

Acknowledgement: *The authors acknowledge funding support from the European Commission, DG SANTE (Grant decision SI2.726352). The authors also would like to thank Shimadzu for the instrumentation facilities.*

P9
PESTICIDE RESIDUE ANALYSIS IN CANNABIS USING MODIFIED QUECHERS AND LC-MS/MS

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The legal cannabis industry is exploding in the United States as more states adopt medical and recreational use laws. Cannabis is still federally illegal so the legal cannabis market is managed by local state governments. While systems for growing, production, and sale of cannabis and cannabis related products are somewhat established, regulation and enforcement of quality and safety testing have lagged behind. Now state governments and private labs are focusing on product safety testing with special emphasis on pesticide analysis. This is the result of various product recalls, media attention and concern from patient advocacy groups. Herein, a modified QuEChERS LC-MS/MS method for the analysis of multiresidue pesticides was developed. The AOAC QuEChERS method was used for a reduced 1.5 g amount of plant material and processed with Universal dSPE. LC-MS/MS analysis used constant polarity switching ESI and monitored at least two transitions per analyte. Matrix-matched calibration was used for quantitation and both method and instrument internal standards were used. Analyte recovery validation was performed according to FDA guidelines by testing three matrices at three fortification levels in triplicate for over 200 pesticides. For the large majority of pesticides, in all three matrices and at all three fortification levels, recovery was between 70-120%.

Acknowledgement: *Shimadzu scientific instruments, trace analytics*

P10
INVESTIGATION OF CHLORPYRIFOS AND ITS TRANSFORMATION PRODUCTS IN FOOD SAMPLES

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Chlorpyrifos (CPF), an anticholinesterase organophosphate insecticide, is commonly used to control pests in agricultural sectors. In recent years, it is one of the most frequently detected residues in fruits and vegetables [1]. On the other hand, pesticides including chlorpyrifos undergo extensive abiotic (industrial processes, waste treatments, and photodegradations) and/or biotic (metabolism and microbial activities) processes which lead to transformation products (TPs) with different toxicity [2]. Furthermore, lack of representative standards and complexity of transformation mechanisms make monitoring of TPs in real samples difficult. The aim of this work was to investigate CPF and its TPs in selected food matrices. Representative standards of TPs were synthesized by electrochemistry coupled online to liquid chromatography-mass spectrometry (EC/LC/MS) that equipped with a follow-through and/or synthesis cell with boron doped diamond working electrode [3]. The TPs were characterized by LC-MS/MS and high resolution mass spectrometry (HRMS) and used for real sample investigations. Different fruit and spice samples (fortified by TPs standards and blank) were extracted by dispersive solid phase extraction (dSPE) and analyzed by LC-MS/MS.
Recoveries were obtained ranging between 94–101% (with matrix effect 85–97%). The method limit of detection (LOD) and quantification (LOQ) for CPF were 1.9 and 5.7 µg/kg, respectively. Among investigated samples, CPF was detected in fresh lemon, black pepper and fenugreek seed with a content of 104, 31 and 4 µg/kg, respectively. Coriander and cinnamon samples also contained trace levels of CPF (<LOD). Transformation products of CPF mainly diethylthiophosphate (DETP), chlorpyrifos oxon (CPF oxon) and trichloropyridinol (TCP) were detected alongside the parent compound in different samples. Hence, by synthesizing reference standards using EC/LC/MS we were able to detect the main TPs of CPF in real food samples. The results show that not only parent residues but also monitoring of TPs is vital to ensure future food safety.

[1] RASFF, *Annual report* 2015.
[2] M. Supreeth and N. S. Raju, *Applied Microbiology and Biotechnology* 2017, *101*, 5961-5971.
[3] T. F. Mekonnen, U. Panne and M. Koch, *Analytical and Bioanalytical Chemistry* 2017, *409*, 3359-3368.

Keywords: *transformation product, electrochemistry-mass spectrometry, LC-MS/MS, chlorpyrifos*

P11
DETERMINATION OF MULTICLASS OF ORGANIC CONTAMINANTS IN RED CHILI AND TURMERIC POWDERS IN A SINGLE METHOD OF ANALYSIS USING AGILENT'S 6495 TRIPLE QUADRUPOLE LC/MS SYSTEM AND EMR-LIPID SAMPLE CLEANUP

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Analysis of agrochemical residues in spice samples such as Red chili powder and Turmeric samples is always considered difficult because of its complex nature. During sample preparation, matrix components may also get co-extracted along with the target compounds, leading to poor sensitivity, robustness and loss in precision. Further, reduced column performance and more periodic source cleaning would also be required leading to loss in productivity. In this study, a fast UHPLC/MS/MS method has been developed for the estimation of multiple organic contaminants in Red Chili and Turmeric powders in a single method of analysis. Using this method, the determination of multiple organic contaminants such as around 235 pesticides, 20 pyrrolizidine alkaloids, 9 mycotoxins and 6 illegal dyes have been estimated in spice samples in a single extraction. The method benefits from the increased chromatographic resolution of the Agilent 1290 Infinity II UHPLC system as well as the versatile ionization capabilities of the Agilent Jet Stream ionization source and the innate sensitivity of the Agilent 6495 triple quadrupole LC/MS system. Matrix effects associated with electrospray ionization were controlled by effective sample cleanup before injection. The results presented demonstrate that the increased sensitivity of the 6495 triple quadrupole LC/MS system enables the accurate and robust quantitation of targeted Pesticides, Mycotoxins, Pyrrolizidine alkaloids and Illegal dyes in the sample extracts with high precision and robustness. Choosing matrix free transitions from Agilent MRM Databases offered accurate determination of the residues. A simple Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) -Enhanced Matrix Removal-Lipid (EMR-Lipid) technique was used for extraction of analytes of interest from the sample. In this study, matrix matched calibration standards were used for accurate quantitation of analytes. Most of these analytes were detected below the maximum residue limits (MRLs) specified by the European Commission.

P12
MULTI-RESIDUE METHOD BASED ON QUECHERS FOLLOWED BY NON-TARGETED UPLC-QTOF-MS ANALYSIS APPLIED FOR PESTICIDE CONTROL AND MONITORING OF THEIR FATE IN AGRICULTURAL AREA

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Residues of current-use pesticides in agricultural crops represent a possible risk for humans due to their various toxic effects such as neurotoxicity or immunotoxicity. Moreover, a cocktail of pesticide compounds enters during field application to plants and soil and their subsequent transport to water is assumed. Therefore, we developed a multi-residue and sensitive analytical method for determination of wide range of pesticides and their metabolites in many matrices including plants, soil and lysimetric water in order to control the pesticide usage and monitor their fate.
Sample preparation procedures were based on QuEChERS approach (plants, soil) and solid phase extraction (SPE) utilized for water samples. Ultra-high performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-QTOF-MS) was used for separation, detection and quantification of pesticides. Samples were analyzed in positive and negative mode under different chromatographic conditions. Non-targeted analysis approach was chosen, i.e. 6 mass spectra per 1 sec. were scanned in m/z range 100-1000 in full scan and after fragmentation of molecular ions using three collision energies, all ion MS/MS scan was performed and m/z of all fragments was monitored. The identification of correct mass formulas for the ion fragments was done using general knowledge of fragmentation mechanism assisted by e.g Isotope Distribution Calculator. The calculated fragment masses were verified by measured masses of analyzed pesticide standards. Concentrations of targeted pesticide residues were evaluated using matrix-matched standard calibration curves. In non-targeted screening, pesticides were qualitatively evaluated using spectral library.
The established method was applied on plant and soil samples which were sampled from each site together in order to find a possible link. Additionally, the basic monitoring of agricultural soils in the Czech Republic and the lysimetric water were evaluated using this method providing the significant advantage in retrospective evaluation of these data for detection of non-targeted compounds which can be performed without re-injection of the sample.

Keywords: *current-use pesticides, UPLC-QTOF-MS, multi-residue analysis, agriculture, non-targeted screening*

P13
MULTI-RESIDUE PESTICIDES SCREEN AND QUANTITATION IN FOOD MATRICES USING ULTIVO TRIPLE QUAD LC/MS

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Pesticides are vital to crop production, but can be toxic to humans if consumed in harmful doses. Regulatory agencies have set maximum residue levels (MRL) for hundreds of pesticides and their metabolites in foods. Most MRLs are set at low ppb levels, which poses significant challenges for screening and quantifying hundreds of analytes simultaneously in complex food matrices. Here, we demonstrate a method for the screening and quantitation of 246 pesticides using Ultivo triple quadrupole LC/MS. The new instrument features robust performance and easy maintenance. Orange, avocado, broccoli and black tea matrices were chosen to represent most fruits, vegetables, and dried herbs. Matrices were extracted using Agilent QuEChERS kits. A new EMR-Lipid kit was used for avocado to remove excess amounts of fat. Most analytes could be accurately quantified below the MRL in each matrix, highlighting the excellent sensitivity of the new instrument. The chromatographic method consisted of using 3.0 x 150 mm, 1.8 µm C-18 column, with 4.5 mM ammonium formate, 0.5 mM ammonium fluoride, and 0.1% formic acid as additives in water-methanol mobile phases. A dynamic MRM method with fast polarity switching was employed to have optimal dwell times for analytes in a narrow time window, with 2-4 MRMs per analyte. This could be accomplished due to retention time stability provided by the high-quality LC system and analytical column. Most of the pesticides could be accurately quantified far below MRLs with high precision (RSD < 20%).

Keywords: *pesticides, Ultivo*

P14
EXTRACTION AND CLEANUP OF PESTICIDE RESIDUES FROM TEA PRIOR TO ANALYSIS BY LC/MS/MS AND GC/MS/MS

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The analysis of pesticide residues, particularly in dry foodstuffs such as spices and teas, is often challenging due to the presence of concentrated matrix interferences. If not removed, these undesired oils and pigments can produce ion-suppression or matrix enhancement effects and also contaminate both GC/MS and LC/MS systems. To this end, implementation of an effective cleanup method prior to the injection of extracts onto chromatographic systems is necessary. For high background samples such as these, standard QuEChERS methodology often does not provide the required cleanup capacity. A novel adsorbent combination in a dual-layer solid phase extraction (SPE) cartridge was recently developed for an efficient cleanup of acetonitrile extracts from dry commodities and difficult matrices (spices, tea, etc.) prior to pesticide residue analysis. The top bed consists of a mixture of C18, primary-secondary amine (PSA), and a unique graphitized, spherical carbon. This specialized carbon was engineered to sufficiently remove pigments while allowing for better recoveries of planar pesticides without the use of toluene for elution. A zirconia-coated silica in the bottom layer of the cartridge aids in the removal of oily matrix components and various pigments. Analysis of a wide range of pesticides of different polarities and classes will be demonstrated by both GC/MS/MS and LC/MS/MS in green tea matrices. Specifically, analyte recovery, reproducibility, and background removal using a novel dual-layer SPE cartridge will be the focus of this presentation.

Keywords: *pesticides, dry samples, spices, ginger, tea*

P15
QUANTITATIVE ANALYSIS OF GLYPHOSATE IN REGULAR AND INFANT GRAIN-BASED FOODS VIA LC-MS/MS USING AN ANION EXCHANGE HPLC COLUMN

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While glyphosate remains one of the most commonly used pesticides in the world, it recently came into the focus of the analytical community due to renewed interest from regulatory agencies. This work describes a method for direct analysis of glyphosate in grains after extraction based on the QuPPE methodology using 50:50 methanol:water as an extraction solvent. Cleanup of samples not containing particulates was done using solid phase extraction with HLB. Samples that were cloudy, including wheat flour extracts, required ultrafiltration through membranes with a 3kDa molecular weight cut off (MWCO). The LC-MS method for glyphosate, aminomethyl-phosphonic acid (AMPA) and glufosinate employed an aminopropyl-bonded polymer-based HPLC column used with carbonate buffer as the mobile phase. The polymer-based column exhibited excellent stability at pH 9, and the carbonate mobile phase was fully compatible with MS detection. The method was validated using spiked samples of organic wheat flour and organic oatmeal in which glyphosate was not detected. The method was applied to both regular and infant cereal and flour samples. Glyphosate was detected in non-organic infant oat cereal at 1 ppm, in infant mixed cereal at 0.25 ppm, in instant oatmeal at 1.2 ppm and in bleached wheat flour at 0.8 ppm. These values fall within the regulatory limits for glyphosate in cereal grains in the USA.

Keywords: *glyphosate, QuPPE, LC-MS, cereal grains*

P16
A NEW STRATEGY FOR THE DETERMINATION OF CAPTAN AND FOLPET IN FOOD MATRICES

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Global regulations and consumer concern make pesticide residue analysis an important component of ensuring food safety. With hundreds of pesticides commercially available and approved for use on crops, it is desirable to rapidly and reliably analyse samples for a large number of pesticides in as few methods as possible, typically using liquid chromatography (LC) and gas chromatography (GC), coupled to mass spectrometry. In order to cover a full suite of regulated compounds, several LC and GC methods are often required to incorporate large suites of compounds, single residues and 'troublesome' compounds. Phthalimide fungicides, including captan and folpet, are compounds that are considered GC amenable pesticides, but are troublesome to analyse via standard GC analysis. In matrices containing chlorophyll, captan and folpet rapidly degrade in the injector port, making repeatable and robust analysis of these compounds very difficult.
In this project, two LC ionisation techniques were assessed as to their ability to incorporate these problematic GC compounds into an LC method. Electrospray ionisation (ESI) and a novel LC-MS ionisation technique (UniSpray or USI) were investigated to determine whether captan and folpet could be successfully analysed in relevant challenging food matrices, including celery and kale. Initial results show that captan and folpet can be analysed using an LC method using both ionisation techniques. Method optimisation was performed independently on each ionisation technique prior to sample analysis. Linearity was assessed with the matrix samples having R^2 values of > 0.99 for both compounds. The methods were also shown to be reproducible (%RSD; n=25) 15%. The data presented demonstrates that the notoriously troublesome GC compounds captan and folpet can be analysed reliably and accurately by LC-MS/MS, avoiding the problems observed using GC analysis.

P17
THE PESTICIDE EXPLORER COLLECTION – FAST SCREENING AND QUANTIFICATION OF PESTICIDE RESIDUES USING A COMPREHENSIVE LC-MS SOLUTION

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Pesticides are chemical or biological agents meant to control, kill or repel plants or animals considered to be pests. Although, pesticides have benefits such as improved productivity and protection of crop losses, some pesticides have drawbacks too such as potential toxicity to humans and other species. – Due to the widespread use of agricultural chemicals in food production and the difficult control of international food chain, people are exposed to low levels of pesticide residues through their diet. Identification and quantification of pesticide residues if foods and food products is therefore an important part of routine food control. Routine pesticide residue analysis remains one of the most challenging tasks in mass spectrometry. Their chemical diversity, the sheer number of potentially used pesticides (>1100), and the wide range of matrices are the key challenges in their analysis. Reliable and validated multi methods using the latest technical developments are therefore required for the analysis of pesticides.
Here we present a complete workflow solution for the analysis of more than 600 pesticide residues by liquid chromatography-triple quadrupole mass spectrometry. The analysis was performed on a Thermo Scientific™ Ultimate3000™ RSLC system coupled to a Thermo Scientific™ TSQ Quantis™ mass spectrometer. Chromatographic separation was carried out on a Thermo Scientific™ Accucore aQ™ column. The QuEChERS extraction procedure was applied on all investigated samples. Leek representing vegetable with high pigment content was chosen as matrix for validation.
The method was tested according to SANTE11945/2015 requirements. Analytical parameters such as linearity, specificity, LOD, LOQ, precision and accuracy were evaluated using the Thermo Fisher TraceFinder 4.1 software. The validation outcome showed satisfactory results since LOQs ≤ 10 µg/kg were reached for a large number of the ~600 target compounds in leek. The RSD values for most of the compounds were lower than 20%. This poster will discuss the data in detail.

Keywords: *pesticides, multi method, QuEChERS, LC-MS/MS*

P18
DIRECT MULTI-RESIDUE DETERMINATION OF HIGHLY POLAR PESTICIDES IN FOOD MATRICES USING HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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Glyphosate together with many of the target polar pesticides and plant growth regulators included in this method are some of the most frequently used pesticides worldwide. The popularity of non-selective high polar herbicides, especially glyphosate, has continued to increase due to low costs and high effectiveness.
Determination of these compounds is very difficult due to their physical-chemical properties. They are very soluble in water and insoluble in most organic solvents and they have low molecular weights. Although no adverse effects have been observed on humans, these compounds and their metabolites, which have sometimes similar toxicological characteristics, have been detected frequently in water, soils and crops. Maximum residue levels (MRLs) have been established for fruits, vegetables and cereals by the European Union and other countries.
Evaluation of these compounds and their metabolites is a big challenge for analytical chemists. So far, the determination of these compounds has been carried out using single residue methods (SRMs). A multi residue method with 15 compounds (parents and their metabolites) using hydrophilic interaction liquid chromatography and mass spectrometry has been evaluated in this study.
An extraction method based on the QuPPe method from the EURL-SRM [1] was developed and used to validate the method according to SANTE Guideline [2]. UHPLC (Nexera LC) coupled to a hybrid quadrupole/linear ion trap mass spectrometer system (Sciex 6500+ QTRAP®) was used for analyzing the samples. Isotopically labeled internal standards for all compounds (except for N-acethyl-AMPA) were used for recovery and matrix effect correction. The extracts were diluted 50 to 100x depending of the matrix and then directly injected into the LC-MS/MS system. In the method developed, no derivatization process was required for detection of glyphosate.
The performance of the method was tested by participating in two proficiency tests EUPT-SRM12 (strawberry) and FAPAS 09109 (oats). The z-scores obtained were all acceptable, e.g. for values for glyphosate, -0.1 and 0.8, respectively.

[1] Anastassiades, M.; Kolberg, D.I.; Benkenstein, A.; Eichhorn, E.; Zechmann, S.; Mack, D.; Wildgrube, C.; Sigalov, I.; Dork, D.; Barth, A. Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method), version 9.2, http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf (accessed 17/01/2017).
[2] Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11945/2015.

P19
PROFICIENCY-TESTING SCHEME FOR PESTICIDES IN VEGETABLES

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BIPEA (www.bipea.org) organizes regular proficiency-testing schemes (PTS) in many analytical domains, including the analysis of pesticides in fruits and vegetables. As an example, in December 2016, one test was conducted using a vegetable matrix - carrot - characterized first to be free from any pesticides residues, and then spiked with 34 pesticides, at levels between 20 and 200 µg/kg.
The production of the samples was performed using a specific equipment to ensure homogeneity between all the samples, and the immediate freezing of the samples after their production allowed to ensure their stability.
This test was intended for the quantification of 33 molecules of pesticides residues as for the identification and the quantification of another one. 42 laboratories took part in this test. The techniques used by the laboratories were GC-MS-MS, LC-MS-MS, GC-MS and GC-ECD, according to the molecules.
Participating laboratories were required to return their results on a dedicated website after a period of one month, and a statistical treatment of the data was as usual performed by BIPEA according to ISO 13528 [1]. Assigned (consensus) values were calculated from the participants' results and the performances of the laboratories could then be evaluated individually and collectively according to ISO 17043 [2].
This test allowed participating laboratories to draw up a general inventory of their analytical skills, and were a very useful tool to detect bias or non-compliant results; they act as a warning signal for the implementation of corrective and/or curative actions in the laboratory.

[1] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, *2015*.
[2] ISO 17043:2010, Conformity assessment - General requirements for proficiency testing, *2010*.

Keywords: *proficiency testing schemes, pesticides, quality control, laboratory performance, food contaminants*

P20
MINIMIZED AND AUTOMATED SAMPLE PREPARATION FOR MULTI-RESIDUE ANALYSIS IN DIFFICULT MATRICES

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It is getting more and more important to control food for pesticides and other undesirable substances. But some foods like tea, spices and hops are very difficult matrices to analyze. There are different methods of preparing samples: the QuEChERs method [1], which is rarely suitable for these complicated matrices or the DFG S19 method [2], which is predominately used for unpolar pesticides („GC-pesticides") and the modified QuEChERs [3], which are time and solvent consuming methods.
We optimized the sample preparation so that we were able to analyze more than 600 analytes by LC and GC- both taken from the same extract- in difficult matrices and in less than 1 hour per sample from weighing to the final evaluation. With sample weights of 0.5-1.0 g a QuEChERs extraction is used. After that there is a cleanup with a combined mini SPE (PSA, NH_2, $MgSO_4$ from Agilent and UCT) [4,5].
The new method was validated with the special matrices tea, curry and hops. In accordance with the SANTE criteria [6] most of the pesticides show LOQs of 0.01 mg/kg (80%) and the other can be measured with a LOQ of 0.02 mg/kg (10%) or 0.05 and 0.1 mg/kg (10%).
With the development and optimization of this method it was possible to prepare samples more quickly and with less solvents. Another advantage is the possibility to measure the sample with both kinds of equipment using only one extract.
This validated method was used as the basis for developing a preparation method, which uses even less material and manpower. For this purpose the cleanup was minimized and automated.
For minimizing of the cleanup procedure, we used our own SPE columns with commercially available SPE materials. Using an automated SPE (Freestyle) we were able to develop a sample preparation method, which give reproducible results with a minimum of costs.

[1] 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; 2014-02
[2] Official collection §35 LFBG: modular multi-method to determine plant protection substances residues in food (extended new version of DFG method S19), L 00.00-34
[3] Multi-residue Pesticide Analysis in Green Tea by a Modified QuEChERS Extraction and Ion Trap GC/MSn Analysis
[4] New Sample Preparation Method for Multi-residue Pesticide Analysis in Tea and Herbs with GC-MS/MS, oral presentation, Romanotto A., Pestizid InfoTag, 29. September 2015 · Frankfurt am Main
[5] Analysis of 600 + pesticides in difficult matrices in 1 hour, poster presentation, Romanotto, A., Langner, J., Muetze, F., EPRW 2016
[6] Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE /11945/2015

Keywords: *SPE, pesticides, automation*

P21
ENVIRONMENTAL IMPACT ON ANTHRAQUINONE FINDINGS IN ORGANIC DARJEELING TEA

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The goal of this study is to locate the sources of AQ on the basis of extensive testing and analysis of the teas and their environment, including different parts of the plants, soil and dust, as well as fertilizers and fortifiers. The study began with planting and went through all the production steps to determine when and where there is a significant growth in the amounts of AQ.
From October 2015 to October 2016 hundreds of different samples were taken and analyzed for AQ and PAHs. The results have shown that, in growing as well as in production there are inputs of AQ. In the environment, factors such as the distance to cities, roads and the railway play an important role and together they build a so called AQ initial finding.
Also the altitude of tea plantation has given us the first evidence of the environmental impact. The lower plantations located near more inhabited regions (<1500 m) show a higher AQ numbers in their tea leaves.
Furthermore, the old hypothesis that AQ is endogenously produced has been refuted. The analysis of AQ in moss and other ambient plants from tea plantations shows the same and higher amounts of AQ in them. Moss is known as a good absorber of fine dust from the air because of its very big surface area [2]. This clearly proves, that only air can be the source of the contamination of both of tissues.
We also tested air from the same places during the three different harvesting periods and found out that, in rain free periods, winter and spring, the AQ contamination of the air is higher than during the rainy seasons. Fine dust is consequently the real source for AQ in the environment.
On the basis of all results of our study, we could differentiate three types of environmental input of AQ in tea in the Darjeeling area:
Global environment: contamination caused by worldwide anthropogenous influences e.g. accumulation and current release of global contaminants due to the melting of glaciers as a result of global warming (findings of highest altitudes)
Distant environment: influence of cities and factories or coal mines from Tibet, cigarette factories, etc. within a circle of five thousand kilometers (findings of middle altitudes)
Immediate environment: influence of local traffic and cities in the region (findings of lowest altitudes)
In summary, AQ is a contaminant in the environment (present in fine dust in air). It is definitely not a pesticide in tea cultivation. This study shows that it is necessary to regulate AQ in tea as a contaminant and with a higher level than 0.02 mg/kg.

[1] Dr. Jan-Peter Frahm, Nees-Institut für Biodiversität der Pflanzen, Universität Bonn,
https://www.uni-bonn.de/neues/moose-reduzieren-die-feinstaubbelastung/
[2] Photooxidation of anthracene in particulate matter, SCIENCE, VOL. 205, 10 AUGUST 1979.

Keywords: *anthraquinone, oxy-PAHs, tea, air contamination*

Acknowledgement: *To Teekampagne (Projektwerkstatt) for financing and Initiation of the study.*

P22
DETERMINATION OF FIPRONIL AND ITS METABOLITE FIPRONIL SULFONE IN EGGS BY LIQUID CHROMATOGRAPHY-TANDEM QUADRUPOLE MASS SPECTROMETRY USING A MODIFIED QUECHERS METHOD

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Millions of eggs were pulled from supermarket shelves in more than a dozen European countries in late July 2017 – and as far as Hong Kong and South Korea – after it was discovered that some had been contaminated with the insecticide fipronil. On July 20, 2017, a notification from Belgian officials via the EU RASFF portal alerted that fipronil had been found in eggs produced by some Dutch farms at concentrations from 0.0031 to 1.2 mg/kg (above the EU MRL). Fipronil is an insecticide intended for professional pest control use to combat infestation of insects such as cockroaches; as well as in veterinary medicine to combat fleas, mites, and ticks on dogs and cats. It is a highly toxic compound and it is not authorised for use as a veterinary medicine, biocide or pesticide around food producing animals. Hence it should never have found its way into a chicken coop.
Fipronil and its metabolite fipronil sulfone in eggs can be determined using LC-MS/MS with electrospray ionisation (ESI) in negative ion mode. However, matrix effects from coeluting co-extractives can impact reproducibility of the method. Therefore suitable sample preparation is needed to achieve a sensitive and robust method. The method validation for the determination of fipronil and fipronil sulfone in eggs using liquid chromatography-tandem quadrupole mass spectrometry will be presented. Discussed results will follow the SANTE criteria (SANTE/11945/2015) set by EC, including a minimum of 5 replicates at the targeted LOQ of the method (0.002 mg/kg) and at least one other higher level (0.02 mg/kg). In this method, the extraction of analytes was performed by modified QuEChERS. For co-extracted lipids removal, simple pass-through solid-phase extraction (SPE) was involved using Oasis PRiME HLB syringe filters. Mean recovery and repeatability (RSDr) for fipronil and fipronil sulfone was 95% (1.2% RSD) and 9% (1.4% RSD), respectively. The benefits of this simple and quick sample preparation will be discussed with the method validation results.

Keywords: *fipronil, UPLC-MS/MS, QuEChERS, egg*

P23
ACCURATE QUANTIFICATION OF FOLPET AND PHTHALIMIDE IN FOOD

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Folpet is a fungicide commonly used in agriculture. Its accurate quantification can be problematic because it degrades into phthalimide during sample preparation and also during analysis by gas chromatography (GC). Consequently, in 2016 the EU authorities modified the definition of the residue for folpet to include phthalimide [1]. It was recently shown that phthalimide could arise from other sources such as the degradation of other pesticides (i.e. phosmet) [2], and could also be generated in the GC injector not only from folpet degradation, but also from the reaction between phthalic anhydride (a ubiquitous compound) and primary amino compounds from food matrices at high temperature [3]. While GC is the technique of choice for both analytes, they could be amenable by liquid chromatography (LC) using atmospheric pressure chemical ionization, thus avoiding the formation of phthalimide on the GC injector.
Three different techniques were used for the quantification of folpet and phthalimide: i) GC with negative chemical ionization and single quadrupole mass spectrometry (GC-NCI-MS), ii) GC with electron ionization and triple quadrupole mass spectrometry (GC-EI-MS/MS) and iii) LC with atmospheric pressure chemical ionization and high resolution mass spectrometry (LC-APCI-HRMS). Sample preparation consisted on an extraction with acidified water and acetonitrile, followed by phase partitioning with NaCl and $MgSO_4$.
The three methods were validated in four food commodities including high water content (e.g. apple puree), high acid and water content (raspberry puree), high starch content (rice flour) with a limit of quantification (LOQ) set at 0.01 mg/kg, and dairy products (e.g. infant formula) with an LOQ set at 0.03 mg/kg. The three methods showed linearity, trueness and precision in accordance with SANTE/11945/2015 criteria for phthalimide and folpet. GC-EI-MSMS was the only technique fulfilling also the selectivity criteria. No residues of phthalimide were detected by LC-APCI-HRMS while trace levels were found by GC, which could be attributed to the GC injector. However, those levels only affected the trueness of phthalimide in high water and acid content commodity.

[1] EURL-SRM ERL-SRM. Quantification of Residues of Folpet and Captan in QuEChERS Extracts. 2017 (Version 3.1): http://www.crl-pesticides.eu/userfiles/file/EurlSRM/meth_CaptanFolpet_EurlSRM.pdf. Accessed July 2017.
[2] A. Berthet, M. Berode, M. Bouchard. Anal. Bioanal. Chem. 400 (2011) 493-502.
[3] Relana, position paper No. 16-03 (Version 2016/07/22): http://www.relana-online.de/position-papers/. Accessed on July 2017.

Keywords: *folpet, phtalimide*

P24
APPLYING 'MRM SPECTRUM MODE' AND LIBRARY SEARCHING FOR ENHANCED REPORTING CONFIDENCE IN ROUTINE PESTICIDE RESIDUE ANALYSIS

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Introduction:
To help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple-reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ion transitions to increase specificity and reporting confidence. In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectrum which could be used in routine library searching and compound verification using reference library match scores. This 'MRM Spectrum mode' was applied to quantify and identify 193 pesticides using 1,291 MRM transitions without compromising limits of detection, linearity or repeatability.
Method: The method includes a total of 1,291 MRM transitions for 193 pesticides in a run time of only 15 minutes. On average 7 MRM transitions were applied to each compound, with more than 10 MRM transitions applied to 34 compounds. A dwell time of 3 msecs was applied to every MRM transition. Samples were analysed in ESI +/- using a polarity switching time of 5 msec. Pesticide spiked samples, extracted using established QuEChERS based methods, were provided by Scientific Analysis Laboratories, UK. Tested matrices included; turmeric, plum, peppermint, parsnip, cherry, lime, pumpkin, tomato and potato. Calibration curves were prepared in the range 0.01 to 0.2 mg/kg. Repeatability of the method was tested using avocado matrix at 0.1 mg/kg.
Preliminary data: Acquisition of the MRM Spectrum method (1,291 MRM transitions in total) did not compromise data quality when compared to a conventional 2 MRM per compound method (386 MRM transitions in total) with consistent signal response and repeatability in both methods. The highest number of overlapping MRM's acquired was 151. Even at such a high data sampling rate the response was in agreement with a conventional 2 MRM method with peak area variation less than 5.2% (n=5) for the 22 target pesticides that eluted during this period. The MRM product ion spectrums were demonstrated to be consistent across the linear range and to be in close agreement with results from the 2 MRM method. Even at the reporting level concentration MRM spectrum mode was able to acquire several transitions for each compound. For example, all MRM transitions for carbendazim were detected at the reporting level of 0.010 mg/kg with a signal to noise for all fragment ion transitions greater than 10. New data processing software (LabSolutions Insight v3.0) was used to review quantitative data and MRM Spectrum mode library
searching with advanced filtering tools to review by exception and to reduce false detect reporting.
Novel aspect: Application of MRM spectrum mode and software workflows to reduce false positive reporting through enhanced data quality.

Keywords: *pesticides, MRM spectrum mode, LC-MS/MS*

P25
PESTICIDES RESIDUE MONITORING ON IMMATURE CITRUS FRUITS AND THE CHARACTERISTICS BY PROCESSING METHODS

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The pesticide residues were monitored in 112 samples of 60 immature citrus fruits (Putgyul) collected directly from orchards during cultivation, 40 putgyul products and 12 citrus beverages. A multi-residue method was applied for the analysis of 320 pesticides, and dithiocarbamates (mancozeb and propineb) were analyzed by patented Rapid Quantitation. 30 kinds of pesticides were detected in 100 putgyuls and a few carbendazim was detected slightly in some citrus beverages, but there were no products exceeding the MRL. The 20 samples with 30 pesticides were pre-treated to remove the residues using three washing and two juicing methods, and the reducing rate of residue was evaluated. 11 pesticides were decreased by 16.7-100% in washing treatment, and 16.0-94.0% in whole fruit juicing, while the other 19 pesticides were not significantly different in the rate of decrease. However, all 30 pesticide residues were largely decreased in juice after removing citrus peel. Consequently, it was concluded that the residual pesticides in Putgyuls were properly controlled. The monitoring yearly for the pesticide residue in immature citrus fruits may provide a basic safety information for the management of pesticides to producers and processing companies as well as the present results.

Keywords: *immature citrus fruit, putgyul, pesticide residues, washing method, citrus peel*

Acknowledgement: *This study was carried out by the agricultural safety research project of National Agricultural Products Quality Management Service (NAQS) in 2016.*

P26
COMPARATIVE DETERMINATION OF FIPRONIL IN EGGS USING QUECHERS METHOD BY GCMSMS

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Fipronil is an insecticide that belongs to the phenylpyrazole chemical family. This insecticide has a moderate acute toxicity to people and mammals. It is used in veterinary remedies for fleas, mites and ticks (MRL for Fipronil in bird eggs is 0,005mg/kg [1]).
Within the flexible scope of accreditation, SP LABORATORIJA is accredited for the determination of Fipronil in food of plant and animal origin. The aim of this study was the validation of Fipronil with modified QuEChERS method in eggs [2]. Samples were prepared with three different ways of extraction. We used the QuEChERS method with the $MgSO_4$/PSA [3] and $MgSO_4$/PSA/C18 cleanup columns as well as the EMR-Lipid dispersive column. This study was performed using an Agilent 7890 GC coupled to an Agilent 7010 Triple Quadrupole GCMS with a mid-column backflushing system. The matrices used for the analysis were non-contaminated laboratory control samples with different fat content: eggs (8,8%), eggs in powder (39%), yolk (56%) and whites (0.2%). The matrix matched calibration was used with PCB 31 as an internal standard. For all samples, the value of the recoveries was between 88-118%, which satisfies the required criteria. We observed slightly higher recoveries at the samples which were prepared with PSA column on all matrices and the highest sensitivity and the best peak symmetry. A slightly smaller peak area of Fipronil was in spiked samples which were purified by the C18 column and the EMR-Lipid dispersive column. According to obtained results, we concluded that all three ways of purifying satisfies the required criteria, but QuEChERS method with the PSA cleanup column is more suitable for Fipronil determination in eggs, due to higher sensitivity, and accuracy, as well.

[1] REGULATION (EC) NO 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.
[2] SANTE/11945/2015: Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed.
[3] European Union Reference Laboratory for Pesticides in Food of Animal Origin and Commodities with High Fat Content: Analysis of Fipronil and metabolites with modified QuEChERS method in Egg.

Keywords: *fipronil, eggs, QuEChERS, GCMSMS*

P27
QUANTIFICATION OF ETHYLENE THIOUREA AND PROPYLENE THIOUREA IN FOOD USING A SOLID PHASE EXTRACTION CLEANUP AND LC-MS/MS

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Ethylene thiourea (ETU) and propylene thiourea (PTU) are toxicologically relevant degradation products of the respective fungicides ethylenebis- and propylenebis-dithiocarbamates, that should be considered in a consumer risk assessment. Their analysis is known to be problematic due to their polar characteristic and their low molecular weight. This work presents a new fast analytical method for the quantification of ETU and PTU, as free or as degradation products of their dithiocarbamates precursor, in food by liquid chromatography tandem isotopic dilution mass spectrometry (LC-MS/MS). The method covers a broad range of food matrices belonging to different commodities such as cereals (high starch content), fruits and vegetables purees (high water content), fruit juice concentrate, dry fruits and honey (high sugar content), powdered milk- and soy-based infant formulae, herbs and spices. To ensure the degradation of dithiocarbamates to ETU and PTU, a heating step at 90 °C for 30 min was needed previous to the sample preparation. It consisted then in an extraction with a mixture of water and acetonitrile containing 1.25% of ammonium hydroxide, followed by a liquid-liquid partitioning using sulfate magnesium and sodium chloride and a further clean-up using Oasis MCX SPE cartridge (Waters). Limits of quantification were below the European Maximum Residue Level (< 10 µg/kg). The method was fully validated and performance parameters including linearity, precision and trueness were compliant with the analytical requirements stipulated in the SANTE/11945/2015 document.

Keywords: *ethylene thiourea, propylene thiourea, dithiocarbamates, LC-MS/MS, food*

P28
DETERMINATION OF GLYPHOSATE, AMPA AND GLUFOSINATE IN SOYBEAN MEAL BY HPLC-QTOF

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Glyphosate (Gly) and glufosinate (Glu) are broad-spectrum systemic herbicides. They are usually used for weed control on crops fields. According to forecasts, by the end of 2017, the global glyphosate market will reach 1.35 million metric tons and above 5000 tons of glufosinate. AMPA (aminomethylphosphonic acid) is the degradation product of glyphosate. The approximate metabolisation percentage of the glyphosate into AMPA in plants can reach 70 %. The literature data suggest that these compounds are usually present in plant samples simultaneously. There are several reasons to control Glu, Gly and AMPA residues: Stable increase in usage of Gly and related compounds in agricultural countries, which cultivate GM crops such as soy, maize and others. A confirmed possibility of Glu, Gly and AMPA to enter the food chain with active use of GM crops during the fattening stages of animal farming, as well as entering with water, meat and milk. The adverse effects of Gly and its metabolites on human health are evident from high degree of correlation between the amount of Gly used and cases of autism, dementia, thyroid and liver cancer, renal failure, Parkinsons disease, and hypertension.
In our study, we are focusing on the efficient extraction of Gly, Glu and AMPA from soybean meal followed by the derivatization step and determination of reaction products by HRMS in the negative-ion mode. The sample preparation procedure is the following: 1 g of homogenized soybean meal is mixed with 6 ml of deionized water and extracted for 20 minutes. Then 40 µl of hydrochloric acid are added and extraction continues for 30 minutes. The mixture is centrifuged at 4750 rpm, an aliquot is transferred into another tube with ACN and centrifuged again. The upper layer is passed through Oasis HLB SPE cartridge and the eluate is collected for derivatisation step. 1 ml of borate buffer and 1 ml of FMOC-Cl are added to 1 ml of purified extract for derivatisation at 50 degrees. Diethyl ether is added to separate the organic phase. The extract is concentrated to 1 ml and diluted to 2 ml with deonized water, then 10 µl of hydrochloric acid is added. Following SPE on MCX sorbent, the extract is concentrated to 0.7 ml and diluted to 1.5 ml with 0.5 % formic acid.
HPLC separation was carried out on ACQUITY BEH column (1x100, 1.7 µm) in gradient mode. Detection was performed on Xevo G2 QTOF mass-spectrometer in negative ionization mode. The characteristic ions were *m/z* 168.02 and 150.01 for Gly-FMOC; *m/z* 135.99 and 110.018 for AMPA-FMOC; *m/z* 206.04 and 180.06 for Glu-FMOC. The described method was applied for naturally contaminated samples of soybean meal. The LOD for this matrix was 25 µg/kg.

Keywords: *glyphosate, soybean, meal, AMPA, HPLC-MS/MS*

P29
FAST ROUTINE ANALYSIS OF POLAR IONIC PESTICIDES IN FOOD SAMPLES BY SUPPRESSED ION CHROMATOGRAPHY AND MASS SPECTROMETRY

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In the last two decades there has been a huge increase of pesticide residues that can be analysed all together in one or more multi methods using both GC-MS and LC-MS techniques. However, some compounds due to their very special chemical properties such as high polarity or ionic character still have to be measured separately. Traditionally used methods include derivatization of the target pesticides to enable their better extraction and separation or utilization of special chromatographic columns such as Hypercarb (with graphitic carbon phase) or HILIC type columns. The drawbacks arising from the effort to use conventional LC-MS/MS approaches can be minimized or even avoided by developing a method employing ion chromatography (IC) separation with its ability to separate effectively very polar compounds. Thanks to the use of a triple quadrupole mass spectrometer as a detector the method is very sensitive and selective.
Presented method allows identification and quantification of ten polar pesticides including emerging compounds like glyphosate, glufosinate and AMPA in three different food matrices (lettuce, oranges and wheat flour). Sample preparation is very easy and was adopted from the Quick Polar Pesticides (QuPPe) method developed by the European Reference Laboratory responsible for single residue methods EURL-SRM [1]. The method was in-house validated according to the European SANTE guidelines 11945/2015. Analytical parameters including linearity, specificity, LOD, LOQ, precision and accuracy were evaluated using fortified blank food materials at three different concentration levels as well as commercially available certified reference materials. All tested parameters showed satisfactory results with LOQs below required legislative limits. The recoveries were in the range from 70 – 120 % for all compounds.

[1] www.quppe.eu

P30
PHTHALIMIDE RESIDUES IN WINE. POSSIBLE SOURCES AND A DOUBTABLE ASSESSMENT

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Folpet is widely used in conventional wine cultivation and is controlled in wine grapes with an MRL of 20 mg/kg [1]. In wine folpet is completely converted to phthtalimide (PI) during fermentation [2]. Among other products like Sanvino Adama, UNIVERSALIS Syngenta, VINCARE Spiess-Urania, VinoStar Adama are in use in wine cultivation. On the other hand, most wines and other spirits contain high amounts of phthalates [3], because of their migration from plastic parts during filling or from the plastic seals in the caps. The typical phthalates in wine are DEHP, DOP, DiNP, DiDP, DiBP and DBP. During microbiological activities in wine they can be degraded to phthalic acid (PA) and PI [4].
In the current study 2 red wines, 2 white wines, one sparkling wine and one organic rose wine were analyzed on PI, phthalic anhydride (PSA) and PA. In all samples except for organic wine high amounts of PI, PA and PSA were detected. For the analysis we used GC-MSMS measurement after the extraction of analytes. PA was determined indirectly through dimethyl phthalate (DMP) after methylation. All samples were analyzed twice, before and after the methylation and DMP was recalculated into PA.
The PI/PSA proportions varied considerably within the various wine types (between 2 and 16). Although the PI/PSA proportions in white wines and rose organic wine are comparable, they are not consistent overall. On the other hand, the PA/PI factor seems to be much more constant with the exception of rose organic wine, which suggests a completely different origin for PA/PI/PSA.
One question remains, is the PI-finding in the organic wine a residue or an artifact and should it be considered in the assessment?
PI was clearly detected in wine (conventional and organic). The presence of PSA is not sufficiently clear in the case of wine to exclude the use of folpet. Moreover, PA as well as PSA are substances that can act as starting materials/intermediates but also as degradation products.
PI can have different origins. The two main sources of PI in wine are folpet applications and phthalates contaminations. Both sources provide PI findings at ppm levels. False positive results for folpet are possible especially in organic wines. That is why the folpet residue definition in wine should have a separate reformulation after a throughout evaluation and concrete determination as well as consideration its processing factors.

[1] Annexes Reg. 396/2005, Annex II, Reg. (EU) 2016/156, Applicable from: 26/08/2016.
[2] Folpet und Weinhefen, Jürg Gafner, Forschungsanstalt Agroscope Changins-Wädenswil, ACW, SCHWEIZER ZEITSCHRIFT FÜR 6 OBST- UND WEINBAU 9/12.
[3] Contamination of wines and spirits by phthalates: types of contaminants present, contamination sources and means of prevention, *Journal of Food Additives & Contaminants*: Part A Volume 31, 2014 - Issue 9.
[4] Biochemical pathway and degradation of phthalate ester isomers by bacteria, Gu JD, Li J, Wang Y, Water Sci Technol. 2005;52(8):241-8.

Keywords: *pesticides, phthalates contaminants, folpet, phthtalimide, wine*

P31
SHORT STUDY TO VERIFY THE FORMATION OF PHTHALIMIDE FROM PHTHALIC ACID AND AMMONIUM IN PEPPERMINT PLANT

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The definition of the residue of the fungicide folpet has changed to the sum of folpet and phthalimide [PI] expressed as folpet [1]. The consideration of the metabolite PI is new. Besides folpet, PI is also a metabolite of other pesticides like phosmet, ditalimfos und thiochlorfenphim.
Especially in dried matrices like tea, herbs and tea infusion commodities, PI is often detectable without any findings of folpet. This is equally true for both organic and conventional sectors. So it is implausible to deduce these findings from the use of folpet or other pesticides. Besides that, in all samples containing PI, Relana® member labs aslo detected phthalic acid anhydride [PSA] [2] with a constant proportion. Both can be formed from phthalic acid, which is a common contaminant [3] and is known as a precursor of PSA and PI.
We tested the formation of PI from phthalic acid and a nitrogen source, ammonia. Nitrogen is generally very important for the growth of every plant, but in agriculture it is absolutely necessary for fast growing leafy commodities like teas, herbs etc. [4]. To avoid the influence of possible contaminants, we worked with deutered phthalic acid (PA). As a source of ammonia we used urea. The application was done through the soil. Five days after the application, we harvested the peppermint leaves and analyzed a part of them for PI D_4. We could only find small traces of the PI D_4 (< 0.01 mg/kg) in fresh leaves. Another part of the leaves was dried by 60°C for ca. 1 hour. We found 0.18 mg/kg PI D_4 in dried peppermint leaves.
One possible source of PI in dried leafy commodities is phthalic acid. With intensive nitrogen fertilizing and additional drying (dehydration), phthalimide formation is possible.

[1] Regulation (EU) 396/2005, Reg (EU) 2016/156 of 18 Jan. 16.
[2] Relana®, position papers 17-04, "phthalimid: metabolite or unavoidable artefact".
[3] John R. Dorney, Jerome B. Weber, Michael R. Overcash, Harry J. Strek, Plant uptake and soil retention of phthalic acid applied to Norfolk sandy loam, J. Agric. Food Chem., 1985, 33 (3), pp 398–403.
[4] Fertilizer schedule, basis of nitrogen fertilization, 2011-2012, www.agrar-press.de.

Keywords: *phthalimide, folpet, phthalic acid*

Acknowledgement: *Colleagues from Thermo Fisher Scientific GmbH for the HR-GC-MS (Orbitrap) for structure confirmation*

P32
ANALYSIS OF 648 PESTICIDES IN FOODS BY LC-MS/MS UTILIZING THE RAPTOR BIPHENYL COLUMN

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There are more than 1,000 pesticides used globally on soil and crops. With the ever-increasing international trade within the food industry, regulatory bodies around the world have increased the number of regulated pesticides and the maximum residue levels (MRLs) allowed in food commodities. National pesticide monitoring programs create new challenges for food safety laboratories as the number of pesticides required for analysis is increasing together with an expanded range of food products. In this poster we present the development of an LC-MS/MS method using a Raptor Biphenyl LC column for screening and quantifying over 648 pesticides in a single analysis. To evaluate the method, QuEChERS extracts of mint, tomato and apple were provided by a commercial laboratory as raw acetonitrile extracts and spiked with 648 pesticides prior to analysis. The method was evaluated in matrix to ensure that the reporting limits were in agreement with recognized MRL's.

Keywords: *pesticides, QuEChERS, LC-MS/MS*

P33
PESTICIDE TRANSFORMATION PRODUCTS: MARKERS OF UNAUTHORIZED APPLICATION IN ORGANIC WINE PRODUCTION

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Modern pesticides can degrade after their application to variety of transformation products (TPs). For this reason the parent compounds might not be detected in the tested matrices / final products. Contrary to conventional agriculture, in organic farming the usage of synthetic pesticides is restricted by the directive 834/2007/ES and 889/2008/ES where only a few, mainly natural pesticides, are permitted. In this case the analysis of pesticide residues belongs to the important tools used in the authenticity control of organic farming products. The analysis of TPs seems to be a promising alternative enabling to prove the application of (unauthorized) pesticides or even to distinguish between its unauthorized use from any unintentional contamination of crops.
The presented study was focused on the determination of pesticide residues and their TPs in vine products (Vitis vinifera - leaves, grapes, musts and wine). An extraction procedure was based on the original version of the QuEChERS method. Crude extracts were analysed by ultra-high-performance liquid chromatography coupled to high resolution mass spectrometry (Quadrupole-Time of Flight spectrometer; Agilent Ion-Mobility Q-TOF 6560, USA) in positive and negative mode. We aimed to describe the dynamics of degradation of parent compounds and formation of TPs during vine growing and maturation and wine making process. Several transformation products originating from dimethomorph, fenhexamid, iprovalicarb, metrafenon, pyraclostrobin, spiroxamine and tebuconazole were identified in vine leaves, grapes, musts and wine. The main identified pesticide transformation routes were oxidation, hydrolysis and conjugation.

Keywords: *pesticides metabolites, organic farming, LC-HRMS*

Acknowledgement: *This work was supported by the Czech National Agency for Agricultural Research (project no. QJ1530272). This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

P34
REDUCING MATRIX EFFECTS IN LC-MS/MS ANALYSIS USING A FULLY AUTOMATED COLUMN SWITCHING SYSTEM

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Analyzing chemical residues in complex sample matrices using LC-MS(/MS) the impact of chromatography is often underestimated. However, co-extracted matrix components from previous LC runs can also collect on the separation column. Even after long flushes with organic solvent, lingering matrix may lead to unexpected irreproducible signal suppression or enhancement during subsequent chromatographic runs. As a result, quantification could become almost impossible, particularly in the case of using Electrospray Ionization (ESI). Not only does the presented column switching system contribute to an accurate quantitation removing lingering components entirely, but also the fully automation increases the sample throughput enormously. Furthermore, a successfully applied use of paired identical analytical columns allows chromatography on the first column while simultaneously rinsing a second separation column after a run had been finished. As an additional outcome a consequently implemented back-flushing extends the LC column lifetime significantly. Moreover, the independent selection of up to 9 LC columns (plus an additional bypass) accompanied by a free choice of different mobile phases enables a fully automated workflow. Therefore, nearly unlimited use of different LC methods is feasible. Thus, manual operation exchanging columns or eluents is no longer needed as well as unattended automated operation overnight and on weekends is achievable. Conclusively, the described column switching system fulfills the requirements of analytical chemists to analyze a large number of samples with a high performance, efficiency and simplicity. The universal use for food, environmental, proteomics and metabolomics analysis completes the field of applications.

Keywords: *LC-MS/MS, pesticides, matrix effects, quality, automation*

P35
PESTICIDES RESIDUES MONITORING IN FRUITS AND VEGETABLES IN REPUBLIC OF MOLDOVA

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The laboratory for determination of pesticides residues from National Center of Verification and Certification of Plant Products and Soil was created with the support of the Romanian Government in 2012.
In 2014 the laboratory was accreditate by the national accreditation body MOLDAC according to ISO 17025.
Since 2015 the laboratory is providing testing for the determination of pesticides in fruits and vegetables for the national monitoring plan.
At the moment the analytical capacity of the laboratory is 70 active substances for GC-MS/MS method and 40 active substances for the UPLC- MS/MS method.
In 2015-2016 the laboratory was provided testings regarding the monitoring of pesticides residues in fruits and vegetables. The most frequently found pesticides was chlorotalonil and propiconazole.
The results shown how important is for Republic of Moldova to do the monitoring of the produts and knew where are the problems.

P36
HRMS BASED STRATEGIES FOR SCREENING OF PESTICIDE METABOLITES

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Nowadays, one of the most common methods of protecting plants from harmful organisms in agriculture is the application of pesticides. Modern pesticides can degrade after their application and produce a variety of metabolites. For example, during biotransformation pesticides can transformed via hydrolytic reaction, oxidation and reduction or can form conjugates (especially β-D-glucosides in plants). These reactions usually leading to a conversion of pesticide into a less toxic compounds, nevertheless metabolites could show similar toxicity or even higher than parent pesticide.
Analysis of pesticide metabolites can be challenging. Unfortunately, only a limited number of their standards is available. In general, these compounds occur in food at very low levels and a spectrum of conceivable target compounds can be wide since different pathways and mechanisms of degradation can be followed. Further, products of transformation processes typically show high polarity and low volatility compare to parent compounds. Considering all these facts, liquid chromatography coupled to high-resolution mass spectrometry (LC–HRMS) is a suitable technique for identification and determination of pesticide metabolites.
In this study, a strategy for identification and determination of pesticide metabolites using LC–HRMS/MS (Quadrupole-Time of Flight spectrometer; Agilent Ion-Mobility Q-TOF 6560) has been described. Their identification in samples was based on accurate mass (*m/z*), isotopic pattern matching and accurate mass of MS/MS fragments. The acceptable mass error of potential elemental composition for molecular ion was ± 5 ppm. LC–HRMS/MS mass spectra (acquired in full-scan mode) have been processed with database of metabolites that was created based on a survey in available literature on pesticide metabolism in plants and consideration of common metabolic reactions. Final identification of potentially present metabolites was based on accurate mass of fragment ions. In the MS/MS spectrum of metabolites the occurrence of 3 groups of fragments has been searched: (i) diagnostic ions, (ii) fragments detected in the MS/MS spectrum of the parent pesticide and as well as its metabolites and (iii) fragments characterizing a part of molecule with metabolic modification, not detected in the MS/MS spectrum of the parent pesticide. For identification of conjugates of parent pesticides or their metabolites searching of neutral losses (e.g. hexoses) in fragmentation mass spectra has been performed. Using this approach 44 metabolites originating from 24 pesticides were identified in samples of grapevine (vine leaves and grapes) and wines. Since pesticide residues occur in treated plants as well as their metabolites, the strategy based on simultaneous analysis of pesticide metabolites and pesticide residues could represent a new approach for verification of adherence the principle of organic production (e.g. analysis related to certification or inspection of the organic products).

Keywords: *pesticide metabolites, LC-HRMS/MS, pesticide residues, grapevine, organic farming*

Acknowledgement: *This work was supported by "Complex strategies for effective detection of food fraud in the chain production-consumer" (QJ1530272) from the Ministry of Agriculture of the Czech Republic. This work was also supported by the "Operational Programme Prague – Competitiveness"*

(CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). Financial support from specific university research (MSMT No 20-SVV/2017). Authors thank to Central Institute for Supervising and Testing in Agriculture (Brno, Czech Republic) for the provided samples.

P37
THE USE OF A DEACTIVATED (BAFFLED) PTV INJECTOR LINER AND GCMS/MS METHOD FOR THE QUANTITATIVE DETERMINATION OF CAPTAN, FOLPET AND THEIR METABOLITES IN ETHYL ACETATE EXTRACTS OF FRUIT SAMPLES

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SASA (Science and Advice for Scottish Agriculture) is one of the United Kingdom's (UK) official laboratories and we participate, on behalf of the Scottish Government, in the annual UK and EU statutory surveillance programmes that monitor various UK and imported food & drink for residues of pesticides, their metabolites and other degradation products. It is mandatory for official UK laboratories to analyse all pesticides listed in the EU multiannual control programme and to also participate in UK and EU proficiency testing schemes.
SASA routinely screen for captan and folpet and over 100 other pesticides using an ethyl acetate multi-residue extraction followed by GCMS/MS analysis. These pesticides are notoriously challenging to analyse since they can degrade to their metabolites tetrahydrophthalimide (THPI) and phthalimide particularly during GC injection. However, use of a deactivated baffled PTV injection liner minimises degradation.
The EU pesticide residue definitions for captan and folpet changed in 2016 to include their metabolites tetrahydrophthalimide (THPI) and phthalimide, respectively. Consequently, THPI and phthalimide were included in the SASA GCMS/MS multi-residue method as a screen approach only. Separate THPI and phthalimide standards were used to monitor degradation and quantify any residues.
In the poster we will demonstrate the success of this new approach by providing quantitative results from retail samples of cherries which were analysed as part of the 2017 UK annual surveillance programme. Preliminary results from EU proficiency test EUPT-SRM12-strawberry will also be presented.

Keywords: *pesticides, GCMS/MS, PTV, captan, folpet*

P38
A RAPID METHOD FOR THE ANALYSIS OF CHLORATE AND PERCHLORATE BY TWO DIMENSIONAL (2D) LC-MS/MS IN FOOD PRODUCTS

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The presence of chlorate and perchlorate residues in food has been an ongoing concern in Europe. Chlorate enters the food chain through washing or disinfecting products using chlorinated water. Perchlorate occurs naturally in the environment and also can be formed in the atmosphere and precipitate into soil. The EFSA published a Scientific Opinion on the risks to public health related to the presence of perchlorate in food, in particular fruit and vegetables. Subsequently the UK Food Standards Agency (FSA) recommended monitoring chlorate and perchlorate levels in the food supply chain. Of the European Reference Laboratory methods published by the EURL-SRM website that are available for chlorate and perchlorate analysis the EURL methods (QuPPe-Method Version 9.2) suggest two options that use either the Hypercarb column (M 1.3) or the Acclaim Trinity P1 column (M 7), although for the latter method there are as yet no example chromatograms. We were interested in the new method on the Acclaim Trinity P1 column as it appeared to offer a rapid analysis, however the method was heavily influenced by matrix and was not sufficiently robust for our purposes in our initial investigations. To overcome these limitations we introduced some modifications to the method most significantly a second chromatographic dimension for the analysis. This approach utilises a simple heart-cutting technique that initially separates the analytes on a HILIC-type phase before transferring on to the Acclaim Trinity P1 column. This significantly reduces the matrix that would otherwise accumulate on the second column and allows for rapid analysis. The method has now been developed and validated according to the SANTE guidelines and is suitable for delivery fast and accurate results, which is key to high volume testing laboratory. The developed analytical method was applied to fruits, vegetables and dairy products using ^{18}O stable isotope labelled standards. Samples were selected based on risk assessment and likelihood of contamination which was indeed confirmed for some. The changes in the analysis procedure helped with eliminating the mass trace chlorate impurity peak which was observed in the perchlorate standards in the reference method. Most of the extracts can be injected directly on the instrument; this is however dependent on the expected residue levels. The lower injection volume decreased the amount of the matrix injected on the instrument, eliminated interferences and contamination problems which led to improved robustness and productivity. The quick extraction in combination with the shorter run time means that results can be ready within hours of sample receipt. The linear range used was 0.005–0.02 mg/kg. Average recoveries are within the SANTE advised range of 70-120%. The method has been successfully implemented and allowed the laboratory to provide fast and reliable results which is critical when dealing with high volumes of perishable products.

Keywords: *chlorate, perchlorate, 2D LCMS/MS, residues, contaminants*

P39
DEVELOPMENT OF A CERTIFIED REFERENCE MATERIAL FOR SELECTED PESTICIDES IN CUCUMBER

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One of the most difficult elements during analytical method validation is the bias estimation. This difference between the measured value and the true value can be evaluated by analysis of a suitable Certified Reference Material (CRM).
In the field of pesticide residue analysis the availability of matrix CRMs is very low for a number of reasons. Consequently laboratories are applying other alternative laborious approaches such as spiking of the different commodities to estimate pesticide recoveries. The European Commission; Joint Research Centre, accredited for the production of reference materials, is working to provide CRMs in this area, as they are ideal tools to assess and control the quality of the analytical methods.
Cucumber was chosen as a matrix representing high water content commodities of plant origin. The CRM material was spiked with 16 different pesticides and was processed to significantly reduce its water content and stored at -70 °C to preserve the stability of the pesticides in the matrix over time. An interlaboratory comparison was recently conducted to establish the certified values for the pesticides in the cucumber material. Seventeen laboratories were carefully selected fulfilling quality requirements for their participation in the campaign. The selection of the target pesticides and the results for the interlaboratory comparison will be presented and discussed, as well as the process for assignment of the certified value.

Acknowledgement: *Prof. Amadeo R. Fernandez-Alba and the EURL for pesticides in fruits and vegetables are acknowledged for their contribution during the selection of target pesticides for this project.*

P40
ROUTINE QUANTITATIVE METHOD OF ANALYSIS FOR PESTICIDES USING GC-ORBITRAP MASS SPECTROMETRY IN ACCORDANCE WITH SANTE/11945/2015 GUIDANCE

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The analysis of pesticide residues in food is challenging because of the high number (typically >800) of substances that need to be analyzed in a diverse range of complex matrices, at low cost and with a fast reporting time. In order to achieve this within a routine environment, sensitive and selective LC and GC triple quadrupole MS systems are used. However, when such large numbers of compounds need to be analyzed it is an advantage to employ a system with full scan acquisition providing the performance is similar to that of triple quadrupole techniques. A generic acquisition based on full scan MS is more straightforward and provides additional information compared with multiple reaction monitoring by triple quadrupole mass spectrometry. It also increases the scope of the analysis, as target compounds are selected post acquisition. In order to obtain sufficient selectivity in the full scan mode, high resolution/high mass accuracy MS instruments are required.
In this study, the quantitative performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS system was evaluated for the routine analysis of GC-amenable pesticides in leek, orange and tomato matrices. In total 99.3% of the 153 pesticide/matrix combinations were detected below the respective maximum residue limits (MRLs) with excellent linearity and in compliance with the SANTE/11945/2015 method performance criteria. Quantitative linearity was assessed using matrix matched standards across a concentration of 0.5-500 µg/Kg. In all cases, the coefficient of determination (R2) was >0.99 for each pesticide from its LOD to 500 µg/Kg in the three matrices. Importantly, the scope of the analysis is increased by acquisition in full scan with targeted data processing with a compound database. Acquisition using 60,000 FWHM resolving power (at m/z 200) reduces matrix interferences and increases confidence in results when screening for pesticides in complex sample matrices. Sub ppm mass accuracy was achieved for all compounds over a wide concentration range ensuring that compounds are detected with confidence at low and high concentration levels. Repeated injections of a tomato matrix at 10 µg/Kg showed that the system is able to maintain a consistent level of performance (RSD% <6%) over an extended period of time as is demanded by the routine testing laboratories.

Keywords: *Orbitrap, pesticides, HRAM, GC-MS, non-targeted*

P41
RESIDUE ANALYSIS OF INSECTICIDE IN HONEY AND MELLIFEROUS PLANTS USING A MODIFIED QUECHERS METHOD WITH LC/MS/MS

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Chemical protection of plants is an indispensable part of modern agriculture. However, it may cause side effects, such as poisoning of beneficial organisms or occurrence of residues of plant protection chemicals in melliferous plants and indirectly in apiculture products. The development of rapid and reliable method to determine insecticide residues in melliferous plants and honey are essential for determining their role in problem of incidental cases of honeybee poisoning by pesticides. The aim of this work was to develop and validate a rapid, sensitive and selective method for determination of 132 insecticide residues in melliferous plants and honey samples using modified QuEChERS/LC/MS/MS. Different parameters affecting the extraction performance of the method such as amount of sample, type of the extraction solvent and the type of clean-up sorbents were thoroughly investigated. The effectiveness of the clean-up was tested for various sorbents (Florisil, alumina, PSA, C18, C8). The method was validated through linearity, LODs, accuracy and precision and the matrix effect on the honey and melliferous plants.

Keywords: *honey, melliferous plants, insecticides, QuEChERS, LC/MS/MS*

Acknowledgement: *This work was supported by Ministry of Science and Higher Education (Ministerstwo Nauki i Szkolnictwa Wyższego), project ID:SBI-01.*

P42
DISSIPATION KINETICS AND HAZARD QUOTIENT (HQ) ASSESSMENTS OF IMIDACLOPRID AND ITS METABOLITES IN FRUITING VEGETABLES UNDER GREENHOUSE CONDITIONS ESTIMATED BY MODIFIED QUECHERS/LC–MS/M

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Fruiting vegetables represent a broad variety of plants, including peppers, tomatoes, cucumber which forms an important component of the human diet. They are rich sources of minerals, vitamins, essential amino acids, sugars and dietary fibers. However, pests are still one of the leading factors threatening *Solanacea* or cucurbits with edible peel production. Therefore, a large number of pesticides are applied to control these pests, however increasing the potential risk for human exposure.
The degradation behavior and dietary intake risk of imidacloprid and their metabolites (imidacloprid, imidacloprid-guanidine hydrochloride, imidacloprid-olefin and imidacloprid-urea) in leaves and fruiting vegetables grown in greenhouse were investigated. A simple, rapid analytical method for the quantification of neonicotinoids residues in fruiting vegetables was developed using modified QuEChERS/LC–MS/MS. The effectiveness of the clean-up was tested for various sorbents or mix of sorbents: GCB, C8, PSA, C18, diatomaceous earth, VERDE, ChloroFiltr and Chitosan.
The dissipation of imidacloprid and its metabolites was described according to a first-order (FO) kinetics equation with R^2 between 0.9155–0.9868. The results showed that the time after 50% (DT_{50}) of the substance degraded was different for leaves and vegetables. The results of dietary intake assessment indicated that the dietary intake of the imidacloprid and its metabolites from tomatoes and cucumbers for consumers were acceptable. This study would provide more understanding of residue behavior and dietary intake risk by imidacloprid used under greenhouse conditions.

Keywords: *pesticide and metabolites, fruiting vegetables, sorbents, LC/MS/MS, QuEChERS*

Acknowledgement: *This work was supported by Ministry of Science and Higher Education (Ministerstwo Nauki i Szkolnicwa Wyższego), project ID:SBI-01.*

P43
ACHIEVING FAST AND EFFICIENT SEPARATIONS USING SUPERFICIALLY POROUS PARTICLE COLUMN TECHNOLOGY

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Superficially porous particles (SPP) have been proven to provide fast and efficient separations. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. When combined with highly selective stationary phases, the result is significant improvements in efficiency and sensitivity over fully porous particles (FPP) of similar dimension.
Through various experiments we hope to demonstrate the advantages of SPP columns over columns packed with traditional 5 µm and 3 µm FPP particles. When used in the development of new assays, SPP particles allow fast run times and excellent method performance without changes in instrumentation.
The performance of SPP particle columns will be demonstrated on a variety of applications, including aflatoxins, pesticides, and veterinary drugs. Run time, signal to noise ratio, and resolution will be evaluated in these experiments.
The versatility of these columns makes them ideal for separations in animal health, food safety, and other testing areas. For example, when it comes to analyzing toxic substances in agricultural matrices, such as aflatoxins in wheat, speed is of paramount importance. Four aflatoxins can be eluted in less than 1.5 minutes using Ammonium formate and formic acid modified mobile phases and mass spectrometry detection.
Restek LC columns offer the speed of superficially porous particles with the resolution of the highly selective stationary phases, allowing peak separation and faster analysis times to be achieved without expensive UHPLC instrumentation.

Keywords: *Restek, pesticides, veterinary drugs, aflatoxins, Raptor SPP, superficially porous, LC, HPLC, UHPLC, LCMSMS*

P44
RAPID LC-MS/MS METHOD FOR THE ANALYSIS OF INSECTICIDES AND ASSOCIATED METABOLITES IN EGG AND OTHER POULTRY PRODUCTS IN RESPONSE TO A CONTAMINATION CRISIS

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During August 2017, fipronil was detected in eggs produced from poultry farms and Belgium and The Netherlands. 15 other European countries were also affected prompting the recall of millions of eggs from human consumption. Fipronil, a broad spectrum insecticide which belongs to the phenylpyrazole chemical family, is used in the application of red mite, flea, cockroach and ant control. It is the main active ingredient in many flea prevention pet care products. The World Health Organisation (WHO) has classed Fipronil as class II moderately hazardous pesticide. For eggs, European legislation sets the maximum residue limit (MRL) in Regulation (EC) No. 396/2005 at 0.005 mg/kg. The European Food Safety Authority (EFSA) defines an Acute Reference Dose (AFrD) of 0.009 mg/kg body weight. AFrD refers to the maximum amount of a substance that can be ingested with no health hazard. At 0.72 mg/kg, the EU Commission proposes this level of contamination of fipronil could present an acute health risk.
Herein, we describe a fast and sensitive multi-component single method using LC-MS/MS for the detection and quantitation of fipronil and its associated metabolites, along with amitraz and its associated metabolites in eggs and other poultry products. Both compounds are used as insecticides on control of fleas and ticks. The developed assay uses a modified QuEChERS sample preparation method for the extraction of the egg and poultry matrices. Chromatography was performed using a reversed phase water/methanol gradient at a complete runtime of 7 mins injection to injection, using a Phenomenex Kinetix Polar C18 column. Mass Spectrometry is performed on a SCIEX 6500+ Triple Quadrupole LC-MS/MS instrument using electrospray ionization, scheduled MRM detection with simultaneous positive/negative ionization switching throughout the run. Results from the assay easily meet the EU regulation MRL in terms of LODs, LOQs, signal to noise, MRM ion ratio accuracy and CV and we will show data to highlight such on both spiked samples and 'real' samples where positive results were found.

Keywords: *residues analysis, LC-MS/MS, QuEChERS, scheduled MRM, egg matrix*

P45
A NOVEL DATA-INDEPENDENT ACQUISITION STRATEGY FOR NON TARGETED, ACCURATE MASS CONTAMINANT SCREENING

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Companies and environmental regulatory authorities continue to investigate High Resolution Mass Spectrometry (HRMS), non-targeted, screening techniques to expand the scope of their screening methods. Improvements in mass spectrometer sensitivity and highly selective acquisition techniques, alongside advancements in the informatics used to process and review data, are facilitating the task.
Several fruit and vegetable samples, previously characterized by a collaborator, were screened against a pesticide library using several Data-Independent-Acquisitions (DIA). DIA strategies for complex mixture analysis, particularly within a contaminant screening environment offer significant efficiency advantages in that they enable a generic, non-biased strategy for data acquisition. The work will discuss the use of a novel DIA method – SONAR, in which precursor and product ion data are acquired with a sliding quadrupole window - for use in accurate mass screening applications and its use alongside more traditional DIA strategies such as full scan low and high collision energy acquisition (MS^E), and its ion-mobility enhanced variant ($HDMS^E$).
A comparison of the outcomes from MS^E, $HDMS^E$ and SONAR screening sets will be presented.
All techniques were able to detect contaminants present. However, the comparison of each technique with increasing matrix complexity shows that the ability of non-selective full scan experiments (MS^E) to generate clean product ion spectra is reduced when compared to $HDMS^E$ and SONAR datasets.

Keywords: *data-independent-acquisition, non-targeted screening*

P46
OVERCOMING THE CHALLENGES OF ANALYSING IONIC POLAR PESTICIDES IN FOOD

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Glyphosate is a non-selective broad spectrum herbicide, which accounts for more than half of global herbicide sales. While discussions on the toxicological concerns of glyphosate and associated compounds continue, maximum residue limits (MRLs) are enforced globally, requiring continued analytical testing to ensure consumer safety. The chromatographic analysis of glyphosate, its metabolites and similar compounds can prove challenging due to the lack of retention by reverse phase techniques. Common alternatives include derivatization and ion chromatography. However, due to time-consuming sample preparation, MS incompatible solvents and the need for specialized equipment and/or reagents, the underivatized LC-MS/MS approach is still preferred. Due to the physicochemical characteristics of these compounds, repeatable peak shape and robust methodologies can be challenging to run routinely.
In the poster, a simple UPLC-MS/MS method will be presented for the direct analysis of highly polar pesticides, which gives sufficient retention and provides excellent sensitivity, robustly, to exceed enforced MRLs. A panel of representative anionic polar pesticides, including aminomethylphosphonic acid (AMPA), glufosinate and glyphosate have been targeted in a selection of relevant foodstuffs prepared using a modified version of the Quick Polar Pesticides (QuPPe) extraction method. Chromatographic separation was achieved on a novel weak ion exchange/ hydrophilic interaction liquid chromatography (HILIC) column, applying an ammonium formate mobile phase gradient.
Method performance was evaluated, in the absence of isotopically labeled internal standard, by assessing chromatographic repeatability, linearity, accuracy and sensitivity. Satisfactory linearity was found for all pesticides over a range of 0.0001 to 0.250 mg/kg in all matrices (R^2 > 0.995, residuals < 20%). The repeatability (%RSD) of the method was determined using spiked matrices, prepared in replicates. Incurred residues detected in certain samples were quantified using standard addition calibration curves and identified in accordance with the SANTE guidelines 11945/2015.

Keywords: *polar pesticides, glyphosate, UPLC-MS/MS, HILIC*

P47
CLEAN-UP OF OAT EXTRACTS FOR GC-MSMS PESTICIDE RESIDUES ANALYSIS BY PSA, C18, Z-SEP AND/OR EMR-LIPID

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The level of co-extracted matrix in cereal extracts obtained by the QuEChERS method (EN 15662) is relatively high. Recently we found that dSPE with PSA (primary secondary amine) efficiently reduce the amount of co-extracted matrix in cereal extracts and that more PSA (150 mg/ml extract) is needed for efficient reduction of the matrix in oat extract than in e.g. wheat extracts (25 mg/ml extracts), thus significantly more than according to CEN 15662. Though, even though oat extracts are cleaned with 150 mg PSA/ml extract interfering matrix still occur in such amounts that it compromises the robustness of the GC-MSMS analysis for some analytes. Recently new products, using other types of sorbents, have become increasingly popular for clean-up of problematic matrices, i.e. zirconium based sorbents (Z-sep) and more recently the product Enhanced Matrix Removal–lipid (EMR-lipid).
We have therefore studied whether a modified QuEChERS method, including an optimised clean-up procedure employing more than one sorbent, could be suggested for the analysis of pesticides in oat by GC-MSMS. Various combination of the sorbents PSA, Z-sep, EMR-lipid were tested for their ability to provide acceptable analytical results for around 100 pesticides in oat.
To test the effect of the individual clean-up sorbents, but also the effect of combining two or more sorbents, a four factor 2-level factorial experiment (full resolution) was setup. Such a full factorial experiment involves 16 different combinations of the four sorbents. Sixteen samples of oat flour in double determinations were spiked with 101 pesticides at a level of 0.02 mg/kg. For PSA, C18 and Z-sep sorbent the standard extraction and clean- up procedure at EURL-CF was employed. For EMR-lipid, the procedure described by the producer was employed. Analyses of the final extracts were performed on GC-MS/MS. The two clean-up procedures that in this factorial experiment were found to provided acceptable recoveries for the highest number of pesticides were selected and validated for more than 100 compounds at one spike level in oat employing 6 replicates for determination of recovery and RSDr. The performance of these two alternative clean-up procedures were compared with that of the CEN 15662 (i.e. 25 mg PSA/ml extract) and the procedure suggested in our recent study (i.e. 150 mg PSA/ml extract).
The study setup and the obtained results will be presented on the poster.

Keywords: *dispersive solid phase extraction, difficult matrices, pesticide residue analysis*

P48
CONTROL OF PESTICIDE RESIDUES IN ONION AND CABBAGE SAMPLES ON THE MARKET OF THE REPUBLIC OF SERBIA IN 2016

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Republic of Serbia has "Regulation of the maximum residue limits in pesticides in food and feed and about food and feed which set the maximum residue limits of pesticides" which adapted the MRLs values to the valid MRLs in the European Union. Our studies comprised the determination of the pesticide residues content in 54 cabbage and 50 onion samples from the market of the Republic of Serbia taken during 2016, by liquid chromatography coupled to tandem mass spectrometry – LC-MS/MS in the ESI+ mode. The samples were tested regarding the content of 90 pesticides. The validation procedure for the method was in accordance with SANTE/11945/2015 and it was carried out using blank onion and cabbage samples spiked with a pesticide mix solution at four levels: 0.01, 0.02, 0.1 and 0.2 µg/mL, with carbofuran–D3, acetamipride-D3 and carbendazime-D3 as the internal standards. The obtained R^2 values for all the investigated pesticides (in onion and cabbage) were higher than 0.99. The recovery data were obtained by spiking blank samples at two concentration levels (0.01 and 0.10 mg/kg) in five replicates, yielding recoveries in the range of 70–125%. The precision values expressed as a relative standard deviation (RSD) were lower than 20% for the intraday precision. The LODs were calculated using the Agilent MassHunter B.04.00 software, and the LOQs were experimentally set at 0.01 mg/kg. The most frequently detected pesticides in cabbage samples were clothianidin, pyrimethanil, metalaxyl, azoxistrobin and thiamethoxam. In onion samples, the most abounded pesticides were metalaxyl-M, dimetomorph, spinosad and spirotetramat. The detections were below the MRLs. These results indicate that the producers have started to apply GAP, but not in all the segments since the detection of pesticides which are not registered for use in the Republic of Serbia were applied.

Keywords: *pesticide residues, cabbage, onion, LC-MS/MS*

Acknowledgement: *The authors acknowledge the financial support of the Ministry of Education and Science, Republic of Serbia, Project Ref. TR31036.*

P49
MULTIRESIDUE PESTICIDE ANALYSIS OF KALE AND GRAPES USING QUECHERS AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY

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Kale and Grapes are included in the USDA's national pesticide residue monitoring program for produce and are in increased demand and consumption globally. The use of a QuEChERS technique followed by LC-MS/MS has proven to be an effective approach for screening multiresidue pesticides from various produce items including kale and grapes. Following the EN 15662 pesticide residue method using roQ™ QuEChERS extraction kit and clean-up dSPE kit, along with the use of a high efficient and wide polarity Kinetex®5 µm Biphenyl Core-Shell HPLC column, a pesticide screening method was developed using LC-MS/MS in under 16 minutes. Over 100 Pesticides were screened at concertation ranges between 0.05 ppm to 1 ppm with a recovery range of 70-130%. The method produced excellent recoveries and selectivity for the earlier eluting polar pesticides owing to the biphenyl phase functionality of the Kinetex Biphenyl column.

P50
IDENTIFICATION AND DETERMINATION OF 153 PESTICIDES IN FRUITS AND VEGETABLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY

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Modern production is almost impossible to imagine without chemical means of protection. A large number of different pesticides are used to protect agricultural products during production, storage and transportation. Now the list of pesticides that can be used includes more than 1700 different active substances and more than 350 of their esters and salts. On the territory of the Eurasian Customs Union, there are MRLs for 573 pesticides, while for many products it is necessary to determine the residual quantities of 100 or more pesticides. So, for example, for grapes it is established MRL for 154 pesticides.
Currently, the determination of residual amounts of pesticides is increasingly carried out using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). The extraction of the desired components in this process is carried out with acetonitrile in the presence of buffering salts. Purification of extracts from lipids, fats and proteins is carried out with bulk sorbents Bondesil-PSA, C18, graphite black, ion-exchange resins and their combinations.
The use of QuEChERS sample preparation does not allow the use of a unified approach for different types of products and when analyzing extracts on mass spectrometric detectors, a strong matrix effect is revealed - from 12 to 2490%.
A method for the determination of 153 pesticides in fruits and vegetables by the method of high-performance liquid chromatography tandem quadrupole-time-of-flight mass spectrometry using acetonitrile extraction without the use of QuEChERS is proposed. The ranges of determined pesticide contents were 0.01-2 mg/kg. The relative standard deviation of the analysis results does not exceed 4-18%. The effect of the matrix is practically reduced to zero. The duration of the analysis is 20-30 minutes.

Keywords: *pesticides, HPLC, time-of-flight, fruits and vegetables*

P51
DEVELOPMENT OF MULTI-RESIDUE PESTICIDES DETERMINATION METHOD IN BROWN HARE KIDNEY

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Agricultural intensification has resulted in the increased mechanization, the use of chemical compounds, and in dramatic changes of landscape characteristics such as the decrease in permanent vegetation cover, the increase in field size and the reduction of habitat diversity. The occurrence of pesticide poisoning in wildlife is a matter of species susceptibility (hazard of a compound) and likelihood of exposure. The aim of this work was to develop a liquid chromatography tandem mass spectrometry method (LC-MS/MS) for the simultaneous identification and quantification of 70 pesticides in brown hare kidneys. The validation was done in accordance with SANTE/11945/2015 by triple quadrupole mass spectrometer (Agilent 6410B Triple Quad Mass Spectrometer, USA) in positive electrospray ionization using multiple reaction monitoring mode (MRM) with the QuEChERS extraction of pesticides. The method was validated for accuracy, precision, linearity, limits of detection and limits of quantification (LODs and LOQs). The calibration was performed as MMC. The calibration range was from 0.01 to 0.1 µg/ml. The obtained R^2 was higher than 0.99 for all the studied pesticides. The LOQs were set on 0.01 mg/kg. For the recovery, the samples were spiked with the analytes at three concentration levels (0.05, 0.1 and 0.2 mg/kg). The average recoveries for all analytes were in the range from 64.7 to 117.8% (RSDs 8.24–19.14%). The obtained mean values of the responds were with RSD <20%. An efficient, sensitive and reliable LC-MS/MS method has been developed which can be applied in the analysis of real samples.

Keywords: *method validation, pesticides, brown hare kidney, LC-MS/MS*

Acknowledgement: *The authors acknowledge the financial support of the AP Vojvodina, the Republic of Serbia, Project Ref. 104-401-4794/2016.*

P52
DETERMINATION OF THE GLYPHOSATE RESIDUE IN BEER ON SERBIAN MARKET

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According to the National Beer Law ("Official gazzette RS", no. 30/2010) hops, barley malt, unsweetened raw materials and water used in beer production are subject to the compulsory quality testing in the view of physical, chemical and microbiological parameters and the presence of residues of plant protection products, biocides or contaminants. The pesticides can also be found in beer mostly as contaminants in brewing water and grains. In particular the herbicide glyphosate has to be monitored carefully since it is widely used on crops such as wheat, oats, edible beans and other crops. Glyphosate [*N*-(phosphonomethyl)-glycine] is a polar herbicide used in agriculture and horticulture but also commonly used around homes and gardens. Its usage was approved by the European Commission in 2002. Glyphosate is a total, non-selective and post-emergent herbicide, developed in 1971 by the Monsanto Company. This herbicide is among the most sold single crop-protection chemical products on the market. Glyphosate is a topic with an extraordinary degree of public attention and concerns since the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, classified glyphosate as a probable human carcinogen. The chromatography of glyphosate is challenging because of its high polarity. A well-established method including a rapid extraction with water (with 0.1 M KOH) and aliquot derivatisation that involves the step with 9-luorenylmethylchloroformate (FMOC) in borate buffer and detection based on liquid chromatography coupled to electrospray tandem mass spectrometry (LC–ESI-MS/MS). This method made a rapid and sensitive analysis [limit of quantification (LOQ) 10 μg/kg] of the glyphosate in daily routine analysis possible. During 2017 we have analyzed 14 beer samples and none of the samples have been contaminated by glyphosate.

Keywords: *glyphosate residue, beer, LC-MS/MS*

P53
THE MULTI-RESIDUE ANALYSIS OF 14 PESTICIDES IN STRAWBERRY, BELL PEPPER, KOREAN CABBAGE BY QUCHERS AND LC-MS/MS

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This study was conducted to develope for the QuChERS method of the 14 pesticide residues in strawberry, bell pepper, Korean cabbage by LC-MS/MS.
The 14 pesticides were spiked at two levels of 0.01 and 0.1 mg/kg. The pesticides were extracted from each sample by using acetonitrile, a QuEChERS EN 15662 method packet, and analyzed by LC-MS/MS in ESI mode. Standard calibration curves were made by matrix matched standards and their correlation coefficients were higher than 0.995. The recovery of 11 pesticides were in the range of 70-130% with <20% CV except 3 pesticides, pymetrozine, propamocarb, and bifenazate. The recovery rates of pymetrozine in strawberry, bell pepper, Korean cabbage were 8, 67 and 63%, respectively. Propamocarb in strawberry was 61% and bifenazate in bell pepper was 40%. The recoveries of 3 pesticides are influenced by the pH of the extract and QuEChRES method was modified in various ways. AOAC 2007.01 method packet was used for the extraction instead of EN 15662 method packet to rase the recovery rates of pymetrozine and propamocarb. The recoveries of two pesticides were 113, 106, 92 and 101%, respectively. Bifenazate in bell pepper was extracted with acetonitrile and conc. sulfuric acid 0.1 ml and the recovery was 92%.
The results indicated that the method using QuEChERS and LC-MS/MS could be applied for 11 pesticide residues in strawberry, bell pepper and Korean cabbage. But the QuEChERS was needed to be modified for some pesticides.

P54
DEVELOPMENT OF AUTOMATED SAMPLE PREPARATION METHOD FOR PESTICIDE ANALYSIS IN BABY FOODS USING SOLID PHASE MICROEXTRACTION WITH ANALYSIS BY GC-MS

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Solid phase microextraction (SPME) is a technique that can be used for the analysis of a wide variety of analytes in many different sample matrices. For analytes of low volatility, such as pesticides, immersion of the SPME fiber into sample is required for extraction. High background samples, such as many foods, pose a challenge due the presence of fats, sugars, pigments and other macromolecules. These can stick to the fiber and reduce its usable life and/or be transferred to the GC, where they may interfere with chromatographic analysis. If the challenges of the immersion technique are resolved, sample preparation via SPME may be simplified and streamlined with the use of automation.
In this work, data illustrating the development of an SPME method for the GC/MS analysis of pesticide residues from baby food, a heavy background food sample, will be presented. Specifically, this presentation will focus on the advantages of using an overcoated versus a standard SPME fiber. Ruggedness of the SPME method was found to be improved through the use of the overcoated SPME fiber in combination with a post-extraction wash step. Optimization of parameters in the SPME method, such as sample pH adjustment, salt addition, sample dilution, and extraction temperature will be described. Results will be presented showing the final SPME method applied to samples of pureed prune baby food spiked with pesticides included on the list described as part of EU directive 2006/125/EC.

Keywords: *SPME, pureed prune, pesticides, baby food*

P55
TRANSFERRING ANALYSIS OF MULTIPLE PESTICIDES TO A NEW GC-MS/MS PLATFORM WITH AN EFFICIENT ION SOURCE

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The use of pesticide preparations aimed at food crops protection has become, unfortunately, a common practice in conventional farming. In spite of the effort to minimize the extent of treatments by using various complementary measures, pesticide residues might be transferred into human food chain, thus posing a health risk for consumers. With regards to a high number of residues potentially occurring in foodstuffs, the availability of analytical method for screening of multiple analytes representing various pesticide classes is urgently needed. To assure meeting reliable pesticide residues analysis, criteria defined in SANTE/11945/2015 document have to be met.
In our study, the routine multiresidue GC-MS/MS method employing Agilent 7000 series MS detector was transferred to a new instrumental platform employing new 7010 series detector with a high efficiency electron ionization (EI) source declared by producer to be significantly more sensitive, approx. by one order of magnitude. A comprehensive validation study with more than 300 pesticides in plant matrix was performed. To eliminate the need to prepare matrix matched standards, analytical protectants (D-sorbitol, D-Glucurono-6,3-lactone, 3-Ethoxy-1,2-propanediol) were used. The generated validation data, when compared with those obtained by the previous system, showed superiority in most performance characteristics. Specifically lower LOQs were achieved, although the equivalent of matrix introduced into system was lower compared to until that time used routine method. Worth to notice that the dynamic MRM data acquisition mode (as compared to segment MRM) resulted in a further improvement of peak shapes, thus more reliable quantification. This presentation will critically assess the obtained data against criteria laid down in ´Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed´ mentioned above.

Keywords: *pesticide residues, GC-MS/MS, new EI source, multiresidue method, validation*

Acknowledgement: *This work was supported from specific university research (MSMT No 20-SVV/2017)" and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

P56
DETERMINING PESTICIDE RESIDUES IN EXPOSURE VIAS FOR BEES IN A SEMI-FIELD STUDY

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In this study, two field experiments were designed to evaluate PPP (plant protection products) residues for examining honey-bees exposure via pollen and nectar from treated apple-trees. The onus was to assess potential exposure vias and concentration levels of systemic insecticides, under field-scale application of foliar treatment. Field experiments were carried out considering the common agronomic practices applied. One goal was to evaluate residues of four treatments with acetamiprid, flonicamid, imidacloprid and thiacloprid. And, under a tent test, the exposure for bees, using mini hives, was evaluated after treatment with imidacloprid; whether this systemic insecticide is persistent and detected in pollen and nectar after spray application. A battery of samples was collected periodically depending on the stage of flowering when aprox. 10, 50 and 80% of the flowers have opened.
For monitoring of PPP residues, extraction procedures were optimized and a validated analytical method with LC-TQ-MS. With the extraction procedures based on QuEChERS, acceptable recoveries were obtained at 5 µg Kg^{-1}, within the 70-120% range, and the instrumental LOQ was mostly 0.5 µg kg^{-1}. In the tent test, consisting of 3 plots, one of them acts as a control, residues of Imidacloprid were evaluated after 17 days of spray application corresponding to the stage of full flowering when aprox. 70% of the flowers were opened. No residues of imidacloprid were detected in nectar. In the 2 treated plots, a concentration between 3.1-4.7 µg kg^{-1} was detected in pollen. And, at this stage, the level of residues found inside the beehive, in beewax, was 1 µg kg^{-1}.
The field study was also carried out with 3 replicates of 30 trees per plot. The foliar treatment was applied before the flowering of the apple-tree. The level of residues was evaluated at different stages of the flowering period, observing a significant reduction of the residues. At the stage of full flowering when aprox. 80% of the flowers have opened, no residues of imidacloprid, acetamiprid, thiacloprid and flonicamid were detected in nectar. At this stage of full flowering, the reduction of residues in pollen collected from the flowers, was of 72% for acetamiprid, 75% for flonicamid and 84% for imidacloprid. The concentration detected in the pollen at this flowering stage was of 10.3; 11.5 and 4.7 µg kg^{-1}, for acetamiprid, flonicamid and imidacloprid, respectively.
The objective of this study was to determine the level of residues in nectar and pollen, as source of food for bees, and not the estimation of their consumption rates and potential effects. The aim was to contribute in the empirical determination of exposure vias and residue levels when foliar treatment is applied with conventional agronomic practiques.

Keywords: *pesticide residues, neonicotinoids, honeybee, LC-TQ-MS/MS, plant protection products*
Acknowledgement: *The authors wish to thank the financial support given by the national programme for research of INIA, project: RTA2013-00042-C10-02/ 01 and 04.*

P57
ANALYSIS OF GLYPHOSATE IN FOOD AND BEVERAGE SAMPLES BY ELISA AND LATERAL FLOW IMMUNOASSAY

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Samples of baby food, beer, cereals, grains, honey, pancake and corn syrup, soy milk, soy sauce, tofu, and wine, purchased in the Philadelphia, US metropolitan area were analyzed for glyphosate residue using ELISA and a field test lateral flow immunoassay.
The ELISA limit of quantification (LOQ) and range of the method were determined for baby food to be (7.5-400 ppb); beer (0.375-20 ppb); cereals and grains (7.5-400 ppb); honey, pancake syrup, and corn syrup (5 to 800 ppb); soy sauce, soy milk, and tofu (75 to 4,000 ppb); wine (0.375-20 ppb). Glyphosate residues above the limit of quantification were not found in pancake and corn syrup, soy milk, and tofu.
Results of a survey of the various samples tested will be presented, as well as comparison between the ELISA and the lateral flow immunoassay.

Keywords: *ELISA, glyphosate, honey, immunoassay, soy*

P58
APPLICABILITY OF BIOCHIP BASED IMMUNOASSAYS TO THE DETECTION OF PESTICIDES

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Introduction
Pesticides are broadly applied to kill pests and their application to agricultural crops have a negative impact on bees, which can contribute to the loss of bee colonies. The widespread release of pesticides into the environment can also contribute to the occurrence of pesticide residues in the food supply. Pesticides are globally regulated by various governmental agencies. The availability of screening methods allowing the detection of pesticides is then beneficial in test settings to monitor their presence to ensure food safety. This study reports the applicability of biochip based immunoassays to the detection of the pesticides, acetamiprid/thiacloprid, azoxystrobin, carbaryl, clothianidin, coumaphos, fenpyroximate, imidacloprid, and thiamethoxam.
Methodology
Immunogens were developed for acetamiprid/thiacloprid, azoxystrobin, carbaryl, clothianidin, coumaphos, fenpyroximate, imidacloprid and thiamethoxam. They were then administered to adult sheep on a monthly basis to provide target-specific polyclonal antisera. IgG was extracted from the antisera and evaluated via competitive chemiluminescent biochip-based immunoassays. The selected purified antibodies were immobilised on a biochip platform, which is also the vessel for the immunoreactions, and analysed using the semi-automated analyser
Evidence Investigator. Analytical parameters were evaluated.
Results
Initial evaluation of each immunoassay showed the following sensitivity values, expressed as half maximal inhibitory concentration (IC_{50}): 0.121 ppb (acetamiprid), 0.073 ppb (azoxystrobin), 0.335 ppb (carbaryl), 0.086 ppb (clothianidin), 0.172 ppb (coumaphos), 1.34 ppb (coumaphos-oxon), 0.060 ppb (fenpyroximate metabolite), 2.164 ppb (fenpyroximate), 0.017 ppb (imidacloprid), 0.062 ppb (thiacloprid), and 0.141 ppb (thiamethoxam). The intra-assay precision values, expressed as CV (%), were <15% for all the immunoassays when different concentration levels were assessed.
Conclusions
The data of this initial evaluation indicate that these new biochip-based immunoassays are applicable to the detection of the pesticides, acetamiprid/thiacloprid, azoxystrobin, carbaryl, clothianidin, coumaphos, fenpyroximate, imidacloprid, and thiamethoxam. Biochip array technology offers flexibility to incorporate multiple assays on the same biochip, thus up to 44 pesticides could be simultaneously detected, leading to consolidation of the screening process in test settings.

P59
MULTIMETHOD FOR HIGHLY POLAR PESTICIDES AND METABOLITES BY IC-MS/MS - FACING THE REQUIREMENTS FOR BABY FOOD AT 0.010 MG/KG REPORTING LEVEL

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Pesticides and their corresponding metabolites of highly polar and/or ionic nature are typically extracted by the QuPPe method (Quick Polar Pesticides Method) and the measurement accomplished by independent modules directed to the specific requirements of the pesticides of interest with regard to chromatographic and mass spectrometric conditions.
Though, the published methodology for the determination of several highly polar pesticides using stationary phases based on porous graphitic carbon, anion exchange or HILIC columns does not meet the requirements for baby food products according to the Regulation (EC) No. 609/2013 in compliance with the maximum residue level of 0.010 mg/kg specified in the German Ordinance on Dietetic Foodstuffs. Moreover, for the acquisition of a wider scope of polar pesticides the sample extract has to be analyzed multiple times using different chromatographic conditions and columns leading to an extended demand in analysis time and costs.
Therefore, we developed a new sensitive, robust and fast multimethod using IC-MS/MS for the determination of parameters like fosetyl, phosphonic acid, chlorate, perchlorate, ethephon, and 2-hydroxyethylphosphonic acid in plant-derived matrices in a single chromatographic run. For compensation of matrix effects and differences in recovery rates isotopically-labelled internal standards (ILIS) were used. The optimized method was fully validated at levels of 0.010 and 0.100 mg/kg-for all compounds in representative commodities like fruit and vegetables, cereals and baby food produce of different complexity according to the actual EU guidance document SANTE/11945/2015. By the means of this method, it was achieved for the first time to quantify target analytes like fosetyl and phosphonic acid at a reporting level of 0.010 mg/kg essential for organic and infant food commodities of plant origin.
The presented analytical method is suitable for LC-MS-MS systems without the need for extra devices such as ion electrochemical suppressor systems. So, the method can be easily implemented on usual LC-MS/MS systems for the use in routine food analysis.

Keywords: *phosphonic acid, fosetyl, IC-MS/MS, baby food, highly polar pesticides*

P60
ANALYSIS OF ETHEPHON RESIDUES BY A NOVEL 2-DIMENSIONAL LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY APPROACH

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A consequence of improvements in instrumentation over the last decade has been a trend to reduce sample clean-up steps to the point where there are effectively none, except perhaps dilution. In a contract laboratory environment there is also a requirement for ever faster analysis, which generally challenges the chromatographic separation. However sensitive a mass spectrometer instrument may be, if an analyte of interest is not ionized efficiently its detection will be problematic. The difficulty in analysing the highly polar pesticides, which have limited or no retention on conventional reverse phase chemistries, is quenching of the signal by matrix co-extractives that suppress ionisation particularly when there is no clean-up. For pesticides that were traditionally analysed by Single Residue Methods (SRMs) it is now common to use the so-called Quick Polar Pesticides method (QuPPe); a technique underpinned by the extensive work undertaken by the EU Reference Laboratories. This has encouraged laboratories to move to suggested solutions from the EURL such as specialised ion chromatography methods and the graphitic carbon columns, approaches that can have their drawbacks in a laboratory with many individual analyte tests on a single instrument. For ethephon analysis there are now many lquid chromatography solutions in the literature but none that appear to utilise 2-dimensional chromatography. To achieve a fast analysis with sufficient peak resolution from matrix, we have adopted a different approach and applied a simple heart-cutting 2-dimensional liquid chromatography method for the routine analysis of ethephon on QuPPe extracts. This approach allows us to reduce the analysis time to less than 9 minutes, using a reverse phase and anion exchange column combination. Critically only acetate buffers that do not contaminate the source are used. This approach, using orthogonal column chemistries for the chromatography, although not immune from some matrix effects, is robust and can be platform independent. Using an Agilent 6460 LC-MS/MS instrument with an additional pump a typical calibration ranges from 2ng/mL to 2µg/mL using solvent standards. Any recovery losses and matrix suppression is compensated for by using ethephon D_4 internal standard. The method is validated to ISO 17025 at 0.02mg/kg for all commodity types and has been in routine use for several years. The development along with the pros and cons of this novel approach will be detailed.

Keywords: *QuPPe-method, 2D-LC-MS/MS, pesticides, fruit matrix*

P61
ANALYSIS OF CANNABIS FOR PESTICIDE RESIDUES BY GC/Q-TOF AND GC/MS/MS

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In the US, 29 states have approved the use of medical cannabis and eight states, representing 65 million people, have approved recreational use by adults. Canada allows medical use and is on a path to full legalization. Because the US government still classifies cannabis as a schedule 1 drug, all legislation controlling the growing, testing and use of cannabis products is done at the state level. Many countries in Europe have decriminalized marijuana use and possession while others have legalized its medical uses. Pesticide residues on cannabis continues to be a hot issue in the press and among users of marijuana products. In the US, state regulations and their enforcement is a patchwork quilt with little uniformity among the states. Some states do little to enforce their pesticide regulations and many growers are not skilled in the use and application of pesticides. There have been many recalls and some growers have gone out of business because of pesticide contamination. Therefore, pesticide residue testing is important for the quality control of a product that may be eaten or inhaled. This paper describes the analysis of cannabis extracts for pesticide residues using an accurate mass high resolution GC/Q-TOF and an ultra-sensitive GC/QQQ. The GC/Q-TOF chromatograms were analyzed by applying a "Find by Fragments" approach using a personal compound database and library (PCDL) containing locked retention times and exact mass spectra for about 850 pesticides and contaminants. Sample preparation is a major challenge, but with sufficient dilution of extracts and instrument selectivity, pesticide residue analysis is possible. Sixteen confiscated marijuana samples were analyzed by GC/Q-TOF and were found to have an average of more than six contaminants in each. These included 22 different pesticides, three PAHs and two fire retardants. But, the preferred instrument for the analysis of GC-amenable pesticides is the GC triple quadrupole because of its sensitivity and selectivity. A 500-fold dilution prevented matrix interferences during the analysis.

Keywords: *cannabis, marijuana, GC/Q-TOF, GC/QQQ, pesticide residues*

Acknowledgement: *The project described was partially supported by National Institute on Drug Abuse, contract # N01DA-15-7793.*

P62
THE SEARCH FOR A ROBUST METHOD FOR GLYPHOSATE, AMPA AND OTHER POLAR PESTICIDES IN FOOD BY LC/MS/MS

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Glyphosate is the active ingredient in the popular herbicide roundup and is used throughout the world. Recently, its safe use has come into question. This has heightened the demand for a sensitive method at the low ppb level for food and even lower levels for environmental water analysis. LC/MS/MS using triple quadrupole technology can provide both the sensitivity and selectivity needed. Methods include derivatization with FMOC, derivatization with trimethylorthoacetate and then reversed-phase chromatography with triple quadrupole MS detection. Methods without derivatization include strong cation exchange and strong anion exchange with and without ion suppression technology after the separation. Additionally, mixed mode and ion pairing chromatography has been reported and even capillary electrophoresis. Each separation combination with mass spectrometry has advantages and pitfalls and each dictates their own requirements for sample preparation. This is further complicated by the phosphinic and zwitterionic chemistries of these compounds. The development of a method with a wide range of applicability to both polar pesticides and food samples of very different composition has been elusive. In this presentation, we will discuss these methods and our efforts to develop a singular robust procedure that will provide the sensitivity needed for food samples from acidic fruits to relatively neutral grains and spices. The advantages and disadvantages of the various methods that have been reported will be described along with the issues related to the chemistry affecting both the sample preparation and measurement technique.

Keywords: *LC/MS/MS, glyphosate, aminomethylphosphonic acid, glufosinate*

P63
CHALLENGES IN GC-MS ANALYSIS OF PESTICIDE RESIDUES IN CANNABIS PLANTS

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In the recent years, Cannabis has been a subject of interest not only for medical experts but also for food producers due to a high content of biologically active substances. At the same time, the need to control its quality and safety has grown significantly. Alike in case of many other crops, pesticides are applied for pre-harvest protection against pests. However, residues of these chemicals may have harmful effects on human's health. Pesticide residues analysis of hemp is a challenging task due to high matrix complexity and a long list of potential target analytes. In this analysis a crucial step is a clean–up procedure. The co-extracts could lead to various chromatographic problems, inaccurate results and also increased instrument downtime.
Within this study, the effectiveness of five clean-up procedures conceivable for involving in multiresidue determination of more than 200 pesticides in Cannabis sativa plants was critically assessed. The target analytes were extracted using a QuEChERS method, removing of abundant matrix co-extracts from acetonitrile phase was then realized by (i) freezing out, (ii) freezing out combined with dSPE using PSA and $MgSO_4$, (iii) liquid-liquid extraction using hexane, (iv) dSPE using PSA and (v) dSPE using PSA, C18 and GCB. Finally, identification/quantification of target analytes, new instrumental platform consisting of Agilent 7890B gas chromatograph coupled to high sensitive Agilent 7010 GC-TQ mass spectrometer (GC-MS/MS) was employed. The performance characteristics achieved were assessed against criteria stated in SANTE/11945/2015 document on analytical quality control and method validation for pesticides residues analysis in food and also quantification limits of the individual target analytes. The best results in terms of purification effect was achived by procedure (iv) above. Recoveries were in range 70–120% for 89% of analytes and LOQ ranged between 0.01–0.2 $mg.kg^{-1}$. 81% was less than 0.05 $mg.kg^{-1}$.

Keywords: *pesticide residues, multiresidue method, GC-MS/MS, hemp*

Acknowledgement: *This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 – No.: MSMT-43760/2015). This research has also received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692195.*

P64
FULLY AUTOMATED DERIVATIZATION AND QUANTIFICATION OF GLYPHOSATE AND AMPA IN BEER USING A STANDARD UHPLC-MS/MS SYSTEM

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Introduction
Glyphosate is currently one of the most common pesticides used worldwide. In spite of its approval by regulatory bodies all over the world, the concern about its harm to humans and the environment persists. Therefore, the strict control of Glyphosate and its metabolite Aminomethylphosphonic acid (AMPA) in food and environment is mandatory.
The chromatography of glyphosate is challenging due to its high polarity. In order to overcome this, there exists a well-established method including a derivatization step with 9-fluorenylmethyl chloroformate (FMOC) followed by LCMS analysis.
Here we report a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment but uses the built-in pretreatment function of the autosampler.
Methods
After precipitation with methanol and centrifugation the beer samples were set into the autosampler. The addition of internal standards as well as the derivatization of Glyphosate and AMPA with FMOC was done fully automatically by the autosampler within 15 min. After derivatization the sample was injected directly to the LC-MS/MS and analyzed accordingly. Due to overlapping sample pretreatment functionality, the next sample was already pretreated during the on-going analysis in order to maximize sample throughput.
Preliminary data
By using the reported instrument set-up, analysis and thus the quantification of Glyphosate and AMPA could be simplified. No additional instrument like for example a liquid handling system was required for sample pretreatment. By staggering the pretreatment and the LC-MS/MS anaylsis the whole method is very time efficient. Calibration curves showed sufficient precision and accuracy and even in a complex matrix like beer we were able to quantify Glyphosate and AMPA at or below 10 ng/mL which is below the EU maximum residue levels.
Novel aspect
Fully automated online-derivatization of Glyphosate and AMPA with a simple, standard UHPLC-MS/MS set-up without any further instrumentation

Keywords: *glyphosate, LC-MS/MS, derivatization, automated, beer*

P65
FAST ANALYSIS OF POLAR IONIC PESTICIDES IN WATER BY SUPPRESSED ION CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY

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Frequent occurrence of polar pesticides and their metabolites in environmental and drinking water has become very hot topic in the past couple of years. The most famous representative of this group is a broad-spectrum systematic herbicide glyphosate and its metabolite AMPA. Because of the chemical properties of the compounds, especially high polarity, it is not possible to analyse these compounds directly with the conventional reversed phase columns. Typically the laboratories use the methods that include derivatisation step or special chromatographic columns like the porous graphitic carbon (PGC) based Hypercarb or HILIC. With both approaches varying method robustness and unreliable results are often reported.
We do present here an ion chromatography – tandem mass spectrometry (IC-MS/MS) method for direct analysis of five polar ionic pesticides (fosetyl-Al, glufosinate, AMPA, clopyralid and glyphosate) in water samples. Ion chromatography is the preferred separation technique for polar ionic analytes such as anions, cations or ionic metabolites and thanks to the recent development in the hyphenation of IC and MS it enables its ease to use and trouble free application. Presented method is very easy and fast with no need for a sample preparation. Only for very dirty surface water samples the filtration through the membrane filters is recommended.
The method was validated in-house for three water matrices covering surface, drinking and bottled water. Analytical parameters as linearity, specificity, LOD, LOQ, precision and accuracy were evaluated using fortified blank water samples at three different levels. All tested parameters showed satisfactory results with LOQs well below required legislative limits.

P66
MONITORING OF CYPERMETHRIN CONTAMINATIONS IN MACROBRACHIUM ROSENBERGII

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Insecticides are widely used for agricultural purposesto maintain crop yields by protecting plants from insects. Their contaminations are major concerns since they can persist and translocate in the environmentsas well as accumulate in human food along the food chain. Cypermethrin is a popular pyrethroid insecticide due to its relatively low toxicities to mammals. However, it cannot only be bioaccumulated but also harmful to invertebrates, including shrimps. The freshwater shrimp (*Macrobrachium rosenbergii*) has abundantly been cultured and grown in the Central and Southwestern part of Thailand. Our study was focused on monitoring of cypermethrin contamination in this shrimp. The shrimp were randomly collected from forty sampling sites in Thailand. Their tissues were extracted using modified QuEChER method and then cleaned up with PSA, C18 and florisil. The cypermethrin levels were analyzed using an Agilent GC-μECD system. Investigated, cypermethrin residues were found in 32.50% of shrimp samples (range 0.065–0.610 μg/g). The contaminations found can be used as data for monitoring program of this selected insecticide to prevent its risks to human health.

Keywords: *macrobrachium rosenbergii, cypermethrin, GC-μECD*

Acknowledgement: *This research is supported by Kasetsart University Research and Development Institute, Thailand.*

P67
QUICK AND EASY METHOD IMPLEMENTATION FOR PESTICIDE MULTI RESIDUE ANALYSIS BY ORBITRAP HIGH RESOLUTION ACCURATE MASS SPECTROMETRY

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Pesticide residue analysis in food is still one of the most important and challenging task in the routine food control practice. European Regulation 396/2005 set maximum residue levels of pesticides in different products of plant and animal origin representing significant analytical challenge with respect to the low limits of quantitation, which are required for food matrices. In addition to that, the number of target contaminants is continuously growing with known or still unknown compounds or metabolites stretching laboratory's flexibility to keep pace with changes. For this reason it is important to have easily adjustable (and implementable) methods that can cope with high number of compounds and reasonable selectivity and sensitivity.
In recent years new instrumental developments of high resolution mass spectrometry were introduced enabling higher sensitivity, specificity and robustness. As consequence of that significant higher confidence in measurements can be achieved even at low concentration levels.
Recent presentation put focus on the workflow and key analytical performance criteria of a methodology of the newly released Pesticide Analyser that easily can be implemented for 325 pesticides, get ready to start and even extended according to the current needs. The presented Pesticide Analyser utilizes Thermo Fisher Scientific's Vanquish UHPLC coupled to QExactive Focus benchtop Orbitrap mass spectrometer.

P68
SELECTIVE SPE METHOD FOR GLYPHOSATE&AMPA BASED ON MOLECULARLY IMPRINTED POLYMERS

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Glyphosate is part of a group of herbicides referred to as phospho-herbicides and is the most commonly used herbicide worldwide with around 25% of the global herbicide market. In 2001, the EPA estimated that Glyphosate was the most commonly used active ingredient in pesticides with between 85 to 90 million pounds applied per year. It undergoes rapid microbial degradation in plants, soil and water to the metabolite aminomethylphosphonic acid (AMPA). In Europe, the following limit values were inferred for glyphosate: ADI (acceptable daily intake): 0.3 mg/kg of bodyweight; AOEL (acceptable operator exposure level), systemic: 0.2 mg/kg bodyweight/day. In addition, Codex alimentarius has defined a MRL (maximum residue limit) of 0.05 mg/Kg in meat or milk and 30 mg/Kg in cereals.
As very polar molecules, the analysis of Glyphosate and AMPA is still a challenge. Indeed, they are difficult to extract with organic solvents and common solid phase extraction (SPE) sorbents. A new SPE sorbent based on Molecularly Imprinted Polymers (MIP) was developed for these products. A MIP is a synthetic material with artificially generated three-dimensional network which shows affinity for a target molecule. Based on this technology, a new and powerful SPE clean-up method was developed for the analysis of Glyphosate and AMPA. SPE results have shown a good ability of the sorbent to catch both molecules with good recoveries in elution without salts interference problem in different water matrices (river water; natural ground water). This sorbent has also been tested on food matrices or on urine.
In addition, a POCIS calibration curve was performed in the laboratory over 35 days with continuous flow water exposure system and shows typical accumulation behaviour for a POCIS.

Keywords: *solid phase extraction, molecularly imprinted polymers, glyphosate & AMPA, passive sampling POCIS, food and water analysis*

Acknowledgement: *French ANR project ORIGAMI*

P69
DEVELOPMENT OF A SMARTPHONE-BASED ASSAY USING CHOLINESTERASE STRIPS FOR THE ON-SITE DETERMINATION OF ORGANOPHOSPHATES

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The use of smartphones as colorimetric detectors is a concept of increasing popularity and great potential in food analysis [1]. Smartphones provide a broad variety of advantages like rapid testing, on-site measurement, low cost, online data management and minimal instrumentation. In the meantime, pesticide residues analysis remains on the spotlight of food science. To determine accurately pesticides like organophosphates (OPs) laborious, sophisticated and time consuming methodologies are needed. For the purpose of rapid screening, alternative strategies can be used based on the principle that OPs inhibit the action of cholinesterases (ChE), namely acetylcholinesterase (AChE) and buturylcholinesterase (BuChE) [2]. The inhibition is dependent on the concentration of pesticides and can be detected by color change [3,4]. To this end, we have been developing a cheap and fast colorimetric ChE smartphone based assay for the determination of OPs.
To begin with, Ellman's and indoxyl acetate (IDA) assays were selected after a comprehensive review of the literature. Cotton based strips with immobilized AChE and BuChE were purchased. Dimensions of the strip, substrates for the enzymatic reaction, concentration of the substrates and chromogenic agents, reaction time between enzyme-inhibitor and color development time were checked and optimized. RGB and HSV color spaces were used as analytical signal for OPs concentration, which was measured using free smartphone applications downloaded from Google Play store. The results obtained using smartphone applications were also compared to ImageJ free image processing software as a reference. These preliminary data show the prospective of the colorimetric smartphone assay to be used and validated in real food matrices like apple and wheat.

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Keywords: *organophosphates, acetylcholinesterase, colorimetric detection, HSV color space, smartphone*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 720325.*

P70
A NEW, FAST, SIMPLE, AND ULTRA-SENSITIVE DETERMINATION OF SEMI-VOLATILE ORGANIC COMPOUNDS IN WATER SAMPLES BY GC-MS/MS TRIPLE QUADRUPOLE SYSTEM

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Introduction: The proposed methodology has been developed for the fast and ultra-sensitive analysis of semi-volatile organic compounds (SVOCs) in accordance with current European regulation for the analysis of organic pollutants in water. The new developed method for sample preparation is miniaturized based on the principles of Dispersive Liquid-Liquid Micro Extraction. Quantitation limits below ppt levels have been reached for most of the compounds analyzed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC-MS/MS TQ). The method has been validated, reporting detection limits, linearity, reproducibility, ruggedness, and recovery values for different kinds of water as tap, river and sea. The methodology has been implemented successfully in different routine laboratories working under accreditation quality parameters.
Methods: For sample preparation, 35 mL of water samples were spiked with a suitable amount of internal standards. Samples were vortex mixed in centrifuge tubes for homogenization and then a newly developed solvent extraction mixture was added to each tube to carry out the extraction. All tubes were centrifuged and the drop generated at the bottom of each vial was transferred in a standard autosampler vial for GC-TQ analysis.
Results: The study was performed with 59 different classes of compounds, including all priority substances reported in the European regulation for environmental (2013/39/EU) and water intended for human consumption (2015/1787/EU). The compounds under study included several types of pesticides, Polycyclic Aromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs). One internal standard was used to correct signal fluctuation with a matrix-matched calibration.
The calibration curves were done in different matrices. The compounds of interest showed correlation coefficients (r^2) higher than 0.994. The linear dynamic range studied started with 0.1 up to 500 pg/mL. For most compounds, lower limits of quantitation were between 0.1 – 0.5 pg/mL. In all cases, two different product ions at the limit of quantitation were obtained in Multiple Reaction Monitoring mode (MRM).
Accuracy and recovery values for spiked samples of real tap, river and sea water were in the range of 70 and 125%.
For precision values, each kind of sample was spiked at a low and a high concentration level, and extracted by four different operators over four consecutive days (inter-day precision). The RSD values for all replicates were below 30% with an average value of 15% for the low concentration level and below 18% with an average value of 7% at high concentration.
Conclusion: A simple, fast, innovative, and low-cost methodology for routine water analysis at sub pg/mL level is presented.

Keywords: *GC-TQ, sample preparation, water, SVOCs*

P71
DETERMINATION OF MULTI- PESTICIDE RESIDUES IN BLACK PEPPER POWDER BY MODIFIED QUECHERS AND THE AGILENT 7000 SERIES TRIPLE QUADRUPOLE GC/MS SYSTEM WITH MORE DILUTION ADVANTAGE

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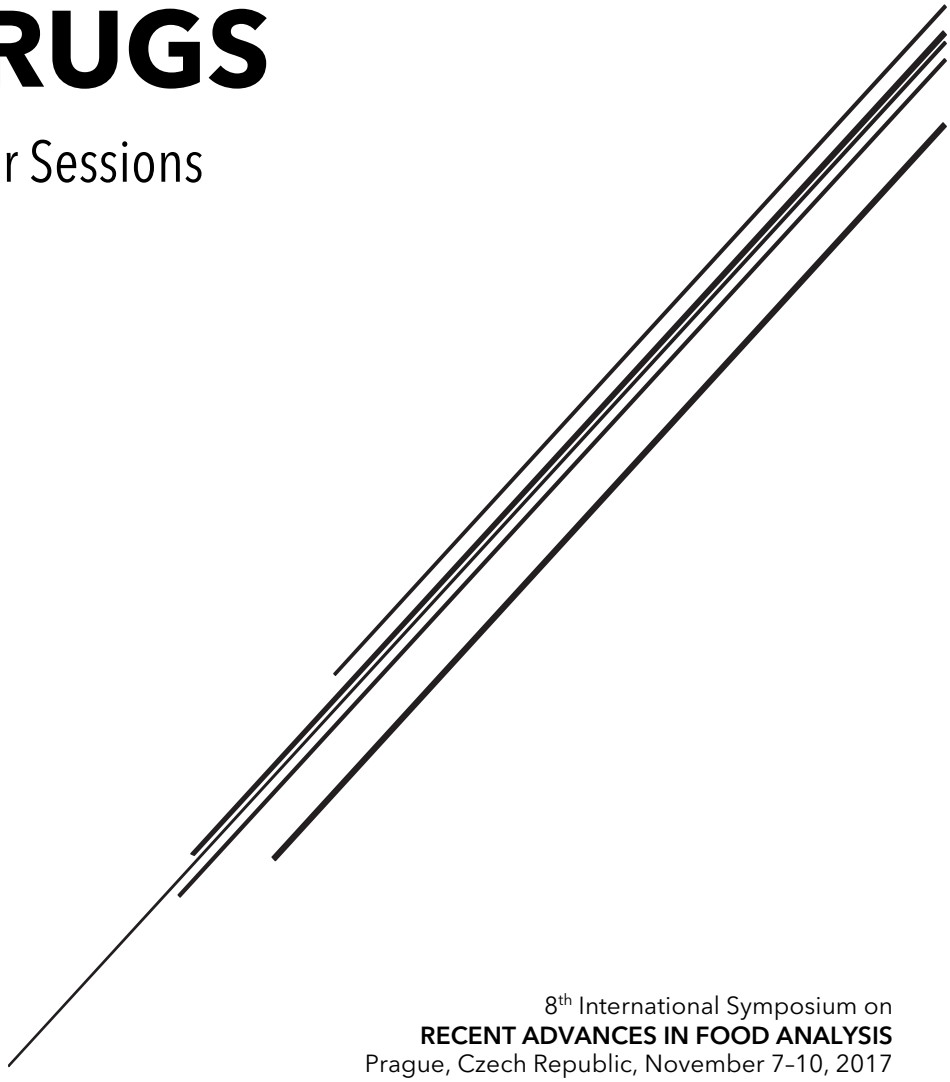
From time, immemorial India has been known as the land of spices. The world consumption of spices is growing steadily year by year. Expansion of India's export of spices to a higher level or even to retain its current share of the world market can be achieved through increased productivity and improved quality. Most of the countries across the world are bringing down the permissible levels of contaminants. Thus, scientific and technological advancement brings forth new challenges in the international trade. The health and food laws which are increasingly becoming stringent reflect the concern of the respective governments for the safety and welfare of their people. Among the wide variety of spices, the major export commodities from India include black pepper, chili and turmeric. Government is implementing various food safety regulations and standards to regulate manufacture, storage, distribution, and control of spices. Consequently, the European Rapid Alert System for Food and Feed (RASSF) alerts for Indian spices came down drastically in the recent years.
Selective analyte signal detection by mass spectrometer is popular in terms of result consistency. QuEChERS sample preparation for spices mentioned above were validated in accordance with SANTE11945/2015 and met all its criteria. However, there was complaint on peak shape distortion consequently frequent liner change, column trimming and ionization source cleaning. Dried black pepper powder was taken for this study to investigate cause.
Using retention time locking and pesticides Multiple-Reaction Monitoring (MRM) database had saved the time to set up the GC/MS/MS instrumental parameters. Due to the availability of more than two MRM transitions for 1100 pesticides, PCBs, PAHs compounds, it was possible to choose matrix free MRM transitions to provide superior selectivity and sensitivity for routine operation.
The co-extractives (especially non-volatile) is being deposited at the inlet part of the GC system are typically responsible for formation of new active sites and cause distortion of peak shapes and shifting of the retention times of target analytes. In addition, interfering ions (formed during the ionization from the matrix co-extracts) with masses close to those of target residue and, even ion suppression, are the main factors that limit achieving low detection limits and reliable analyte identification.
Pepper consists of volatile extract between 0.3–4.2% and non-volatile extract between 3.9-11.5%. Three published modified QuEChERS sample preparation with more dispersive clean up were experimented. The efficiency of the cleanup experiment was investigated by gravimetrically. As there was no significant reduction in co-extractives by these methods, dilution of the final extract was made considering method quantification limit of 10 ng/g.
Targeted list of 133 pesticides were diluted 50 times (0.02 g/mL) were detected with > 10 S/N validated as per SANTE guidelines.

Acknowledgement: *madasamy, chennai mettex labs, chennai.*

RESIDUES - VETERINARY DRUGS

Poster Sessions



8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

RESIDUES – VETERINARY DRUGS

R1
COMPARISON OF EXTRACTION PROCEDURES TO EXPLORE THE FATE OF NICARBAZIN RESIDUES IN EGGS AFTER COOKING BY USING A NON-TARGETED ANALYSIS WORKFLOW

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A robust sample preparation, notably in terms of extraction and cleanup, is essential in food analysis. These steps are typically designed to separate target analytes from interferences. However, these treatments may also eliminate other compounds whose analysis could prove decisive in current or future data interpretation. For example, analytical methods targeting specific contaminants often eliminate key information on adducts, metabolites, breakdown products, chemical tracers or other related toxicants. In the context of food safety, this information may be critical as these "secondary" structures may be equally relevant from a toxicological perspective. There is a need to develop non-targeted workflows, based on comprehensive extraction procedures that allow not only for the detection of the targeted parent compounds but also of their related compounds.
Nicarbazin was the first coccidiostat to have broad spectrum activity and has been in use since the 1950´s in broiler chickens for the prevention of faecal and intestinal coccidiosis The commercial product of nicarbazin is an equimolar complex of N,N'-bis-(nitrophenyl)urea, also known as dinitrocarbanilide (DNC), and 4,6-dimethyl-2(1H)-pyrimidinone. DNC, the marker residue for nicarbazin, was found in more than 40% of eggs in 2013 according to the Canadian Food Inspection Agency, making it the most abundant veterinary drug residue in eggs in Canada. Some studies have revealed that DNC is degraded under cooking conditions. To date though, no comprehensive analysis of its degradation products has been reported. In this work, three extraction procedures were compared to study the fate of DNC after cooking. Compounds were analyzed using an HPLC-QTOF-MS/MS. Extractions were compared for raw and cooked eggs with the objective to detect the broadest range of compounds, including DNC and its possible degradation products.

Keywords: *veterinary drugs, foodomics, food safety, non-targeted analysis, mass spectrometry*

R2
SCREENING METHOD FOR THE ANALYSIS OF ANTIBIOTICS IN MILK, CHEESE AND WHEY BY UHPLC-HRMS

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A screening method for the analysis of antibiotics belonging to seven families in milk, cheese and whey from different animal species by UHPLC-HRMS (Exactive-Orbitrap ™) is proposed.
Several aqueous/organic combinations of solvents were tested for the extraction from the matrix, and Dispersive Solid-Phase Extraction (DSPE) was used as cleanup step. The number of molecules that improved their signal and the number of detected molecules to get a method fit for the purpose were the relevant criteria for the selection of the optimal method, which was the same for the three matrices.
A matrix-effect study was also performed applying the selected method before studying the performance characteristics for each matrix.
The validation was carried out according to the Guidelines for the Validation of Screening Methods for Residues of Veterinary Medicines of the CRLs (20/1/2010). Specificity, CCβ, "Cut-off factor" Fm and "Threshold value" T were determined for the three matrices.
A database of about 90 allowed antibiotics was created to carry out Target and Post-Target analysis.
The participation in several proficiency tests with satisfactory results showed the adequate aplicability of the method.
The UHPLC-HRMS coupling (Exactive-Orbitrap™) can be applied as a suitable tool in the screening for the presence of antibiotics in milk, cheese and whey.

Keywords: *high resolution mass spectrometry, antibiotics, milk, whey, cheese*

Acknowledgement: *The authors would like to thank the Ministerio de Ciencia e Innovación (Spain), for funding the project AGL2013-45147-R.*

R3
CONFIRMATION BY LC-MS/MS OF MACROLIDE, LINCOSAMIDE, PENICILLIN AND CEPHALOSPORIN RESIDUES IN MUSCLE FROM ANIMALS INTENDED FOR HUMAN CONSUMPTION

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Among antibiotics used in animal farming, macrolides and penicillins are in 3rd and 4th place after tetracyclines and polypeptidic antibiotics in terms of frequency of use. Cephalosporins of 3rd and 4th generation are less used but more critical as they can be one of the only alternatives in human medicine that cures some infections. The use of antibiotics in veterinary medicine is related to public health in a worrying context of emergence of antibiotic resistance.
The main aim of this study was to develop a LC-MS/MS multiresidue method for the analysis of antibiotic substances in muscle tissue of slaughtered animals in order to monitor the use of these substances in accordance with the European regulations. The major challenge was to develop this multi residue method on 27 targeted substances (erythromycin, gamithromycin, lincomycin, oleandomycin, pirlimycin, spiramycin and neospiramycin*, tildipirosine, tilmicosine, tulathromycin, tylosin*, tylvalosin* and 3-o-acetyltylosin*, amoxicillin, ampicillin, benzylpenicillin, phenoxymethylpenicillin, cephalexin, cephapirin* and desacetylcephapirin, cefquinome, ceftiofur, cloxacillin, dicloxacillin, nafcillin and oxacillin) which have very different physico-chemical proprieties and cover very wide range of MRLs to target : Maximum residue limits range between 25 µg/kg at least (for penicillin V) and 1200 µg/kg to the maximum for tildipirosin in porcine muscle (Table 1 of the Commission Regulation (EU) No 37/2010 of 22 December 2009).
The developed analytical procedure and results of the validation will be described. Sample preparation is based on a simple extraction by acetonitrile after addition of EDTA, followed by the evaporation of a fraction after addition of DMSO in order to avoid deleterious evaporation to dryness, and a final reconstitution with ammonium acetate. LC-MS/MS acquisition is based on positive mode detection with two MRM transitions per compound. Validation was carried out according to the European decision 2002/657/CE. From the 13 macrolides and lincosamides tested, 9 were successfully validated with respect to the European regulation. For penicillins and cephalosporins, 12/13 were successfully validated. Ceftiofur and cephapirin will require further development due to their special status in terms of MRLs.
Investigations were also conducted on naturally incurred pork and beef samples to check the relevance of the marker residue for tulathromycin which is the fragment obtained after hydrolysis and loss of a sugar moiety. This regulation involved a specific analytical procedure for tulathromycin which prevents inclusion of this compound in a multiresidue method. Results of these investigations will be presented as well.
*Finally not successfully validated according to decision 2002/657/EC criteria

Keywords: *antibiotic residues, muscle, LC-MS/MS, validation*

R4
AMINOGLYCOSIDE AND COLISTIN RESIDUES IN FOOD BY HRMS

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Aminoglycosides (AGs) are bactericidal antibiotics widely used in farm. In European Union the Maximum Residue Limits (MRLs) for AGs in muscle, liver, kidney, fat, milk and eggs of different animal species are set by Regulation 37/2010. These antibiotics are highly polar molecules with multiple ionization sites poorly retained on the traditional reversed phase columns. As consequence their determination at trace levels in food has been always a great analytical challenge. Colistin A and B belong to the important antibiotic class of polymyxins (cyclic peptides). Their administration has been recently restricted in veterinary practice because of the increased evidence of antibiotic-resistance cases. Considering some common properties, the aim of this research was the development of a multiclass method including both these drug families in meat and milk. In this study, 14 compounds were simultaneously analysed, 12 AGs and 2 colistins (A and B), in meat and milk. The determination was carried out using a HILIC (hydrophilic interaction liquid chromatography) column coupled to a Q-Orbitrap mass spectrometer (Thermo LC-Q-Exactive). Sample treatment consisted of an extraction followed by an SPE clean-up based on weak cation exchange (WCX). In the aqueous extraction mixture, trichloroacetic acid (2% TCA) was added because strong acidic conditions and high ionic strength are needed to liberate the protein-bound AGs and extract them, respectively. For meat samples, the more critical factors were TCA and NaCl percentages and the dilution of the extract before the loading onto SPE cartridge. Spectinomycin and colistins showed the major challenges. On the other hand, for milk, the extraction was accomplished simply by an aqueous TCA solution (0.25%). The method was then validated according to Commission Decision 2002/657/EC: for each matrix 72 experiments (four replicates for each spiking level repeated on three different days) were carried out at six different concentrations in a range encompassing the fixed MRLs. The estimated performance characteristics (selectivity, linearity, accuracy, CCα and CCβ) were satisfactory; ruggedness, stability and matrix effect were evaluated, too. To the best of our knowledge, this is the first confirmatory method in food including all regulated AGs and colistins.

Keywords: *aminoglycosides, colistin, high resolution mass spectrometry, food of animal origin, hydrophilic interaction liquid chromatography*

Acknowledgement: *This work was supported by the Italian Health Ministry ("Development of methods for the determination of aminoglycosides in meat and milk" - Ricerca Corrente IZSUM RC022014).*

R5
DEPLETION STUDY AND ESTIMATION OF WITHDRAWAL PERIODS FOR FLORFENICOL IN PACU (PIARACTUS MESOPOTAMICUS)

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The intensive production of farmed fish is at a global all-time high, and the control of bacteria proliferation in fish farms requires the frequent use of antimicrobials. This practice raises important environmental concerns related to the emergence of antimicrobial-resistant bacteria strains. Only a few antimicrobial drugs have been approved for use in aquaculture, one of which is florfenicol. This work studies the depletion and withdrawal period of florfenicol and its main metabolite, florfenicol amine, in pacu (*Piaractus mesopotamicus*), a neotropical characin widely farmed in the southern hemisphere. Juvenile pacu (average weight of 724 g) were stocked in a closed-loop laboratory system with controlled water temperature (25.8 °C), and were fed for 10 consecutive days with a diet containing an intended dose of 10 mg florfenicol kg^{-1} bw. Muscle and skin tissues were collected at 1, 3, 6, 8, 10, 12, and 16 days post-treatment, and florfenicol and florfenicol amine were quantified using a validated ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. The limits of quantitation for florfenicol and florfenicol amine were 10 ng g^{-1} in muscle and 50 ng g^{-1} in skin. Considering a maximum residue limit of 1000 ng g^{-1} for the sum of florfenicol and florfenicol amine in muscle with skin in natural proportions a withdrawal period of five days (water temperature 25.8°C) or 129 degree days was calculated on the basis of the upper limit of the one-sided 95% confidence interval for the 99th percentile derived from the residue depletion study.

Keywords: *florfenicol, fish, withdrawal period, pacu, piaractus mesopotamicus*

Acknowledgement: *The authors gratefully acknowledge financial support provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant #2013/09543-7), CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).*

R6
ANALYTICAL METHODS FOR THE DETERMINATION OF FLORFENICOL AND FLORFENICOL AMINE IN FISH TISSUES, FEED AND WATER

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Florfenicol (FFC) is a broad-spectrum antimicrobial widely used in aquaculture. In Brazil, only a few veterinary drugs are approved for use in fish farms, among them, florfenicol. The recommended oral dose is 10 mg florfenicol per kg of fish biomass, once daily for ten consecutive days. With the aim to conduct a depletion study of florfenicol in pacu (*piaractus mesopotamicus*) analytical methods, using ultra-high performance liquid chromatography (UHPLC), needed to be developed and validated for the determination of FFC and its main metabolite florfenicol amine (FFA) in fish tissues, water from the recirculating water tanks and FFC in feed. FFC was extracted from the feed (pre-mix) using solid-liquid extraction with methanol. Quantitation was performed by UHPLC-DAD, using thiamphenicol as internal standard. The method presented a linear range from 0.4 to 1.8 mg g^{-1}, linearity higher than 0.99, intra-day precision of 0.6 % (n=3), and accuracy of 105%. FFC and FFA were extracted from fish tissues using a modified QuEChERS approach. Clean-up was performed on a C_{18} sorbent. The extraction efficiencies ranged from 96 to 114% (muscle) and 87 to 133% (skin). The quantitation was carried out by an Acquity UPLC™ IClass system (Waters, USA) coupled to a Waters triple quadrupole mass spectrometer (Xevo TQD Zspray), with an electrospray ionization interface operating in the positive and negative mode simultaneously. Quantitation was performed in the selective reaction monitoring mode, using one quantitation and one confirmation ion. Florfenicol-d_3 was used as surrogate. Intra-day and inter-day precisions at 50, 600 and 1000 ng g^{-1} were lower than 21% and 26%, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 3 and 10 ng g^{-1} for FFC and FFA in muscle, respectively, and 15 and 50 ng g^{-1} for skin. For the determination of FFA and FFA in water an on-line solid-phase extraction system to the UHPLC-MS/MS was developed and validated. The samples (50 µL) were loaded in water:methanol (95:5, *v/v*) on a SPE (Oasis HLB) column. Elution was performed with 0.01 % formic acid (35:65, *v/v*) at 0.40 mL min^{-1}. The linear range for both analytes were in the range of 0.5 to 12.5 µg L^{-1} with linearities higher than 0.99. Intra-day and inter-day precisions at 10 and 50 µg L^{-1} were lower than 3.7 and 11.6 %, respectively. The LOD and LOQ were 0.3 and 1.0 µg L^{-1} for FFC and FFA. The validated methods were employed in a depletion study to establish the withdrawal period of FFC in pacu (*piaractus mesopotamicus*).

Keywords: *florfenicol, florfenicol amine, depletion study in fish, SPE-UHPLC-MS/MS, QuEChERS*

Acknowledgement: *The authors gratefully acknowledge financial support provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant #2013/09543-7), CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).*

R7
SOLID PHASE EXTRACTION BASED ON FUNCTIONALISED SILICA NANOMATERIALS FOR THE DETERMINATION OF 64 ANTIBIOTICS IN MILK

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Antibiotics are widely used for therapeutic purpose in food producing animals. In dairy cows, they are administered for diseases such as mastitis and diarrhoea. This prevalent use of antibiotics is a potential source of residues, which could be taken up by consumers. Official control plans are therefore regularly carried out to assure the respect of Maximum Residue Limits [1]. Multiclass analytical methods can monitor more drug classes through a single analysis, so they are faster, less time consuming and cheaper than the traditional single-class ones. Nevertheless, these methods are real analytical challenge, considering the complexity of matrices to analyse and the wide variety of chemical structures involved. This latter aspect requires sample treatment protocols with generic extraction and non-selective purification to keep all the analytes together. In our previous work, a screening and confirmatory method to determine 62 antibiotics belonging to ten different classes was developed and validated in raw cow milk by liquid-chromatography coupled to a high-resolution hybrid mass spectrometer (LC-Q-Orbitrap) [2]. However, as mentioned, due to the lack of a suitable purification step, the final extracts were quite dirty and an "instrumental clean-up" through mass cuttings and SIM experiments was indispensable. On the other hand, this acquisition approach significantly restricted the operational capabilities of high-resolution mass analysers, which are able to allow retrospective analyses when operating in full scan mode. Thus, a new sample preparation strategy was tested for screening purposes involving an additional final clean-up using nanoparticles functionalized with aminopropyl groups [3]. The aim was to obtain cleaner extracts without an excessive increase of the analytical time and cost. Half a gram of milk was processed, testing different amounts of nanoparticles (d-SPE). Results showed that 75 mg of nanomaterials could be the right balance to enable the full scan acquisition and the detection of all the analytes at the levels of interest (2-10 µg/kg). Recoveries were higher than 60%, except for some of the less polar analytes (rifaximin, valnemulin and tylvalosin). The validation study is in progress [4].

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[4] Commission Decision of 12 August 2002. Off. J. Eur. Union. L 221:8-36.

Keywords: *antibiotics, milk, functionalized silica nanomaterials, high-resolution mass spectrometry, dispersive solid-phase extraction (d-SPE)*

R8
ANALYSIS OF NITROFURANS IN FEED AND FEED WATER SAMPLES USING ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY MASS SPECTROMETRY METHOD

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The use of the nitrofurans has been banned within the European Union (Annex IV of Regulation 2377/90/EEC) due to their toxicological influence on the health of consumers of food from animal origin [1]. Previously, these antibacterial agents were added to feeds or water to stimulate growth or/and to prevent and treat several bacterial and protozoan infections, such as fowl cholera, coccidiosis, and blackhead.
In 1994 the absence of safe residue levels, has determined European Union to prohibition for veterinary use [1,2,3] and no maximum residue limit (MRL) has been established for this antibiotic. However, there were 854 notification events reported for nitrofurans, data from the Rapid Alert System for Food and Feed (RASFF) database for the years 2002 to 2017 and indicated that contamination of incidents in various matrices with nitrofurans was still present [4]. The notifications are related to a wide range of food products, particularly the categories of: crustaceans and products thereof, fish and fish products, meat and meat products, honey, feed and feed water.
Unfortunately, there are only few methods were created for determination of nitrofurans in feed and feed water using mass spectrometry techniques [5,6]. That why, the purpose of this study was to create and validate method with good recovery and good purification of extracts for confirmation and quantitation of nitrofurans residues in feed and feed water matrices by UHPLC–MS/MS system.

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Keywords: *nitrofurans, feed, feed water, UHPLC-MS/MS*

Acknowledgement: *This work was funded by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

R9
MULTICLASS METHOD FOR THE DETECTION OF VETERINARY DRUGS IN SURFACE WATERS

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Environmental studies have shown that pharmaceuticals can contaminate groundwater, surface water, sediments, aquatic flora and fauna. Effluents from sewage and wastewater treatment plants, pharmaceutical industries and hospitals have been frequently involved in such contamination. In addition, recent studies have revealed significant concentrations of pharmaceuticals in wastewater from animal facilities in proximal aquatic habitats. A positive correlation between exposure to some veterinary drugs and increased adverse effects in aquatic biota (endocrine disruption, antibiotic resistance, neurotoxicity and genotoxicity) has been documented [1]. It is worthwhile pointing out that now pharmaceutical substances are explicitly earmarked for study within the framework of EU-wide water monitoring in Directive 2013/39/EU amending Directive 2000/60/EC as regards priority substances in the field of water policy.
This work deals with the development of a multiclass LC-Q-Orbitrap method for the determination of over than sixty veterinary drugs belonging to ten different antibiotic classes (amphenicols, beta-lactams, diamino-pyrimidine, lincosamides, macrolides, pleuromutilins, quinolones, rifamycins, sulfonamides and tetracyclines) in surface water. LC-MS conditions were the same developed in our previous paper in which the same substances were determined in meat [2]. On the other hand, the sample treatment was completely changed to allow the detection of compounds in the range of interest, i.e. part per trillions (ng/L). After the optimization, the method was able to detect 64 drugs at levels from 0.1 to 10 ng/L. As part of the validation study, linearity, accuracy and matrix effects were assessed, too. Data-dependent scan, performed in addition to the full scan acquisition, was used for identification purposes.
Fifteen water samples from Nera River (Umbria, Italy) were collected in the spring/summer of 2017. Analyses revealed the occurrence of six compounds belonging to five different antibiotic classes: sulfadiazine, oxytetracycline, florfenicol, florfenicol amine, flumequine and trimethoprim. The found concentrations were lower than 1 µg/L. The detected antimicrobials are frequently used to treat bacterial diseases in aquaculture. Further studies are in progress to evaluate the possible correlations between antibiotic use in Umbrian aquaculture farms and the presence of residues in the Nera River. The main advantage of the here developed procedure was the wide range of determined compounds, which allows the monitoring of most of the antibiotics used in the veterinary practices.

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[2] Moretti et al. Screening and confirmatory method for multiclass determination of 62 antibiotics in meat. Journal of Chromatography A, 1429, 175–188, 2016

Keywords: *veterinary drugs, surface waters, liquid chromatography-high resolution mass spectrometry, Nera River*

Acknowledgement: *This work received financial support by the Umbria Region.*

R10
DEPLETION PROFILE OF ALBENDAZOLE MARKERS RESIDUE IN FISH FILLET AFTER ORAL ADMINISTRATION USING DRUG INCORPORATED BY FEED COATING

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Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people worldwide. The global increase in landing of seafood and fisheries products is a result of the continuing growth of aquaculture, which accounts for half of all food fish consumed worldwide.
All intensive animal production systems, particularly aquaculture, provides a favourable environment for infectious diseases outbreaks, a drawback of high stocking density practices. However, aquaculture industry has not yet justified the development, availability and regulation of specific veterinary drugs.
Albendazole is a broad-spectrum anthelmintic approved for farmed ruminants and recently considered for treatment of fish parasites, therefore subject of careful monitoring because of potential residues in animal products. This study evaluates the depletion of albendazole (ABZ) and its main known metabolites: albendazole sulfoxide – ABZSO, albendazolesulfone – $ABZSO_2$ and albendazole aminosulfone – ABZ-2-NH_2SO_2, in the fillets of the Neotropical Characin pacu, *Piaractus mesopotamicus*, fed diets containing 10 mg ABZ kg^{-1} body weight in a single dose. Fish were euthanized at eight, 12, 24, 48, 72, 96 and 120 hours after medication and the depletion profiles of ABZ, each metabolite and the sum of all marker residues were assessed and evaluated considering methodological variations regarding determination of the Maximum Residue Limits (MRLs) adopted by different international regulating agencies for estimation of the withdrawal period (WP). The estimated WPs ranged from two to seven days.

Keywords: *veterinary drug residues, albendazole and metabolites, drug depletion, withdrawal time, aquaculture*

Acknowledgement: *This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - São Paulo Research Foundation, Grant # 2012/18334-0) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES - Coordination for the Improvement of Higher Education Personnel.*

R11
HIGH SENSITIVE MULTIRESIDUE ANALYSIS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS RESIDUES IN MUSCLE, MILK AND PLASMA USING LIQUID CHROMATOGRAPHY COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY

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The aim of the present work is the development of a fast and reliable analytical multiresidue method for the confirmation of non-steroidal anti-inflammatory drugs residues in muscle, plasma and milk. The samples are extracted with organic solvent and purified with liquid-liquid extraction (LLE). The purified extract was analyzed by LC-HRMS.
The method was validated in accordance with the criteria defined in Commission Decision 2002/657/EC, decision limit (CCα) values and detection capability (CCβ) values have been established for each compound. Recoveries of the methods, calculated spiking the samples in the range 0.1 - 750.0 µg Kg^{-1}, were 80 to 95 % for muscle, 75 to 101% for milk and 87 to 97% for plasma. The precision (CV) ranged between 9.2 and 15.1% for muscle, 9.9 and 14.6% for milk and from 9.4 to 13.4% for plasma. Linearity for the analytes was calculated from 5.0 to 1000.0 µg kg^{-1}.
Under the Official Control Plan activity, several samples were found positive for Flunixin, Meloxicam and Carprofen residues in meat, milk and plasma.
The main advantages of the presented method are its rapidity, the specificity, the good precision and recovery that make it very suitable to the confirmatory determination of non-steroidal anti-inflammatory drugs residues in food and plasma.

Keywords: *anti-inflammatory drugs, validation, residues, mass spectrometry*

R12
RABIES VACCINE BAITS AS A POTENTIAL SOURCE OF WILD BOARS (SUS SCROFA) MUSCLES CONTAMINATION WITH TETRACYCLINE ANTIBIOTIC – PRELIMINARY STUDY

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Tetracycline is a compound used as biomarker for rabies vaccine bait, intended for foxes. This antibiotic is incorporated into bones and teeth and can be detected by fluorescence microscopy for a long time after consumption. Monitoring of the effects of oral immunization of foxes against rabies includes the determination of tetracycline presence in the mandible bones. However, there is a high probability of accidental intake of the vaccine, by the other species living in the forest ecosystem, like wild boars (Sus scrofa) and the residual of TC in animals tissues can occur, what may adversely affect the health of consumers. Control of TC residues presence concerns muscles derived from cattle, swine, sheep, goats, horses, poultry, fish and farmed wild animals. However, conducted monitoring does not include the material intended for human consumption derived from wild boars, while nowadays a noticeable increase in the wild boars meat intake is observed. Venison is a highly valued culinary product due to its nutritional properties. This meat is characterized by a low fat content, high content of whole protein, taste attributes, as well as provide a lot of energy to human. The purpose of this study was to determine the risk of tetracycline residues presence in wild boars muscle tissues from rabies vaccine baits.
In presented study, diaphragm muscle samples from 144 animals were tested for the presence of TC, collected for 4 weeks after rabies vaccine extrusion. For the quantitative analysis of tetracycline and it's 4-epi form, a simple and labour-effective liquid chromatography – tandem mass spectrometry method was developed. The optimal isolation of tetracycline and 4-epi tetracycline was achieved with trichloracetic acid. For the cleaning up, the PVDF filters were found the most suitable. The results of this study show, that tetracycline was found in 53 animals, what represents 37 % of all tested animals. The confirmed concentrations were in the range of 5 – 286 µg/kg. The presented situation suggests, that the control of venison for human consumption, concerning tetracycline residues, should be considered.

Keywords: *rabies, vaccine, baits, wild boars, tetracycline residues, LC-MS/MS*

Acknowledgement: *This work was financially supported by KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

R13
DETERMINATION OF SALICYLIC ACID IN FEED AND FEED MATERIALS USING LC-MS/MS

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Salicylic acid (SA), acetylsalicylic acid (aspirin) and sodium salicylate belong to the best-known non-steroidal anti-inflammatory drugs. Salicylates occur naturally; they are phytohormones regulating growth and act as mediators in plant response to viral and bacterial infections. The content of salicylates decreases as plants ripen, also cooked foods contain less salicylates than fresh and dried ones. Salicylates present in food are released in the digestive system, providing a source of SA. The commonly occurring natural salicylic acid found in plants intended for animal feed may be associated with their presence in food of animal origin and could have adverse effects on the health of the population of most susceptible consumers.
We have developed a simple and sensitive method for the determination of salicylic acid in feed and feed materials using LC-MS/MS. Analyte was extracted using liquid-liquid extraction with 0.1% hydrochloric acid in methanol. The eluate was evaporated to dryness under a stream of nitrogen and dissolved in 50% acetonitrile. The chromatographic separation was performed using a Shimadzu Nexera X2 and a Poroshell C18 chromatography column (2.1 x 150 mm, 2.7 µm) in a gradient elution program using formic acid and acetonitrile. Total instrumental analysis time was 7 min. Detection was made using the Shimadzu LC-MS 8050 mass spectrometer working in negative electrospray ionisation. The method has been validated according to Commission Decision 2002/657/EC. The calculated parameters of the method (linearity, precision, recovery) proved that it was fit for determination of the salicylic acid in feed and feed materials. Feed materials such as wheat, barley, triticale, paprika and selected feeds destined for laying hens were tested. The salicylic acid has been found in trace amounts in all compound feeds (below 0.1 mg/kg). Corn has also been studied as an example of a feed material that could contain higher amounts of SA. The results of salicylic acid in corn were different depending on whether it was young or grown (5.76 mg/kg and 0.20 mg/kg, respectively). The results will be used to plan further research on biotransformation of salicylates and will allow to determine to what extent the salicylates can transfer to food of animal origin.

Keywords: *salicylic acid, feed, feed material, LC-MS/MS*

Acknowledgement: *The research was carried out under the grant of the National Center of Science, Preludium Competition, Grant No. 2015/17/N/NZ7/04105: Biotransformation and residue depletion of acetylsalicylic and salicylic acid in tissues and eggs of laying hens.*

R14
MULTI-COMPONENT ANALYSIS OF MEDICATED FEED AND OF PHARMACEUTICAL RESIDUES IN FEED

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Livestock is commonly medicated via feed. In Finland, over 30 pharmaceuticals and coccidiostats are authorized in medicated feed. The concentration of pharmaceutical must be within certified range in order to achieve desired effect without side-effects. Feed producers may use same production lines for the manufacturing of medicated and non-medicated feed, which may cause contamination of non-medicated feed with pharmaceuticals. Thus the monitoring of concentration of pharmaceutical in feed as well as monitoring of residues in non-medicated feed is important.
The objective of this study was to develop a multi-component method for the analysis of pharmaceutical and coccidiostat residues in feed. At the moments method includes 32 pharmaceuticals and 11 coccidiostats. Second objective was to broad the scope of the method to the medicated feed. After simple extraction, sample was analysed with Waters Acquity UPLC combined with Xevo TQ MS. Concentrations were calculated using standard addition method. Medicated feed samples were diluted 1:100 to 1:1000 depending on concentration in the product.
The composition of feed varies substantially causing fluctuating matrix effect. Thus, standard addition method was considered necessary. The developed method with variable scope is cost-effective and will be used in the official monitoring of feed.

Keywords: *multi-method, medicated feed, pharmaceutical residues*

R15
DETERMINATION OF PLEUROMUTILINS IN FEED AND FOODSTUFFS OF ANIMAL ORIGIN BY AN ENZYME-LINKED IMMUNOASSAY

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Tiamulin and valnemulin are a semi-synthetic pleuromutilin antibiotics derivative, which act by inhibition of bacterial protein synthesis They are used mainly in veterinary medicine, especially for swine and for poultry. The potential contamination of animal origin foodstuffs with pleuromutilins results in a risk of the potential increase in drug resistance in bacteria. The use of antimicrobial substances as feed additives and as growth promoters was prohibited in 2006 by Regulation No 1831/2003 of the European Parliament Now antimicrobial substances can be used only as medicated feedingstuff. Medicated feedingstuffs are produced only on prescription in authorized manufacturing place. In agreement with EU and Russian legislation, both production process and usage of medicated feedingstuff has to be strictly monitored and controlled. The main aims of the control process are determination of active ingredient concentration, homogeneity of the product and detection of illegal use of tiamulin in feed.
The maximum residue levels (MRLs) established by Custom Union for tiamulin and valnemulin in pork, porcine liver, eggs, chiken meat and liver range from 50-1000 µg/kg. To protect the health of consumers, it is necessary to detect pleuromutilins residues in animal edible tissues and feed.In this work, a polyclonal antibody-based indirect competitive enzyme-linked immunosorbent assay (ELISA) was developed for the determination of pleuromutilins. The highly specific antibody was targeted for valnemulin conjugated with keyhole limpet hemocyanin. The antibodies were characterized in the homologous and heterologous antigen-coated competitive indirect ELISA. The 50% inhibition values (IC50) was 0,3 µg/L for valnemulin and 4,1 µg/L for tiamulin.
Developed technique was optimized for screening pleuromutilins in pork, eggs and feed. The sample preparation procedures were developed. The developed method was validated according to the European guideline for screening methods. The detection capability (CC_β) was 10 µg/kg. The cross-reactions towards other families of antibiotics were negligible and there was no masking effect of these antibiotics on the detection of pleuromutilins. Finally, the results suggest that the indirect ELISA is a robust specific, accurate, and sensitive method of detecting pleuromutilins residues in feed and food of animal origin.

R16
A SIMPLE DETERMINATION OF STILBENE RESIDUES IN MULTIPLE MATRIXES BY ISOTOPE DILUTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Stilbenes are intended for occasional therapeutic use in husbandry. They are also injected into animals to fatten them faster, and such practice results in “fast flesh” production. These synthetic hormones are found to be endocrine disruptor and potentially dangerous to human. Their application on food animals is prohibited in Hong Kong and various countries.
A simple but sensitive isotope dilution liquid chromatographic tandem mass spectrometric (LC-MS/MS) method was developed for determining the residue of stilbenes, namely hexoestrol, dienoestrol and diethylstilbestrol, in a variety of matrixes, including animal muscle, offal, aquatic species, milk and animal urine samples. The homogenized samples are extracted with diethyl ether after enzymatic hydrolysis by b-glucuronidase/aryl sulfatase in ammonium acetate buffer solution. The diethyl ether extract is evaporated to just dryness and the residue reconstituted with chloroform. Stilbenes are then extracted into sodium hydroxide solution by liquid/liquid partition. Such aqueous solution extract is subject to solid-phase extraction cleanup by HLB extraction column. Target analytes were eluted with methanol after the extract is washed with water and 5% methanol (v/v) in water, respectively. The extract is filtered and analyzed for stilbenes using liquid chromatography tandem mass spectrometry.
This method was validated according to 2002/657/EC Directive, and the validation was conducted by the analyses of spiked samples with recoveries of the three target stilbenes in the range of 71% to 93% in animal tissue, aquatic species and milk, and 86% to 116% in animal urine. Limit of detection of stilbenes in animal tissue and aquatic species and milk, and animal urine ranged from 0.074 to 0.14 µg/kg and 0.31 to 0.76 µg/L, respectively.

The proposed method is simple, rapid and applicable to multiple matrixes so that monitoring residue of stilbenes in farm samples like animal urine and feed, and various food samples from market surveillance becomes more efficient and cost-effective.

Keywords: *food, drug residues, stilbenes, LC-MS/MS*

R17
SENSITIVE AND FAST MEASUREMENT OF AMINOGLYCOSIDE ANTIBIOTICS IN MILK, MEAT OR EGGS BY HILIC-MS/MS AND IDENTIFICATION USING MULTI-MRM SPECTRUM MODE

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Aminoglycosides are an antibiotic family widely used for the treatment of bacterial infections in cattle, sheep, pigs and poultry. Due to their high affinity for tissues, the consumption of meat, milk or eggs containing aminoglycosides can be potentially hazardous for human health. Regulatory agencies worldwide have set maximum residue limits (MRL) for these compounds with veterinary use.
Aminoglycosides are very polar compounds poorly retained by reversed-phase liquid chromatography and ion-pairing reagents are not desirable when analysts use several methods on a single system.
Here we present a method using HILiC with high sensitivity mass spectrometer to reach limits of quantification as low as 1 fg on column, combined with multi-MRM Spectrum Mode identification.
After crushing, samples were extracted with buffer. An aliquot of the supernatant was then submitted to solid phase extraction using a WCX sorbent. Extracts were diluted prior to analysis.
Analysis was performed in HILiC mode with an Inertsil Amide columns. Two methods were set up. One for high speed screening and sensitive quantification. All compounds were eluted in less than 1.5 minute and two MRM transitions per compounds were recorded. The second one was using the same mobile phase and column with an extended gradient and 15 MRM per compounds.
Total recovery was superior to 85 % for all compounds in all matrices evaluated. Matrices were pig (bacon, muscle and fat), beef (muscle), chicken (muscle, liver), eggs and milk. It was found that losses were due to extraction process and not to ion suppression.
Then, for the quantitative method, it was possible to prepare a calibration curve in neat solutions and to use it for all matrices. The calibration concentration of each antibiotic was set to range from one tenth of the lowest MRL to 50% more than the highest one. Lowest and highest MRL were selected within all possible matrices from the considered regulations (Europe, USA and Japan). In most of the case it was possible to quantify far lower than the requested LOQ. This was found to be of particular interest in the case of fraud, as some aminoglycosides are banned when animal are bred to produce eggs or milk.
Aminoglycosides can share product ions, the possibility of cross-talk increase in high speed methods. Many published methods used lengthy separation time to prevent this. Here, it was shown that even with very low dwell times and fast electronic switches, no cross-talk was measurable.
A second method with increased separation and fifteen MRM transitions per compound was set up. This second method can be used for increased confidence in identification. MRM Signals were merged to generate an optimized fragmentation spectrum that was compared to a library and calculate hit score. No sensitivity difference was noticed when acquiring two or fifteen transitions per compound making this approach suitable for quantitative measurement also.

Keywords: *aminoglycosides, HILiC, tandem mass spectrometry, Multi-MRM identification*

R18
COMBINING NON-TARGETED SWATH® ACQUISITION WITH HIGHLY SELECTIVE MRMHR FOR THE ANALYSIS OF VETERINARY DRUGS IN TISSUE USING THE SCIEX X500R QTOF SYSTEM

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Veterinary drugs are commonly used in livestock breeding to prevent or treat infections of the animals and to ensure their optimal growth. European guidelines require to sensitively control residues of veterinary drugs in animal products to prevent any potential risks for human health. We present a versatile and sensitive workflow on the SCIEX X500R QTOF system which combines a non-targeted screening workflow using SWATH® data acquisition looped with highly selective MRMHR data collection. Following chromatographic separation, samples were analyzed on the SCIEX X500R QTOF system. Data acquisition was performed using TOF-MS mode looped with eight SWATH® MS/MS experiments and scheduled MRMHR acquisition. SWATH® mode is a unique MS/MSALL technology. Variable SWATH® Q1 windows were used, based on the average ion density of the matrix samples. TOF-MS mode is the standard acquisition mode for quantitation, providing non-targeted data collection which can be subsequently processed in SCIEX OS using a list of targeted compounds. For all 27 analytes, TOF-MS mode provided excellent sensitivity in a standard solution at 1 ng/mL. However, in complex matrices such as liver extracts, sensitivity may be disturbed by matrix interferences. In such cases, quantitation could be alternatively performed using the comprehensive MS/MS traces from SWATH® acquisition. If even higher selectivity and sensitivity is needed, true MRMHR provided even better signal-to-noise ratios. Confident identification of the veterinary drug residues according legal requirements can achieve by accurate precursor and fragment mass measurement and their compound specific ion ratios. In conclusion, the SCIEX X500R QTOF system is a unique and powerful instrument for the sensitive analysis of veterinary drugs in complex matrices, combining versatile acquisition modes for different requirements.

R19
THE ANALYSIS OF VETERINARY DRUG RESIDUES, CONTAMINANTS AND NATURAL TOXINS BY UHPLC-HMRS/MS: A STEP FORWARD FOR PUBLIC HEALTH LABORATORIES

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The recent advances in the field of mass spectrometry have led to the availability of benchtop high resolution mass spectrometers (HRMS) for official control laboratories. Particularly interesting is the appearance of the Orbitrap® based instruments since 2005: these mass spectrometers bring special reliability to analytical results, enabling the separation of coeluting isobaric compounds and an outstanding sensitivity when monitoring molecular ions, compared to low resolution mass spectrometers (LRMS).
Although current legislation is not already updated to such available novelties in the field, laboratories must provide results with the maximum confidence, due to the legal consequences for consumers as well as for food producers. In order to assure food safety, some confirmation criteria were internally established in our laboratory, and the use of HRMS has become a basic tool.
The Laboratory of the Public Health Agency of Barcelona has established a confirmation strategy for veterinary drug residues, contaminants and natural toxins analysis using HRMS, (Q-Orbitrap mass analyzer) based on the following issues:
All suspicious results from UHPLC-LRMS/MS are confirmed by UHPLC-HRMS/MS, to avoid false compliant or false-non-compliant results.
The Q-Exactive spectrometer has become a routine instrument in our laboratory: some analytical methods has moved from LRMS to HRMS. Examples of the last include:

- analysis of corticosteroids in liver and urine
- analysis of synthetic hormones in urine and fat
- analysis of chloramphenicol in meat, honey, urine and milk
- analysis of polypeptide antibiotics in meat, and meat products
- analysis of acrylamide in coffee, chips and cereal based food-stuff
- analysis of lipophilic toxins in bivalves

We present a summary of analytical methods in these fields, implemented in the last 2 years using the Q-Exactive instrument, as well as some examples of final positive or negative confirmation of the presence of the substance.
These methods are currently included in the scope of the accreditation of the Laboratori de Agència de Salut Pública de Barcelona following ISO/IEC 17025 requirements

Keywords: *HRMS, veterinary drug residues, contaminants, marine biotoxins*

R20
DEVELOPMENT OF A SCREENING METHOD FOR MULTICLASS VETERINARY DRUG RESIDUES USING HIGH RESOLUTION MASS SPECTROMETRY

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The monitoring of veterinary drugs residues in food of animal origin is mostly based on the implementation of several screening analysis followed by confirmatory methods to cover all the class of compounds. The screening analysis is a crucial step as it most often determines the go/no go to the confirmation step. The challenge for the screening analysis is to have in a single run a maximum of information on the presence of a large number of veterinary drugs potentially present in a food matrice with respect to the required target level in accordance to legislation. In France, a screening LC-tandem MS-based method is already routinely applied for the screening of 75 antibiotics in meat operating in Multiple Reaction Monitoring (MRM) mode. The introduction of benchtop hybrid high resolution mass spectrometers (HRMS) offers an emerging and powerful alternative for multi-residue screening analysis compared to conventional low resolution triple quadrupole mass spectrometry. Indeed, in LCHRMS, there is no limitation in the number of analytes as no pre-targeted optimisation of MRM transition is needed before injection.
The objective of our work was to develop a multi-class screening method using LC-HRMS capable of detecting the presence of the largest number of veterinary drugs simultaneously in a single analysis of meat.
In this work, the optimization of the liquid chromatographic conditions has led to the separation and detection of more than 130 compounds from different drug classes (Antibacterials, NSAIDs, sedatives, coccidiostats, anthelmintics) within 15 min per run. In this poster, we will detail the different acquisition modes tested for the screening of more than 130 compounds (Antibacterials, NSAIDs, sedatives, coccidiostats) using a Q-Exactive plus system. Three combined acquisition modes associating full scan with data-dependent acquisition (FS-ddMS²) or full scan with data-independent acquisition (FS-AIF, FS-vDIA) have been implemented. A compound database containing information on parent ion, fragment ion, retention time was created. The application of a targeted data processing allows the identification/confirmation of the compounds, based on criteria as retention time, parent ions with associated mass accuracy, fragments ions (1 or more ions with associated mass accuracy). The selection of the best acquisition modes for screening was discussed in relation to both false positive and false negatives rates.

R21
RAPID PASS-THROUGH CLEANUP OF BOVINE LIVER SAMPLES PRIOR TO UPLC-MS/MS MULTIRESIDUE VETERINARY DRUGS ANALYSIS

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Tissue samples, such as bovine muscle and liver, are typically extracted with an acetonitrile based solvent for LC-MS determination of veterinary drug residues. Among the most significant co-extracted substances are fats and polar lipids, particularly phospholipids (lecithin). Bovine liver typically contains about 45 mg of fat and about 25 mg of phospholipids per gram of tissue. Fats can be effectively removed from the acetonitrile based tissue extracts by liquid extraction with hexane or with SPE with octadecyl silica (C18). However, these defatting procedures are not effective for removal of phospholipids. Excessive amounts of phospholipids can shorten LC column life, contribute to ion-suppression, and contaminate the mass-spectrometer. In this study a novel reversed-phase sorbent is used for highly effective removal of both phospholipids and fats from bovine liver extracts prior to LC-MS/MS analysis. Greater than 95 % of phospholipids and greater than 85 % of fats were effectively removed from the tissue extracts after the simple pass-through SPE procedure. Recoveries of 45 compounds with published MRLs in beef liver averaged 83% with only a few compounds under 60%.

Keywords: *bovine liver, SPE lipids*

R22
MULTI-METHOD FOR THE DETERMINATION OF RESIDUES OF DIFFERENT ANTIBIOTIC GROUPS IN EGGS BY LC-MS/MS

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The determination of antibiotics of different substance groups with a single multi-method is very important for an effective residue control of food of animal origin.
The antibiotic substance groups included in this validation study - penicillins, amphenicols, macrolides, lincosamides, quinolones, tetracyclines, sulfonamides, pleuromutilines and diaminopyrimidines - are broad-spectrum antibiotics with different activities against gram-positive and gram-negative bacteria. The implementation of maximum residue limits (MRLs) in different food matrices including eggs by European Regulation (EC) No 470/2009 and subsequent modifications was to ensure safer food and consumer protection.
The method for the identification and confirmation of the above-mentioned antibiotic groups in eggs was developed with a specific extraction step, clean-up and ensuing measurement by LC-MS/MS. The method was validated for MRL substances in a concentration range of 0.25 to 2.0 MRL. Substances without MRL were also covered and in these cases the spike concentrations were chosen as low as reasonably achievable.
A validation on the basis of an alternative in-house validation approach was performed in accordance with Commission Decision 2002/657/EC. The impact of different factors was checked to take into account the character of different samples and varying conditions in the laboratory. Using the factor-comprehensive in-house validation concept, the validation of the multi-method was successfully accomplished with a limited number of experiments within a single validation study. The relevant validation parameters, e.g. the critical concentrations CC-alpha and CC-beta, the repeatability, the within-reproducibility and the recovery rate, are reported and discussed.

Keywords: *multi-method, eggs, antibiotics, LC-MS/MS, alternative validation concept*

R23
THE ANALYSIS OF BENZOYLPHENYLUREA ANTIPARASITICS IN FISH - FAST AND ROBUST

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In aquaculture infections with sea lice are a growing problem. Salmon farming, in which the fish are kept in large quantities, is still increasing annually. In Norway it is very important for the export. The fish louse is a parasite that damages the skin of the fish and makes the infested animals vulnerable to diseases. The benzoylphenylureas teflubenzuron, diflubenzuron and lufenuron are used intensively to limit the increase of sea lice attack. These insecticides act as growth inhibitors by blocking the chitin synthesis of the insects and thereby preventing the transformation to the next larval stage [1]. The medication of the fish takes place via the feed. The use has doubled annually between 2011 and 2013 and is an additional problem by the enrichment of the substances in the sediment and its impact on crustaceans [2,3].
For the substances teflubenzuron, diflubenzuron and lufenuron, the EU has established maximum residue limits (MRL) for fish between 500 µg/kg and 1350 µg/kg [4]. Therefore it is necessary to develop a selective and sensitive method for detecting these substances in fish matrix. The poster shows a rapid and robust method for the analysis of the antiparasitics diflubenzuron, lufenuron, teflubenzuron and other benzoylphenylureas with a fast extraction and measurement by LC-MS / MS. All samples are weighed twice. One is spiked with the internal standard (IS), the other with a standard solution containing all analytes and the IS. Since experience has shown that each matrix has a different influence on the intensity of the MS/MS signals, the recovery rate of each sample is determined individually.
The proposed method has been successfully validated corresponding to EU Directive 2002/657 / EC [5]. It is suitable for the analysis of benzoylphenylurea antiparasitics in fish and meat with a limit of quantification of 10 µg / kg without time-consuming sample preparation by SPE. Recovery and repeatability are in accordance with Directive 2002/657 / EC on the implementation of analytical methods and the evaluation of results.

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[4] Commission Regulation (EC) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification in accordance with maximum residue limits, Official Journal of the European Union (L15).
[5] Commission Decision of 12 August 2002 implementing Council Directive 96/23 / EC (2002/657 / EC), Official Journal of the European Union (L221 / 8).

Keywords: *veterinary drugs, food contaminants, residues, benzoylphenylurea antiparasitics*

R24
RESIDUE ANALYSIS OF VETERINARY DRUGS IN FISHERY PRODUCTS BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Veterinary drugs are used to treat bacterial diseases and growth-promoting substances in fishery products. Their use in edible fish results in remaining antibiotics in edible tissues, which poses a potential health risk to human health.
In this study, simultaneous analytical method of 77 veterinary drugs is developed to make sure the safety of the fishery products. We optimized analytical methods for 77 veterinary drugs (69 parent drugs, 6 metabolites, and 2 internal standards). Extraction was performed with EDTA in Ammonium acetate buffer solution and clean-up was done by C18 and primary secondary amine (PSA). Analytical methods were developed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The proposed method was validated for specificity, precision, recovery and linearity. Validation study was applied in three matrices (flatfish, eel and shrimp). As a result, recovery was 70.8~151.5%, 53.8~143.3%, 75.0~147.0% in flatfish, eel and Shrimp. This method is suitable for monitoring study in fishery products.

R25
SEPARATION OF CHLORAMPHENICOL ISOMERS VIA CHIRAL PHASE LC-MS/MS IN HONEY

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Chloramphenicol (CAP) is a widely used broad-spectrum antibiotic which is not authorized anymore for use in food-producing animals in the EU and in numerous other countries, due to several adverse toxic effects. As a relatively quick and inexpensive technique many laboratories and food authorities use an ELISA technique to analyze for chloramphenicol. Confirmation measurements of positive ELISA results are performed by the more specific LC-MS/MS technique. In routine analysis for CAP in honey, occasionally, false negative results from the ELISA test kit were observed. These false negative results are problematic for the laboratories, the merchants and the customer. To address this issue, a new LC-MS/MS method to analyze the four stereoisomers of chloramphenicol, was developed and validated. The four isomers cannot be separated by classical RP-LC-MS/MS. A special chiral reversed phase liquid chromatography is used for this purpose. In an internal survey, honey samples with identified CAP residues were re-analyzed by this new method. In many samples partly or only SS-chloramphenicol was detected, the corresponding ELISA analysis showed low or no results, respectively. In contrast to RR-chloramphenicol the SS-stereoisomer is without antimicrobial activity. This is the first report about SS-chloramphenicol residues in honey and, generally, in food samples. The potential sources for SS-chloramphenicol are discussed and the need for further systematic studies emphasized.
The chiral LC/MSMS technique is described in detail in 'Gerhard G. Rimkus and Dirk Hoffmann, Enantioselective Analysis of Chloramphenicol Residues in Honey Samples by chiral LC/MSMS and Results of a Honey Survey, Food Addit Contam A 2017, http://dx.doi.org/10.1080/19440049.2017.1319073

R26
FEATURES OF THE DETERMINATION OF RESIDUAL AMOUNTS OF AMPHENICOLS IN FOOD PRODUCTS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY QUADRUPOLE-TIME-OF-FLIGHT MASS SPECTROMETRY

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The widespread use of chloramphenicol in veterinary medicine has led to the appearance of its residual amounts in food. The toxic effect of this drug in food products on the human body has been established. In the Russian Federation, this drug is approved for use in veterinary medicine, but should not be found in food. However, there are residual amounts of chloramphenicol in milk and dairy products, meat, honey, poultry meat and fish products. Often, instead of the main drugs, succinate, palmitate, chloramphenicol stearate and thiamphenicol acetylcysteinate glycinate are used. In the animal and human body, chloramphenicol derivatives decompose to chloramphenicol, and thiamphenicol derivatives to thiamphenicol and acetylcysteinate. However, it has been established that undecomposed preparations of chloramphenicol derivatives can be found in food products. In the existing regulatory documents, this fact is not taken into account to determine these antibiotics, which can lead to significant errors in the analysis.
A simple method for the determination of amphenicols (chloramphenicol, chloramphenicol succinate, thiamphenicol, thiamphenicol glycinate acetylcysteinate, florfenicol, florfenicolamine) in food products by the method of high-performance liquid chromatography / quadrupole-time-of-flight mass spectrometry of high resolution is proposed. For food samples, extraction was performed with acetonitrile in the presence of formic acid, followed by dilution of the extract with distilled water prior to analysis. The determination ranges were 0.1-10 ng/g for chloramphenicol and 1-100 ng/g for thiamphenicol and florfenicol. The relative standard deviation of the analysis results did not exceed 10%, the duration of the analysis was 30-40 minutes.

Keywords: *amphenicols, veterenary drugs, time-of-flight, HPLC*

R27
ANALYSIS OF VETERINARY DRUG RESIDUES USING LIQUID CHROMATOGRAPHY COUPLED TO ACCURATE MASS HIGH-RESOLUTION MASS SPECTROMETRY

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In order to protect the public and animal health, EU countries must monitor food and food producing animals, assuring that harmful residues do not exceed regulatory limits. In the implementation of such monitoring programs, routine laboratories use targeted analysis for multi-residue determination with triple-quadrupole mass spectrometric detection. However, neither the monitoring programs are comprehensive enough nor the targeted methods can encompass the enormous number of compounds to be monitored. Therefore, implementation of full-scan screening methods with potentially unlimited scope of application is of paramount importance for official control laboratories. Thus, the use of high-resolution mass (HRMS) analyzers has gained popularity for screening of unlimited number of analytes in complex matrices. As a result, HRMS methods are playing already a central role in routine pesticide residue analysis but their potential in the analysis of banned veterinary drugs is not yet fully explored. The objective of this work was to develop a screening method for analysis of β-agonists with a liquid chromatography coupled to accurate-mass high-resolution mass spectrometry (LC–HRMS) and to assess its applicability at low regulatory levels. To this aim, the exact mass product ion spectra database was generated and the selection of quantifier/qualifier ions for reliable identification was evaluated in matrices like water, hair, retina, urine and liver. Data acquisition approaches on hybrid quadrupole-time-of-flight (QTOF) mass analyzer were also assessed: full-scan MS and a combination of full-scan and broadband CID experiments. A generic sample preparation was used for matrix hair, while a more laborious SPE sample preparation was employed for urine samples. The analyte detection and identification were evaluated in samples fortified at levels ranging from 0.5 to 3 ppb for urine and from 2.5 to 20 ppb for matrix hair. Criteria for confirming the identity e.g. retention time, mass accuracy, number of confirmatory ions and ion ratio were considered for establishing screening detection levels (SDLs) and screening identification levels. In matrix water and hair, the SDLs of beta-agonists were below the established recommended concentrations. In case of matrix urine, a severe matrix effect was observed that hampered the detection of cimbuterol, salbutamol, zilpaterol, terbutaline, fenoterol, while other analytes were detected at or below the recommended levels.

Keywords: *high-resolution mass spectrometry, liquid chromatography, veterinary drugs, residue analysis, beta-agonists*

R28
MAXIMISING THE SCREENING OF CONTAMINANTS IN FOOD WITH BIOCHIP ARRAYS

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Introduction. The presence of contaminants in food is cause of concern due to public health implications. The availability of screening methods for the detection of contaminants in food is then beneficial in test settings. Biochip array technology allows the simultaneous screening of multiple contaminants from a single undivided sample, which consolidates testing and reduces the quantity of samples to be assessed by confirmatory analysis. This study reports the application of different biochip arrays to the multiplex screening of anthelmintic drugs, growth promoters and nitrofurans in food.
Methodology. For each biochip array, simultaneous chemiluminescent competitive immunoassays, which define discrete test sites on the biochip surface, were employed. The assays were applied to the semi-automated bench top analyser Evidence Investigator. The system incorporates dedicated software to process and archive the multiple data generated.
Results. With the anthelmintics biochip array, the following anthelmintic drugs were detected: amino-benzimidazoles (albendazole 2-amino sulphone, amino-flubendazole, amino-mebendazole), avermectins (abamectin, doramectin, emamectin benzoate, eprinomectin, ivermectin), benzimidazoles (albendazole, albendazole sulphoxide, albendazole sulphone, flubendazole, oxfendazole, oxibendazole, parbendazole), cambendazole, levamisole, moxidectin, thiabendazole, 5-hydroxythiabendazole, keto-triclabendazole, triclabendazole, triclabendazole sulphone, triclabendazole sulphoxide. Limits of detection (LOD): range: 0.3 ppb-2.0 ppb in milk and 0.15 ppb-6.5 ppb in beef muscle. With the biochip array for nitrofurans, AHD (1-aminohydantoin hydrochloride), AMOZ (5 methylmorpholino-3-amino-2-oxazolidinone), AOZ (3-amino-2-oxazolidinone), SEM (semicarbazide) are detected simultaneously. LOD range: 0.06 ppb-0.35 ppb in prawn/shrimp, 0.06 ppb-0.4 ppb in beef, pork, poultry tissue, 0.08 ppb-0.5 ppb in honey. With the growth promoter biochip array, beta-agonists (clenbuterol, carbuterol, brombuterol, salbutamol, cimbuterol, mabuterol, mapenterol, terbutaline), boldenone, corticosteroids (dexamethasone, dexamethasone 21 acetate, betamethasone, betamethasone 21 acetate, flumethasone), nandrolone, ractopamine, stanozolol, stilbenes (hexestrol, diethylstilbestrol, diethylstilbestrol glucuronide, dienestrol), trenbolone and zeranol can be simultaneously determined. LOD range: 0.1 ppb-1.4 ppb in tissue, 2.0 ppb-170 ppb in feed.
Conclusion. Biochip array technology is applicable to the simultaneous screening of multiple contaminants from a single sample with biochip arrays applicable to different food matrices. This methodology enhances the scope of tests and the test result output, reducing the quantity of samples to be assessed by confirmatory analysis.

Keywords: *biochip arrays, anthelmintic drugs, growth promoters, nitrofurans, food*

R29
OPTIMIZATION OF A CLEAN-UP CONCEPT FOR THE DETERMINATION OF ANTIBIOTICS AND NITROIMIDAZOLES IN ANIMAL MATRICES USING THE SPE SAMPLE PREPARATION

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Antibiotics are important chemotherapeutics for curing bacterial diseases, and their application is especially common in veterinary medicine. However, in animals intended for human consumption there is the risk that their flesh may contain chemical residues. For this reason, strict regulatory limits were set up within the European Union [1]. For animals intended for human consumption there exist, depending on the matrices, *Maximum Residue Limits* (MRL) for approved agents and *Minimum Required Performance Limits* (MRPL) for banned agents [2]. Based thereupon, a verification of the legal latency times and the MRL is possible. For the determination of antibiotic residuals in animal matrices an intense sample preparation is needed to achieve the regulatory limits. Several new SPE cartridges (CHROMABOND® HR-X, CHROMABOND® HR-X PLUS from MACHEREY-NAGEL) were compared with the Oasis HLB (Waters) which had been used up to now. Furthermore, basic approaches for the optimization of the SPE clean-up were taken into consideration. Exemplarily, antibiotic agents from the groups of penicillins, cephalosporins, makrolides, tetracyclines, sulfonamides, diaminopyrimidine derivates, quinolines, lincosamides, pleuromutilins, amphenicols, and nitroimidazoles were determined in milk and bovine muscle using solid-phase extraction (SPE). Firstly, the performance of the antibiotic agents with the SPE cartridge was tested in the buffer solution (McIlvaine EDTA buffer, pH 4) without the matrix. High recovery rates were achieved especially for the Oasis HLB. However, the CHROMABOND® HR-X and the CHROMABOND® HR-X PLUS led to better results for the matrix filtrate samples. Afterwards, all the agents were added directly to the blank matrix samples and followed by sample preparation. The CHROMABOND® HR-X had an advantage over the other SPE cartridges since almost all the antibiotic agents could be determined and the quantification provided good recovery rates from 40% to 120%. A joining optimization did not increase the agent recovery rates despite a variation in the equilibration time. In consequence of another particle size, it is recommended to use 2 x 3 ml methanol to elute the compounds from the CHROMABOND® HR-X instead of 1 x 6 ml methanol for the Oasis HLB. In bovine muscle samples, a definite increase in the recovery rates could be observed. A variation of the agent concentrations of 25 µg/kg, 50 µg/kg, and 100 µg/kg featured equal recovery rates.

[1] S. Schwarz et al., Use of antimicrobial agents in veterinary medicine and food animal production, International Journal of Antimicrobial Agents 17 (2001) 431–437.
[2] Verordnung (EU) Nr. 37/2010 der Kommission vom 22. Dezember 2009 über pharmakologisch wirksame Stoffe und ihre Einstufung hinsichtlich der Rückstandshöchstmengen in Lebensmitteln tierischen Ursprungs.

Acknowledgement: *The authors would like to thank MACHEREY NAGEL for their financial support.*

R30
TISSUE BOUND FLORFENICOL RESIDUES: REQUIREMENT OF WHOLE TISSUE HYDROLYSIS FOR DETERMINATION OF TOTAL FLORFENICOL CONTENT IN PHYSICO-CHEMICAL DETECTION METHODS

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Florfenicol (FF) is a broad spectrum antibacterial compound licensed for use in animals used for food production. The EU maximum residue limit (MRL) refers to the '*sum of florfenicol and its metabolites measured as florfenicol-amine*' (FFA). Method development and analysis of incurred porcine kidney and muscle samples was carried out at AFBI. The results of these investigations demonstrate that the *major proportion* of florfenicol residues present *are non-solvent extractable* in incurred tissues. At day 0 withdrawal time >80% of the total FF residue content in kidney and >65% in muscle was tissue bound, with the proportion of non-extractable residues increasing with withdrawal time. The incurred study results demonstrate that methods lacking a hydrolysis step can have a 100% false negative or 'compliant' rate. Physico-chemical screening or confirmatory methods *must* therefore include a hydrolysis step to measure total FF related residue content and ensure compliance with the MRL definition listed in Commission Regulation (EU) 37/2010. A robust and sensitive method for the determination of total florfenicol residue content by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS) was developed and validated at AFBI in accordance with EC directive 2002/657/EC.

Keywords: *veterinary drug, florfenicol, LC-MS/MS, florfenicol amine, residues*

R31
MULTI-CLASS VETERINARY DRUG ANALYSIS WITH SELECTIVE LIPID REMOVAL USING CAPTIVA EMR-LIPID

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Methods for multi-class veterinary drugs in food implement an extraction capable of accommodating diverse classes of analytes, but also large amounts of matrix co-extractives, including lipids, requiring cleanup. Traditional cleanup materials struggle to effectively and selectively remove unwanted lipids causing low analyte recovery, poor method reproducibility, and increased instrument maintenance. Captiva EMR-Lipid overcomes the limitations of traditional sample cleanups by providing selective lipid removal for fatty samples without unwanted analyte retention. Captiva EMR-Lipid offers a simple, pass-through cleanup workflow for fatty extracts from QuEChERS, protein precipitation, and other organic extractions. This work describes the validation of a 2-step extraction protocol optimized for 39 representative veterinary drugs from 17 classes followed by cleanup with Captiva EMR-Lipid and analysis with LC/MS/MS. The method demonstrates excellent extraction and cleanup efficiency from meat samples, delivering high recovery (60%-120%) and precision (%RSD <20%) for the vast majority of analytes. Lipid removal efficiency and comparisons with traditional cleanup methods are also shown. The lipid removal efficiency and selectivity of Captiva EMR-Lipid is ideal for those seeking a simple sample preparation workflow that can improve method performance for multi-residue analysis.

R32
APPLICATION OF NON-THERMAL PLASMA TECHNOLOGY FOR ANTIBIOTIC RESIDUES REMOVAL

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Progressing climate change raises concerns regarding both food safety and security worldwide. Increased efforts are directed towards increasing agricultural productivity and more efficient management of current resources while assuring consumers' safety by continuous monitoring of variety of man-made and natural chemical residues in food commodities and the environment itself. In order to limit food wastage through rejection of non-compliant produce, novel technologies enabling food detoxification are being currently explored. The present study aims at evaluating the efficiency of cold atmospheric plasma technology in removal of antibiotic resides in milk. The decomposition efficiently and its optimisation for fifteen β-lactam antibiotics, including penicillins and cephalosporins in both solvent and food systems has been evaluated. Possible decontamination by-products and their persistence have been assessed, as well as the influence of plasma treatment on small molecular mass analytes composition in the milk matrix. Results in solvent based systems show that significant degradation of all compounds can be achieved within first 5 minutes of treatment and was shown to be heavily dependent on plasma composition and analytes' structure. The process proved to be repeatable and reproducible between the exposure days. However, minimal concentration dependence was shown at longer treatment times. Transfer to milk presents analytes' decomposition efficiency dependence on the complexity of the matrix exposed, thus, additional process optimisation and assessment is required. Nevertheless, encouraging degradation observed for the majority of resides assessed suggesting possible alternative applications such as water or sewage treatment.

Keywords: *antibiotic residues, cold plasma, milk*

Acknowledgement: *The research was funded by a BBSRC grant - 'EnvironSafe: Cold Plasma applications for Food Safety and Sustainability' (BB/P008496/1).*

R33
MULTICLASS RESIDUE ANALYSIS OF VETERINARY DRUGS IN ANIMAL TISSUES USING AGILENT EMR-LIPID OR CAPTIVA EMR BY LIQUID CHROMATOGRAPHY COUPLED WITH TANDEM MASS SPECTROMETRY DETECTION

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This application describes a workflow solution that was developed for the screening and quantification of multiclass veterinary drugs in different animal-derived food samples, from sample preparation to quantification of results. These veterinary drugs, belonging to 27 different chemical classes, included macrolides, sulfonamides, tetracyclines, β-lactams, β-agonists, chloramphenicols, nitroimidazoles, benzimidazoles, glucocorticosteroids, nonsteroid anti-inflammatory drugs (NSAIDs), anticoccidiosis drugs, quinolones, imidazoles, androgens, polyethers, triphenylmethanes, phenothiazines, quinoxalines, trematocides, antivirus, pesticides, dapsones, organic acids, nitros, tranquilizers, and so forth. The animal-derived food samples included muscle and liver of swine, cow, and chicken, as well as fish and hen eggs. The sample preparation involved a rapid and efficient protein precipitation extraction by acidified acetonitrile, followed by Agilent EMR-Lipid dSPE or Captiva EMR-Lipid Cartridges for further cleanup. The workflow solution supports the mid-to-high-end of Agilent tandem mass spectrometers (Agilent 6460 Triple Quadrupole LC/MS, Agilent 6470 Triple Quadrupole LC/MS, and Agilent 6495 Triple Quadrupole LC/MS) coupled with the Agilent Jet Stream Ionization Source. With >160 veterinary drugs spanning 27 different classes in one method, our workflow solution would significantly help increase sample throughput without sacrificing data quality, robustness, or sensitivity.

R34
RAPID SCREENING AND QUANTITATION OF Β-LACTAM ANTIBIOTICS IN FOOD BY ACCURATE MASS IONS USING UHPLC-QTOF/MS

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A rapid screening and sensitive quantitation of 19 β-lactam antibiotics in various food matrixes (meat, kidney, liver, pork, milk, eggs, honey) by ultra-high performance liquid chromatography / quadrupole-time-of-flight mass spectrometry of high resolution was proposed. Protein precipitation and extraction of the analytes were carried out with acetonitrile. The extract was used for analysis without purification. Identification was carried out for the accurate masses of analyte ions (± 5 ppm), retention time (± 0.1min) and the pattern of the isotope distribution of ions (mSigma < 20). It has been established that penicillins form positively charged protonated adducts with methanol $[M+CH_3OH+H]^+$, cephalosporins - protonated ions $[M+H]^+$, or adducts with sodium $[M+Na]^+$. A slightly differentiated matrix effect is established for milk, poultry meat, beef, pork, liver, kidney, bacon and eggs, and different for honey. With the aim of leveling the matrix effect, the analysis is suggested to be carried out both by the method of additives and using a single matrix calibration (for milk) for poultry meat, beef, pork, liver, kidney, bacon and eggs, and honey separately. The LODs and LOQs were 0.05-5 ng g^{-1}, 1-10 ng g^{-1}, respectively. Quantitative analysis of the detected analytes was carried out both with the method of standard additives and with the use of matrix calibration (RSD% ≤ 5). Recoveries of β-lactam antibiotics for different matrices and different sample preparation were 75 to 110%; RSD% did not exceed 10% under matrix-matched calibration conditions. The advantage of using the additive method in determining the residual amounts of the identified β-lactams in food products is shown.

R35
RAPID SCREENING AND DETERMINATION OF 150 RESIDUES OF VETERINARY DRUGS IN MILK BY UHPLC-QTOF/MS

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A rapid screening and determination of 150 residues of veterinary drugs in milk by the method of ultra-high-performance liquid chromatography / quadrupole-time-of-flight mass spectrometry of high resolution was proposed. For analysis, 1.0 g of milk was used; protein precipitation and extraction were performed with acetonitrile; the extract was used for analysis without purification and concentration. The identification of veterinary preparations was carried out for the accurate masses of the ions of the analytes formed during electrospray ionization, retention time, and the pattern of the isotope distribution of the ion (mSigma). The quantitative analysis of the detected analytes was carried out by the method of standard additives. The detection limits were 0.1 - 0.5 ng g^{-1}, the ranges of the analytes (0.1) being determined are 1-500 ng g^{-1}; the duration of sample screening is 20-30 min; the analysis is 30-40 min.

R36
SIMULTANEOUS DETECTION OF ANTIBIOTICS FROM TEN FAMILIES IN MEAT AND FISH SAMPLES USING VALIDATED FLOW CYTOMETRIC METHOD

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To guarantee consumer protection and industrial transformation processes, rapid screening analytical methods for detection of antibiotic residues in the food chain are essential. Current tendency in food analysis is implementation of multi-residue technologies which allow simultaneous monitoring of several analytes per sample (multiplexing); thus considerably reducing analysis time and cost thanks to high throughput capability. Flow Cytometric Immunoassays (FCIAs) combine the detection of receptor-ligand interactions by immunoanalysis, with the multi-parametric characterization of individually encoded beads provided by Flow Cytometry.
The aim of this work is to present the validation of a FCIA for the simultaneous analysis of more than 80 antibiotic residues from 10 families widely used in the veterinary field, including aminoglycosides, β-lactams, tetracyclines, macrolides, lincosamides, phenicols, fluoroquinolones, sulfonamides, polymyxins and pleuromutilins in pork, bovine and poultry meat as well as in high and low-fat content fish muscle.
The validated assay is based on a simple and common extraction for all matrices which is particularly adapted for routine analyses. The performance of the method shows Detection Capabilities (CCβ) for most of the antibiotics within the scope at or below European regulatory limits. This test kit BEADYPLEX™ allows the rapid detection of antibiotics in different food commodities, and also their family identification in one single screening analysis.

Keywords: *antibiotics, flow cytometry, meat, fish, screening*

Acknowledgement: *This work fits into the Eurostars project Go-With-The-Flow E!6993 and has received funding from Eureka and European community*

R37
OXYTETRACYCLINE ACCUMULATION IN THE MACROALGAE ULVA LACTUCA: POTENTIAL RISKS FOR IMTA SYSTEMS

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The steep increase in aquaculture production and its negative effects are forcing the development of sustainable aquaculture systems. Integrated multitrophic aquaculture (IMTA) can be a suitable approach to fish production, where several species with independent commercial value are cocultured, with associated economical and environmental benefits. However, intensive productions are often related to the use of antibiotics and other chemicals in order to help solve disease outbreaks in culture sites. Exposure tests were performed with the macroalgae *Ulva lactuca* in order to evaluate the effects of oxytetracycline in growth. Antibiotic concentrations both in seawater and macroalgae were determined at several sampling points after immersion in an oxytetracycline bath at two concentrations, and the effects assessed. These results can help comprehend one of the possible scenarios of farming macroalgae species in IMTA systems for feed and food industry. As biofilters, these organisms are located at the exit point of fishponds or near cages, potentially accumulating pharmaceuticals.

Keywords: *macroalgae, oxytetracycline, aquaculture, IMTA, accumulation*

Acknowledgement: *Financial support was given by FEDER through the Operational Program for Competitiveness Factors – COMPETE, through FCT – the Portuguese Foundation for Science and Technology – under the grant attributed to João Rosa (grant SFRH/BD/102008/2014) and" and to Sara Leston (SFRH/BPD/91828/2012).*

R38
FLORFENICOL AND FLORFENICOL AMINE RESIDUE DEPLETION IN TAMBAQUI MUSCLE (COLOSSOMA MACROPOMUM)

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The residue depletion of florfenicol (FF) and its metabolite, florfenicol amine (FFA), in tambaqui muscle was evaluated in fish farmed under average temperature of 31.1 ^{0}C, which is typical on the Amazonia region of Brazil and responsible for the largest production of tambaqui. FF was administered orally, through the feed, at a dose level of 10 mg/kg body weight, during 10 days. Groups of ten fish were slaughtered at 1, 2, 3, 4, 5, 6, 7, 10, 14, 16, 21, and 28 days after treatment. A high-performance liquid chromatography- mass spectrometer (HPLC-MS/MS) analytical method was developed and validated for quantitation of FF and FFA. Method validation was performed according to the criteria of Commission Decision 2002/657/EC and the validation guidelines of the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH GL49, 2011). The method limit of quantitation (LOQ) was 10 ng/g and 15 ng/g for FF and FFA, respectively. Data indicates low FF and FFA deposition in the tambaqui muscle, since the performed analysis after the 4th and 2nd day posttreatment, FF and FFA residue levels in the tambaqui fillets were below their LOQ, respectively. Also, at the 1st day after treatment the sum of FF and FFA residues were already in a lower concentration (238 ng/g) than the allowed maximum residue limit (MRL) of 1000 ng/g established by Commission Regulation (EU) No 37/2010. Thus, according to Directive 2004/28/EC a 1 day withdrawal period for the use of FF in tambaqui can be considered appropriate to warranty the healthiness of tambaqui muscle and, consequently, to assure that their consumption do not offer risk to the consumers health.

Keywords: *florfenicol, florfenicol amine, depletion study, colossoma macropomun, tambaqui*

Acknowledgement: *The authors acknowledge the financial support received from São Paulo Research Foundation-Agilent Technologies (FAPESP-Agilent Grant 2013/50452-5); and the National Council for Scientific and Technological Development (CNPq Grant 305390/2013-9), Brazil.*

R39
MULTIRESIDUE METHOD FOR DETERMINATION OF SULFONAMIDES AND TRIMETHOPRIM IN TILAPIA FILLET BY LIQUID CHROMATOGRAPHY COUPLED TO QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY

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A multiresidue method for quantitation of sulfonamides (sulfapiridine, sulfamerazine, sulfatiazole, sulfamethazine, sulfadimetoxine, sulfametoxazole, sulfametoxipiridazine) and trimethoprim in tilapia fillet (*Oreochromis niloticus*) using liquid chromatography coupled to mass spectrometry (LC-ESI-QToF/MS) was developed and validated. The sample preparation was optimized using the QuEChERS approach. The chromatographic separation was performed using a C-18 column and 0.1% formic acid in water and acetonitrile as mobile phase in isocratic elution mode. The validation parameters evaluated were linearity ($r \geq 0.99$); limit of detection (LOD) and limit of quantification (LOQ), 1 ng g^{-1} and 5 ng g^{-1}, respectively; and intra- and inter-day precision (CV < 15.0 %). Considering the maximum residue limit (MRL) established for sulfonamides and trimethoprim of 100 and 50 µg kg^{-1}, respectively, the decision limit (CCα 102.6 - 120.0 µg kg^{-1} and 70 µg kg^{-1}) and detection capability (CCβ 111. 7 - 140.1 µg kg-1 and 89.9 µg kg^{-1}) were determined. Analyses of tilapia fillet samples from fish exposed to sulfamethazine through feed (incurred samples) were conducted in order to evaluate the method. This new method demonstrated to be fast, sensitive and suitable for monitoring the sulfonamides in tilapia fillet.

Keywords: *multiresidue analysis, sulphonamides, trimethoprim, Tilapia, QuEChERS*

Acknowledgement: *The authors acknowledge the financial support received from São Paulo Research Foundation-Agilent Technologies (FAPESP-Agilent Grant 2013/50452-5); and the National Council for Scientific and Technological Development (CNPq Grant 305390/2013-9), Brazil.*

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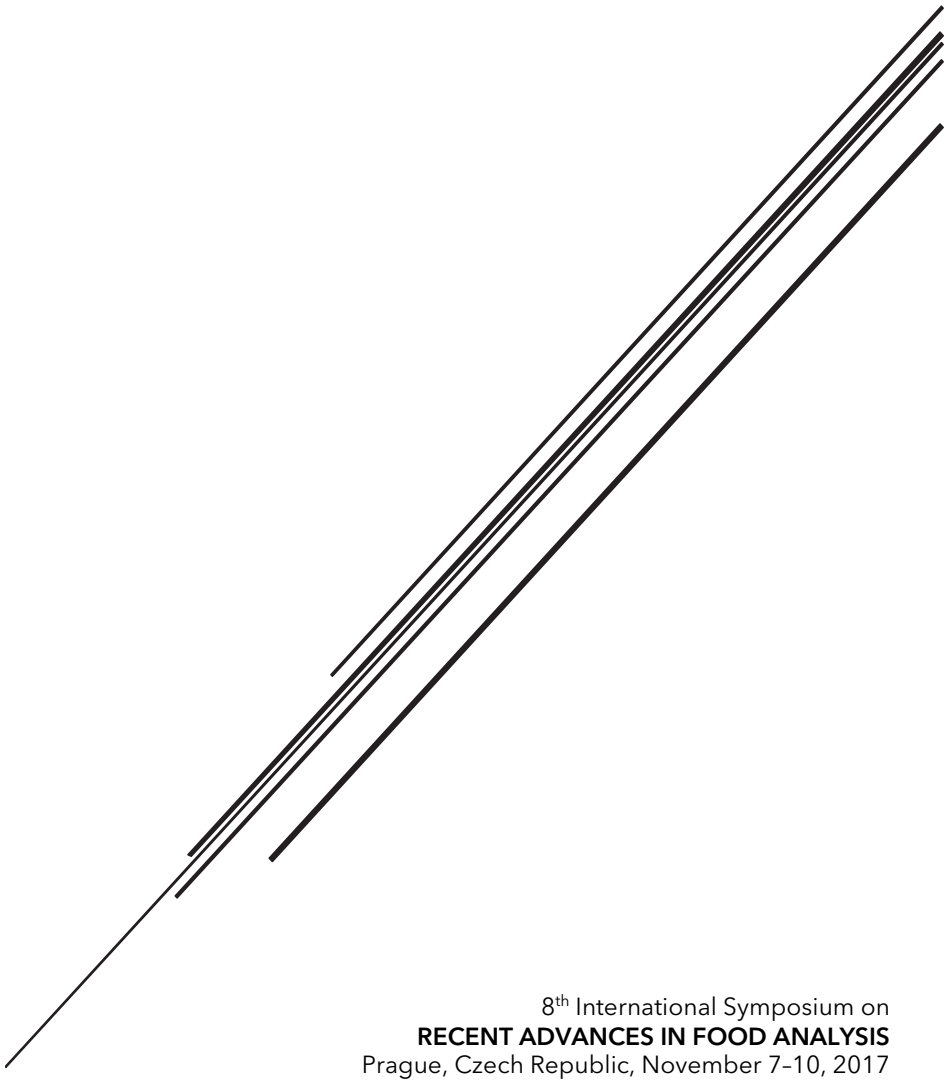

8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

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LAST MINUTE POSTERS

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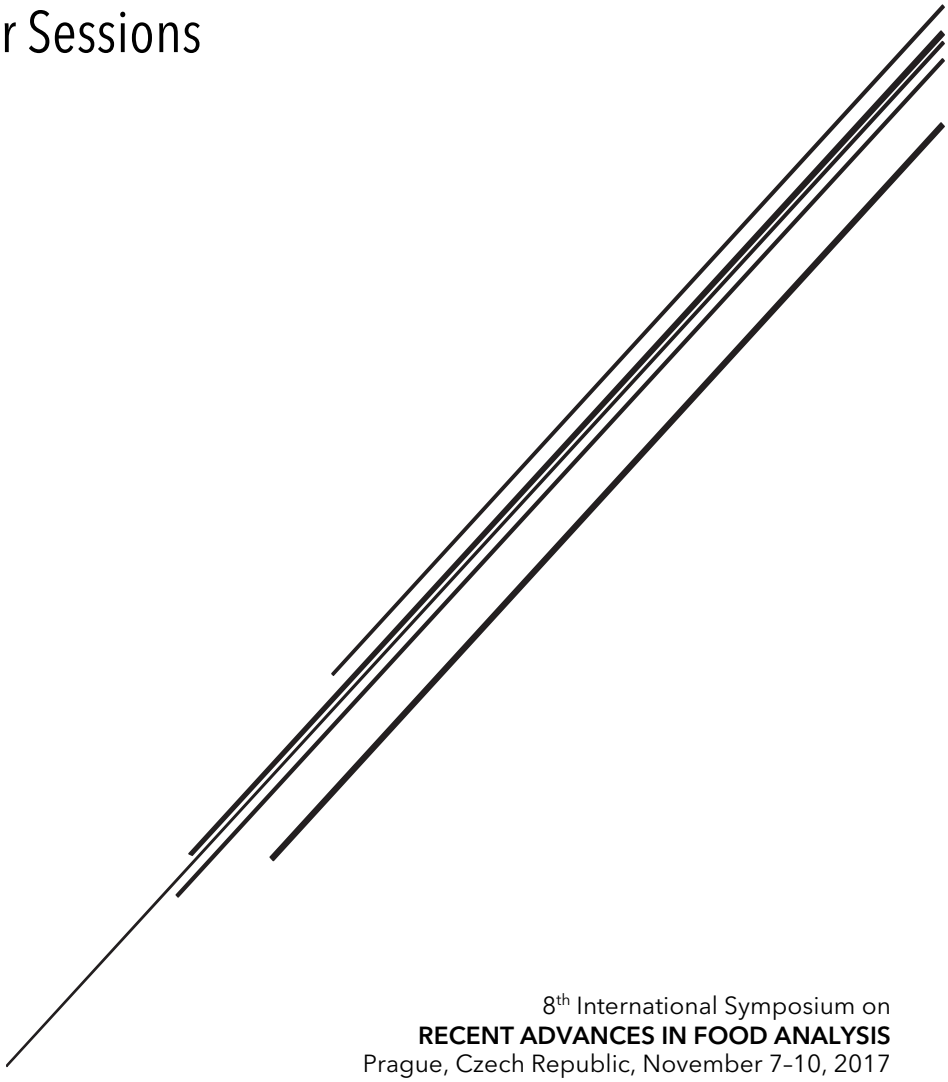

8th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 7–10, 2017

LAST MINUTE POSTERS

LM1
TOWARDS A GENERIC PROCEDURE FOR THE DETECTION OF RELEVANT CONTAMINANTS FROM WASTE ELECTRIC AND ELECTRONIC EQUIPMENT (WEEE) IN PLASTIC FOOD-CONTACT MATERIALS: PRACTICAL ASPECTS

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Beside an intensive food control on persistent organic pollutants, the other way of contaminating food is appearing by using contaminated food preparation/storage materials. The last few years, traces of brominated flame retardants (BFRs) have been detected in black plastic food contact materials (FCMs) indicating the presence of recycled plastics, mainly coming from waste electric and electronic equipment (WEEE) as BFRs are main additives in electric applications. In order to evaluate efficiently, and preliminary in situ, the WEEE presence in plastic FCMs, a generic procedure for evaluation of WEEE presence in plastic FCMs by using defined parameters having each an associated importance level has been proposed. By our experience, this can be achieved by combining parameters like overall bromine (Br) and antimony (Sb) content; additive and reactive BFR, rare earth element (REE: Ce, Dy, La, Nd, Pr, Y) and WEEE relevant elemental content with additionally polymer purity. In most of the cases the WEEE contamination could be confirmed by combining X-ray fluorescence spectrometry (XRF) and thermal desorption/pyrolysis GC-MS at first. The Sb and REE content did not give a full confirmation about the root of contamination, however for Sb the opposite counts: Sb was joined with elevated Br signals. Therefore Br at first followed by Sb was used as WEEE precursors as both elements are used as synergetic flame retardants system. WEEE specific REEs could be used for small WEEE (sWEEE) confirmation however this parameter should be interpreted with care. The polymer purity by FTIR and pyrolysis GC-MS in many cases could not confirm WEEE specific contamination however can be used for purity measurements and suspicion the usage of recycled fractions (WEEE and non-WEEE) as a third line confirmation.
Our proposed generic procedure for the evaluation of WEEE in plastic FCMs, includes an antimony loop for the synergetic confirmation, and levels of importance, all taking into consider principles of Regulation (EU) 10/2011 (plastic FCMs) and (EC) 282/2008 (recycled plastic FCMs).
To the best of our knowledge, WEEE waste adding to plastic FCMs is illegal according to EC 10/2011, however, due to the lack on screening mechanisms there is still breakthrough of such articles on the market, therefore our generic procedure to quickly and effectively screen suspicious samples.

Further reading:
F Puype et al. ``Towards a Generic Procedure for the Detection of Relevant Contaminants From Waste Electric and Electronic Equipment (WEEE) in Plastic Food-Contact Materials: A Review and Selection of Key Parameters ``. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 34 (10), 1767-1783. 2017 Jun 20.

Keywords: *brominated flame retardants, thermal desorption, mass spectrometry*

LM2
COMPREHENSIVE CANNABIS ANALYSIS: PESTICIDES, AFLATOXINS, TERPENES, AND HIGH LINEAR DYNAMIC RANGE POTENCY FROM ONE EXTRACT USING ONE COLUMN AND ONE SOLVENT SYSTEM

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Increased legalization of Cannabis for medical and adult use in the United States substantiates the need for robust and reproducible methods for quantitation of pesticide residues, aflatoxins, relevant psychotropic cannabinoids, and terpene profile in Cannabis products. The SCIEX vMethod demonstrates the capability of the Triple Quad/QTRAP 6500+ in meeting the maximum residual levels (MRLs) for the full suite of pesticides comprising the Oregon Pesticide List in Cannabis flower matrix. The vMethod has undergone validation and verification and is an optimized methodology for pesticide residues and potency analysis. However, quantitation of aflatoxins and terpene profiling are increasingly demanded analyses in this industry. In order to demonstrate comprehensive testing of Cannabis products, these four compounds classes were all measured in flower samples using a total of two acquisition methods for the 6500+, while using a single mass spectrometer and separation configuration. A 16 minute gradient maximizes separation of endogenous isobaric interferences for pesticide and aflatoxin analysis. A seven-minute gradient separates all isobaric cannabinoids from each other and ensures precision of quantitative analysis across the full range of potential concentrations, yielding a single method to assess potency from product concentrations between 0.03-90%. The same gradient effectively separates terpenes to assess the Cannabis flavor profile.

Keywords: *cannabis, pesticides, mycotoxins, terpenes, cannabinoids*

Acknowledgement: *SCIEX acknowledges Phenomenex (Torrance, CA) for providing HPLC columns and expertise for this application note.*

LM3
DETERMINATION OF AFLATOXIN M1 IN MILK PRODUCTS USING AN AUTOMATED IMMUNOAFFINITY CARTRIDGE SYSTEM

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Aflatoxin M1 is a major metabolite of aflatoxin B1 in humans and animals and may be present in milk from animals fed with aflatoxin B1 contaminated feed. Current European Legislation is in place for aflatoxin B1 in animal feed in order to reduce the risk of aflatoxin M1 in the food chain. For aflatoxin M1 legislative levels are often very low therefore suitable methods are required.
R-Biopharm Rhône has developed a patented, online immunoaffinity cartridge which can be used prior to HPLC for analysis of aflatoxin M1. The cartridges are used together with the RIDA®CREST handling system to combine automated online sample clean-up with quantitative analysis. Each cartridge is calibrated and can be used for up to 12 samples before the cartridge is automatically removed and replaced with a new one.
The cartridges contain monoclonal antibodies attached to a polymer making them highly specific and sensitive. A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero.
Here, methods and results will be presented for the automated, rapid determination of aflatoxin M1 in various commodities, including milk, infant milk formula, cream, yoghurt and cheese.

Keywords: *milk, clean-up, aflatoxin M1, infant milk formula, immunoaffinity*

LM4
IMPROVING WORKFLOW AND REDUCING COSTS OF MYCOTOXIN ANALYSIS THROUGH AUTOMATION WITH RIDA®CREST AND IMMUNOPREP® ONLINE

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Increased legislation and scrutiny of the food supply chain has led to more frequent testing of mycotoxins, which has in turn placed additional challenges on food testing laboratories. Laboratories are frequently being asked to increase capacity and to provide a faster turnaround service in order to compete with other labs, without compromising on test accuracy or quality of results. They are also challenged to find innovative and cost-effective ways to improve testing efficiency and workflow and to reduce the time samples are idle during processing and test application. All of this has to be achieved at the same time as eliminating errors, conserving labour and reducing the workload pressure on staff.
In this study, RIDA®CREST together with IMMUNOPREP® ONLINE is shown to automate sample preparation and analysis, saving time and improving throughput by using two cartridges to process and analyse two test samples simultaneously. The system is also demonstrated to improve accuracy, reproducibility and recoveries of results in a wide range of commodities. IMMUNOPREP® ONLINE is recommended to be reused up to 15 times, reducing storage space and transport costs. The system was also found to improve traceability and performance reducing errors with the added advantage for each cartridge to be quality controlled onsite for greater confidence and compliance with accreditation systems such as ISO 17025. In summary RIDA®CREST with IMMUNOPREP® ONLINE was demonstrated to increase processing capacity and significantly reduce manual labour time.

Keywords: *cartridge, immunoprep, HPLC, LC-MS/MS, UHPLC*

LM5
OPTIMISATION OF BIOLOGICALLY ACTIVE COMPOUNDS EXTRACTION FROM CHOKEBERRIES MARC USING RESPONSE SURFACE METHODOLOGY

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Chokeberries (*Aronia melanocarpa) in food industry are widely used for production of juice. P*omace formed as an industrial by-product *is rich source of polyphenols and other compounds with biological activity, including natural antioxidants*. The aim of the current research was to optimize extraction of biologically active compounds from chokeberries pomace by response surface methodology. Box-Behnken design was used for optimization of extraction conditions from frozen pomace. Experiment were performed in two steps. Firstly as variables were selected: time (min), acetone concentration (% v/v), number of extraction cycles, and in the second step: temperature (°C), ethanol concentration (%v/v) and citric acid concentration (% w/v). For extracts as responses total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH, ABTS+ scavenging activity) were determined using a spectrophotometric methods.
The Box–Behnken design for the first experiments showed importance of the acetone concentration in extraction solvent to maximise extraction yield. Strong interactions between acetone concentration and number of extraction cycles were determined. Results of the second step showed importance of ethanol concentration and temperature showing positive effect of lower temperature for TPC and TFC responses. Whether for DPPH scavenging activity higher results were obtained in samples extracted in higher temperatures.
Box-Behnken design is an effective method for optimization of the extraction conditions of phenolic compounds and compounds with scavenging activity but optimal conditions differ.

Keywords: *chokeberries antioxidant polyphenols anthocyanin response surface methodology*

Acknowledgement: *National Research Programme "Sustainable agricultural resources of high quality and healthy food production Latvian (AgroBioRes)" (2014–2017). Project No. 4 „Sustainable use of local agricultural resources for development of quality and healthy food product development (FOOD)"*

LM6
USE OF RESPONSE SURFACE METHODOLOGY FOR THE ASSESSMENT OF THE PHENOLIC COMPOUNDS AND ANTIOXIDANT PROPERTIES OF CROTON LECHLERI SAP CO-PRODUCT EXTRACT FROM AMAZON FOREST, BRAZIL

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The objectives of this study were to optimize the extraction of total phenolic compounds (TPC), total anthocyanin (TA) and to evaluate the antioxidant activity (AA) from *Croton lechleri* sap co-product extract. A factorial 23 design (FD) with three factors were conducted for optimization using response surface methodology (RSM). The temperature (35 and 70 °C), the time (30 and 90 min), and the solvent (ethanol and water) were designed for the extraction. This design was composed of eight trials performed in triplicate. The TPC was determined by Folin Ciocalteu, the TA was performed by the pH-differential method, while AA by scavenging activity of the DPPH• (2.2-diphenyl-1-picrylhydrazyl) radical. The TPC, TA and AA ranged from 192.32 ± 0.56 to 61.44 ± 3.65 mg GAE/g sap co-product (GAE: Gallic acid equivalent), 2.48 ± 0.24 to 21.71 ± 1.83 mg/100 g sap co-product and 4.03 ± 0.31 to 16.13 ± 0.11 µmol de Trolox/g sap co-product, respectively. In the best extraction condition recommended by FD and RSM for the total phenolic compounds content and antioxidant activity, the ethanol was used as solvent at 70 °C for 60 min. However, the aqueous extract at 70 °C for 30 min showed the highest TA content (21.71 ± 1.83 mg/100 g sap co-product) and had no statistical differences between the aqueous extract at 70 °C for 90 min (20.04 ± 2.83 mg/100 g sap co-product). The statistical analysis showed that the most significant factor in the extraction of antioxidant compounds was the solvent, followed by temperature, and time. The phenolic compounds were determined by High-Performance Liquid Chromatography. High contents of gallic acid (94.88 ±2.08 mg/kg), catequin (260.11±18.23), epicatequin (22.05±2.38) and siringic acid (28.68± 2.15) were detected after treatment with ethanol at 70 °C for 60 min. The extract analyzed under these conditions showed inhibitory activity against *Staphylococcus aureus*, *Salmonella bongori*, *Salmonella thyphimurium* and *Candida albicans* at the concentration of 0.005, 0.010, 0.005 and 0.150 mg/mL, respectively. In addition, these extracts showed bactericidal activity against *Salmonella thyphimurium* (0.630 mg/mL). This study illustrated that the optimal conditions for extraction of the phenolic compounds from *Croton lechleri* sap co-product extract have a high potential to produce functional ingredients. Furthermore, these results confirm the possibility to use the Croton lechleri sap co-product as alternative antioxidant and antimicrobial agent from sustainable sources in the food industry.

Keywords: *croton, sap, phenolic compounds, antioxidant aktivity*

Acknowledgement: *The authors wish to thank the National Council for Scientific and Technological Development – CNPq and the UTFPR for the scholarships and financial support.*

LM7
MIXOLAB THERMOMECHANICAL CHARACTERISTICS OF DIFFERENT GLUTEN-FREE BREAD FORMULATIONS WITH RICE FLOUR, CORN STARCH OR POTATO STARCH

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The Mixolab, is a recording dough mixer used to measure the rheological properties of dough subjected to dual stress of mixing and temperature changes. Mechanical changes due to mixing and heating simulate the mechanical work as well as the heat conditions for the bread baking process. It measures torque (Nm) produced by dough between two mixing blades. Parameters obtained from Mixolab curve were: C1 torque (water absorption required for dough to produce target torque), C2 torque (related to protein weakening as a function of mechanical work and temperature), C3 torque (related to starch gelatinisation during heating), C4 torque (hot gel stability), C5 (related to starch retrogradation). The aim of this study was to determine the Mixolab thermomechanical characteristics of gluten-free bread formulations with different flour:starch ratios and water addition levels.
Rice flour and starch (potato or corn) mixtures were prepared at different ratios (70:30 or 60:40). Mixolab (Chopin Technologies, France) was used to obtain the curves either at target torque (1.1±0.05Nm) or different water addition levels (80%, 84%, 88%, 92%). ICC method No:173 and Chopin+ protocol (dough weight 80g) was carried out in the study.
The target torque for rice flour:potato starch at 70:30 or 60:40 ratios were achieved at water absorptions of 62.0% and 61.5%, respectively. Increase in the amount of potato starch resulted in a decrease in water absorption. For rice flour:corn starch mixtures, water absorptions were 60.0% (70:30) and 59.0% (60:40). For the samples with different flour:starch ratios, no marked changes were observed in C2, C3, C4, C5 torques of the Mixolab curves that the target consistency obtained. Increasing the water addition level caused decreases in all of the torque values of all samples.

Keywords: *Mixolab Thermomechanical Characteristics, Gluten-free Bread Formulations, Rice Flour, Corn Starch, Potato Starch*

Acknowledgement: *The authors wish to thank Hacettepe University, Scientific Research Projects Coordination Unit for the financial support (Project No:014 09 602 001-727).*

LM8
ENHANCE CHROMATOGRAPH PERFORMANCE ON PESTICIDE RESIDUES QUANTIFICATION BY DILUTION TECHNIQUE IN GC/MS/MS

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From time, immemorial India has been known as the land of spices. The world consumption of spices is growing steadily year by year. Expansion of India's export of spices to a higher level or even to retain its current share of the world market can be achieved through increased productivity and improved quality. Most of the countries across the world are bringing down the permissible levels of contaminants. Thus, scientific and technological advancement brings forth new challenges in the international trade. The health and food laws which are increasingly becoming stringent reflect the concern of the respective governments for the safety and welfare of their people. Among the wide variety of spices, the major export commodities from India include black pepper, chili and turmeric. Government is implementing various food safety regulations and standards to regulate manufacture, storage, distribution, and control of spices. Consequently, the European Rapid Alert System for Food and Feed (RASSF) alerts for Indian spices came down drastically in the recent years.
Selective analyte signal detection by mass spectrometer is popular in terms of result consistency. QuEChERS sample preparation for spices mentioned above were validated in accordance with SANTE11945/2015 and met all its criteria. However, there was complaint on peak shape distortion consequently frequent liner change, column trimming and ionization source cleaning. Dried black pepper powder was taken for this study to investigate cause.
Using retention time locking and pesticides Multiple-Reaction Monitoring (MRM) database had saved the time to set up the GC/MS/MS instrumental parameters. Due to the availability of more than two MRM transitions for 1100 pesticides, PCBs, PAHs compounds, it was possible to choose matrix free MRM transitions to provide superior selectivity and sensitivity for routine operation.
The co-extractives (especially non-volatile) is being deposited at the inlet part of the GC system are typically responsible for formation of new active sites and cause distortion of peak shapes and shifting of the retention times of target analytes. In addition, interfering ions (formed during the ionization from the matrix co-extracts) with masses close to those of target residue and, even ion suppression, are the main factors that limit achieving low detection limits and reliable analyte identification.
Pepper consists of volatile extract between 0.3–4.2% and non-volatile extract between 3.9-11.5 %. Three published modified QuEChERS sample preparation with more dispersive clean up were experimented. The efficiency of the cleanup experiment was investigated by gravimetrically. As there was no significant reduction in co-extractives by these methods, dilution of the final extract was made considering method quantification limit of 10ng/g.
Targeted list of 133 pesticides were diluted 50 times (0.02 g/mL) were detected with > 10 S/N. validated as per SANTE guidelines.

Keywords: *GC-MS/MS QuEchERS Pesticides quantificaion*

Acknowledgement: *Madasamy. Chennai Mettex Labs, Chennai.*

LM9
SCREENING OF TETRODOTOXIN IN BIVALVE MOLLUSCS FROM GALICIAN RIAS (NORTHWEST OF SPAIN)

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Tetrodotoxins (TTX) are a potent group of natural neurotoxins produced by microorganisms and affecting the aquatic environment. These neurotoxins have been recently found in some species of bivalves and gastropods along the European Coasts (Greece, UK and The Netherlands) linked to the presence of high concentrations of Vibrio, in particular Vibrio parahaemolyticus. This study is focused on the evaluation of the presence of TTX in bivalves from Galician Rias (NW of Spain) following the EFSA recommendation included in the EFSA opinion about TTX. To carry out this evaluation the detection and isolation of the major Vibrio spp and other enterobacterial populations have been carried out with the aim of determining the pathways genes (PKS and NRPS) involved in the biosynthesis of these toxins. The collected samples were screened for Vibrio spp, and the positive samples were analysed by biochemical and genetic test. The selected samples were sent for confirmation by LC-MS/MS, after positive results obtained by neuroblastoma cell assay (N2a). The conditions described by Turner et al and Yamashita et al. have been applied and optimized for the analysis of TTX and some of its analogues, such as trideoxy, dideoxy and deoxy compounds. The method has been in house validated and a good linearity in the range from 0.16 to 10.0 mg of TTX /Kg of shellfish tissue was obtained being the LOD= 0.36 µg TTX/Kg and LOQ= 1.03µg/Kg. Some of the samples previously selected after positive response to the biochemical and genetic tests were found positive for TTX but with low concentrations of TTX (< LOQ). TTX toxin was not detected in extracts from cell-cultured. The possible presence of trideoxy and dideoxy TTX analogues was identified in some shellfish and bacterial extracts but the lack of standards for these analogues did not allow the progress on the determination of these compounds.

Keywords: *tetrodotoxin, LC-MS/MS*

Acknowledgement: *This work has received financial support from the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016-2019) and the European Union (European Regional Development Fund - ERDF)*

LM10
APPLICATION OF CELL BASED ASSAY FOR THE SCREENING OF EMERGING MARINE BIOTOXINS IN EUROPE

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Marine biotoxins are natural contaminants of the marine environment produced as secondary metabolites by bacteria and microalgae and mostly affecting bivalve molluscs. Recently, two groups of marine toxins have been considered the main emerging toxins in Europe, Ciguatoxins (CTX) and Tetrodotoxins (TTX).
The Ciguatera Food Poisoning (CFP) is a food poisoning endemic of tropical and subtropical regions as a result of the consumption of fish contaminated with Ciguatoxins (CTXs). After some outbreaks in some European regions and the occurrence of several intoxications in different European locations due to the consumption of imported fish from third countries, the ciguatera has been considered an emerging risk in Europe.
On the other hand, TTX is a potent neurotoxin, commonly associated with pufferfish, although the presence of TTX in some shellfish harvesting areas of Europe has been recently reported. The biogenic origin of TTX is still controversial, even though TTX biosynthesis by host-associated bacteria has been reported.
The application of Neuroblastoma N2a as screening method to evaluate total toxicity is being widely used in the field of marine biotoxins, being a very sensitive tool. Even though the N2a assay has some disadvantage such as the lack of specifity due to the non-specific toxic effects.
In this work it is shown the application of this method to evaluate the presence of emerging marine biotoxins, CTX and TTX, and it was also evaluated the effect of the matrix complexity on this method, which contributes to the non-specific toxic effects interfering with the detection of these toxins, furthermore it is also show improvements on sample pretreatment in order to improve the reliability on the results obtained.

Keywords: *Tetrodotoxin, Ciguatoxin, neuroblastoma assay*

Acknowledgement: *This work has received financial support from the Xunta de Galicia (Centro singular de investigación de Galicia accreditation 2016-2019) and the European Union (European Regional Development Fund - ERDF)*

LM11
EVALUATION OF BIOACTIVE COMPOUNDS IN DIFFERENT PLANT MATERIALS FOR DEVELOPMENT OF NEW TEA PRODUCTS

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Tea processing has had many modifications over the last 100 years from loose tea to blended, packet teas, tea bags and finally to instant teas, ready-to-drink teas and flavored tea's. Due to its taste, flavor, and many beneficial health effects tea is one of the most widely consumed beverages worldwide. In the first step of experiments consumer's attitude towards new tea products were conducted and it showed that people would like the following ingredients of instant tea – black and green tea extracts, ginger, lemon etc. Ginger and lemon are excellent sources of several bioactive compounds as phenolics, terpenes, tannins including non-volatile pungent compounds such as gingerols, paradols, shogaols, and zingerones in ginger. The aim of current study is to evaluate bioactive compounds in black and green tea, ginger and lemon for development of new instant tea product.
The research was carried out at the Faculty of Food Technology of the Latvia University of Agriculture (LLU). For this study tea (green and black), ginger (India and China origin), lemon (India and Spain origin) was selected as raw materials for new product development. In experiment for ginger and lemon juice, and all tea samples water extracts total phenolic, total flavonoid content and antioxidant activity by DPPH and ABTS assay were determined spectrophotometrically. For uniting of samples with similar composition hierarchical cluster analysis were performed. Correlation analysis was used to determine relationship between phenolic content and antioxidant activity.
The analysis of hierarchical clustering was used in order to group raw materials with regard to total phenols, total flavonoids, antioxidant activity. Samples were divided into three clusters: the first cluster comprised green tea with the highest content of phenolics, the second cluster comprised only black tea, with medium content of phenolics and antioxidant activity, the third cluster consisting of the other samples with significantly lower parameters. The results showed a highly positive correlation between both the content of phenolic compounds and antioxidant activity, as well as all the antioxidant activities of each other which allows to predict that an increase in one indicator results in increase in other indicators ($r>0.9$) and the correlation was significant at the 0.01 level. Tea extracts are the potential source of phenolics and compounds with antioxidant activity and composition of other raw materials should be investigated.

LM12
FAST AND LOW-COST MANUAL SOLID PHASE EXTRACTION OF PCDD/FS, PCBS AND OCPS IN DRINKING WATER

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Solid Phase Extraction of Persistent Organic Pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), biphenyls (PCBs) and organochlorine pesticides (OCPs) has long been an accepted sample preparation technique for EPA 500 series drinking water samples. Both C18 and DVB cartridges have consistently generated excellent recoveries in drinking water matrices.
Although automated SPE offers fast, reliable and reproducible results and limits the possibility of human error, many laboratories have continued interest in manual procedures. In this work we present an easy-to-use manual system consisting of a manifold mounted on a support and operated under vacuum. 1 g C-18 cartridges were used with a simple program that generates data in under 30 min.
250 mL water samples were acidified, spiked with 5 mLs methanol, spiked with 13C labeled PCDD/Fs and PCBs, and with OCPs. 1 g C-18 cartridges were conditioned with dichloromethane, methanol and water. The samples were then loaded under vacuum, the cartridges dried for 5 min with nitrogen and eluted with 20 mLs dichloromethane. Extracts were concentrated and analyzed with GC/MS.
Excellent recoveries were obtained for all analytes - PCDDs, PCDFs, PCBs, and OCPs - with this system. The system provides a low-cost and fast way to collect SPE data for 6 drinking water samples in under 30 min.

LM13
EVALUATION OF A HIGH THROUGHPUT, NO DCM OR CAPITAL EQUIPMENT SAMPLE CLEAN UP FOR POPS ANALYSIS

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The continued interest in Persistent Organic Pollutants (POPs), such as polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs), biphenyls (PCBs) and PBDEs, has led to a variety of attempts to automate both the extraction and cleanup of complex sample matrices. This has resulted in development of automated Pressurized Liquid Extraction (PLE) as an alternative to Soxhlet, and of fully automated sample cleanup instead of manual preparative open column chromatography.
In this contribution, we have developed a semi-automated system which is cheap and effective for fast and reliable sample clean up, using a relatively simple approach. An important feature of this technique is that a minimum amount of solvent is used, and no dichloromethane is used at all. This is important since many laboratories around the world are phasing out this solvent.
The semi-automated system is composed of a laboratory funnel, filled with hexane or toluene, a solvent pump and three pre-packaged columns: high capacity acid-base-neutral silica (lower capacity possible), carbon and alumina. The sample can be injected directly on top of the silica column. The sample is eluted through the silica column onto carbon and alumina and the analytes are eluted from both columns with toluene into separate fractions. Total solvent use depends on the lipid capacity of the silica used and varies between 100 - 250 mLs. Prepackaged column kits for Dioxin & PCB analysis reduce risk of background contamination. Total processing time for sample cleanup is less than 45 minutes. An unlimited number of parallel sample cleanup channels can be used.
A variety of matrices, such as eggs, feed, and oils, showed very good recoveries (70%-110%) for both PCDD/Fs and PCBs. Because the system is mostly composed of disposable parts, the risk of cross-contamination is very low. The system can be set up at low cost. The certified columns and simple semi-automated system guarantee same morning or afternoon POPs analysis.

LM14
AN AUTOMATED TECHNIQUE FOR MULTI RESIDUE ANALYSIS OF PESTICIDES IN AGRICULTURAL SAMPLES

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Recent years have seen an increased interest in the analysis of agricultural products and foods with regards to pesticides. Although not regulated extensively by the US federal authorities (FDA, etc), a number of methods has been developed for quick and effective screening of matrices for multiclass and multiresidue analysis (QuEChERS). Automation of such methods can greatly enhance laboratory efficiency, and provided with the right tools, lead to same day sample analysis with greater accuracy and reproducibility.
Pressurized Liquid Extraction (PLE) was used for extraction of various food matrices (green and black tea, fennel seed, astragalus root, hawthorn, gotu kola berry and green coffee). The various matrices were spiked with pesticides solutions and standards, and layered in a stainless steel extraction cell between Ottawa sand, in-cell clean up material and Ottawa Sand on top. Extraction was done in acetonitrile for 10 min at 1000 psi at ambient temperature. The cells were then flushed with nitrogen to collect the extraction solvent. Aliquots of the final extracts were analyzed without further clean up.
A total of 203 pesticides were analyzed with GC/MS. Excellent recoveries were seen for the various matrices. Chromatographic peaks were good with no interferences. High throughput pesticide analysis is possible with this technique: each individual run only takes 20 min, so with an 8-position PLE system 24 samples can be run in an hour. The automated extraction and in-cell clean up eliminates the need for manual steps and human error, leading to fast, reproducible and consistent results.

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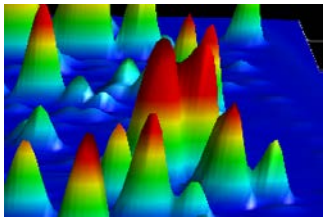

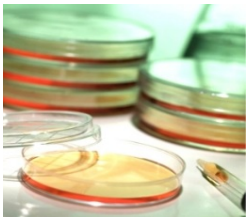


ISBN 978-80-7080-999-0