BOOK OF ABSTRACTS

9th International Symposium on

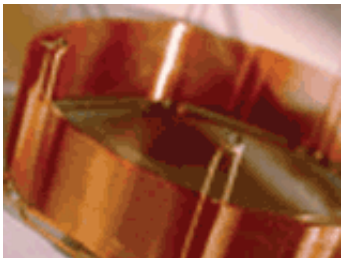
RECENT ADVANCES IN FOOD ANALYSIS

November 5–8, 2019

Prague, Czech Republic

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



UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

WAGENINGEN UNIVERSITY & RESEARCH

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9th International Symposium on

RECENT ADVANCES IN FOOD ANALYSIS

November 5–8, 2019

Prague • Czech Republic

Clarion Congress Hotel Prague

Organized by

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

&

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

MINISTRY OF AGRICULTURE OF THE CZECH REPUBLIC

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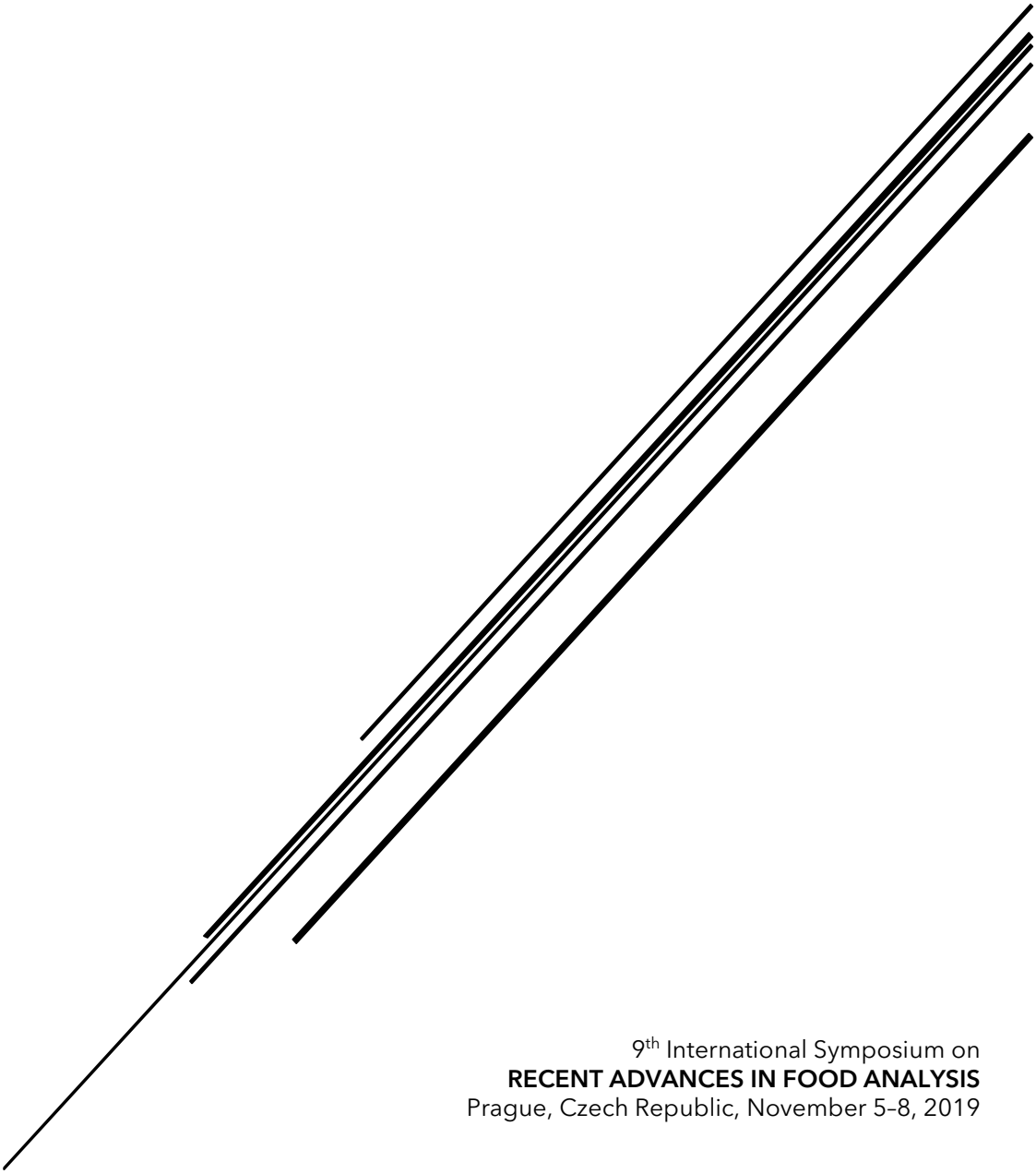

9th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 5–8, 2019

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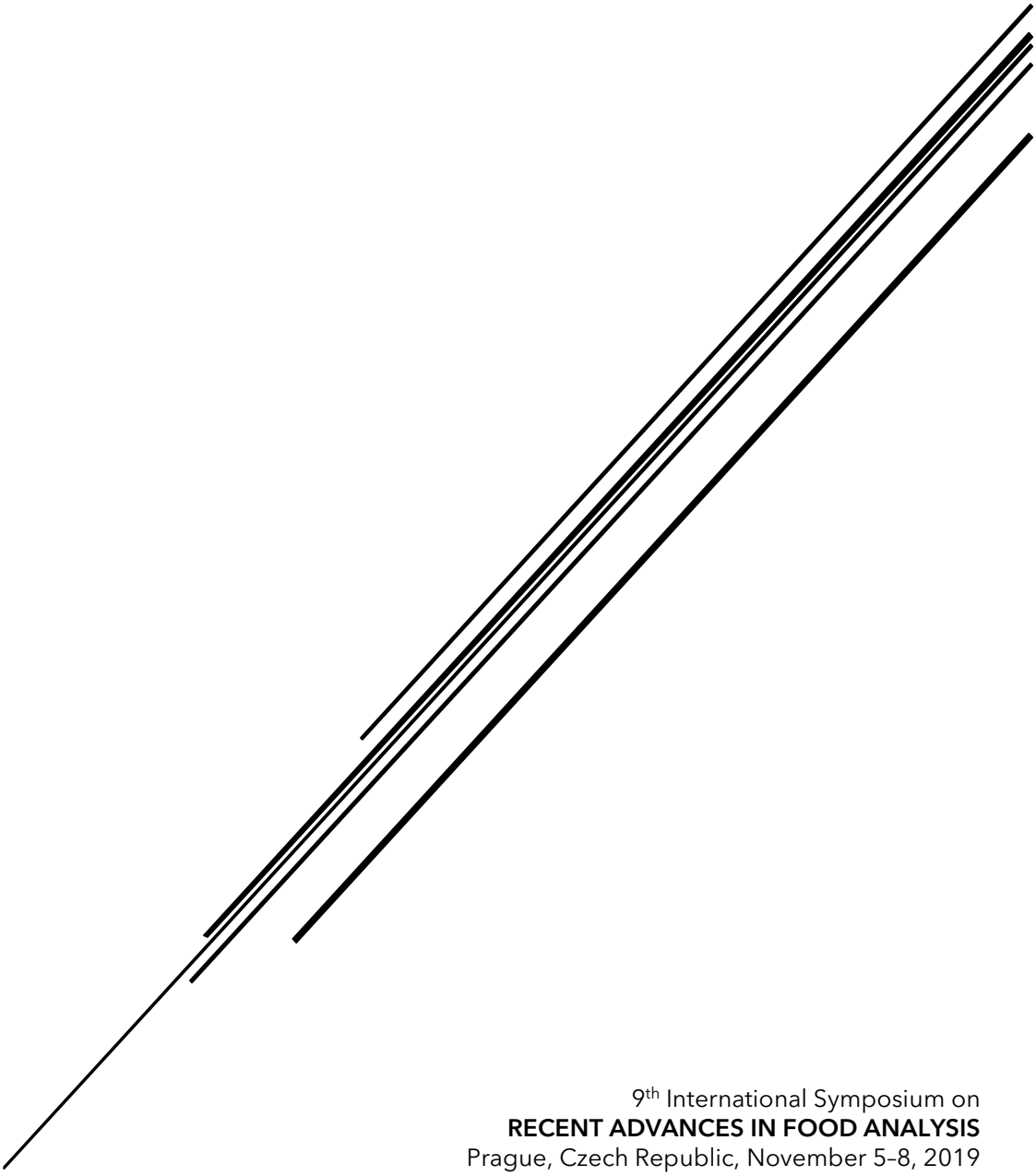

9th International Symposium on
RECENT ADVANCES IN FOOD ANALYSIS
Prague, Czech Republic, November 5–8, 2019

November 5, 2019 (12:45–13:30)

VENDOR SEMINAR:

What Chemicals May Migrate into Your Food?

What chemicals may migrate into your food?

Erich Leitner

TU Graz, Graz, Austria

During this "Lunch and Learn" seminar, Prof. Erich Leitner from TU Graz will give a short introduction to European legislation regarding the migration of chemical compounds from packaging material into food. He will show the impact of NIAS and MOSH/MOAH on food quality and food safety with compelling examples, and he will also compare different analytical approaches for qualifying and quantifying migrated compounds in food samples. In addition, Restek will offer a traditional Prague delicacy–served in sustainable biological packaging material–during this "lunchless" time of the conference.

November 6, 2019 (13:30–14:15)

Axel Semrau®

VENDOR SEMINAR:

Automated Solutions for the Determination of Contaminants in Food – From Sample Prep to Analysis

Is the MO in MOSH-MOAH really Mineral Oil, what about natural occurring Saturated and Aromatic Hydrocarbons?

Wim Broer

NofaLab BV, Schiedam

The analysis of MOSH/MOAH in edible oil and foodstuff is one of the most challenging problems in the analytics of food within the last years. To remove the natural occurring hydrocarbons from the sample a manual clean up step using AlOx is recommended. The presentation describes an automated method for this manual step using an HPLC pump. One topic is the check for robustness in a routine work. Another is to compare different types of edible oils.

New automated methods for the analysis of 3-MCPD in edible oil and fat extracts

Andreas Bruchmann

Axel Semrau GmbH & Co KG, Germany

3-MCPD analysis is a very hot topic in all food labs. There are some systems in the field, which can automate the analysis according the AOCS Cd29c-13 method. This method is well known and works fast and reliable, but for certain requirements it is necessary to use another method. The presentation describes the automation according AOCS Cd29b-13 method and AOCS Cd29a-13 method. During the presentation data for a new method developed by Zwagerman et. al are shown.

The instrumental basis of the presented methods are the CHRONECT Workstations by Axel Semrau.

November 6, 2019 (13:30–14:15)

SHIMADZU
Excellence in Science

VENDOR SEMINAR:

In Food We Trust - Let's Talk Quality

Ancient beverages and analytical chemistry

Erich Leitner

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

The discovery of alcoholic beverages correlates with the period when mankind started to settle down in the Mesolithic around 10.000 BC. This happened incidentally by the spontaneous fermentation of overripe fruits. Since then alcohol is consumed around the globe. The Code of Hammurabi a Babylonian code of law dated back to 1754 BC also contains the first regulations about beer quality.

Since then several laws and regulations for alcoholic beverages were passed over the centuries. Nevertheless, it took until 1820 when the German Friedrich Accum published the first book on food chemistry and analysis entitled "Treatise on Adulteration of Food". Just six decades ago chromatographic methods found their way into analytical laboratories, changing the quality evaluation of food and beverages.

The history and quality of alcoholic beverages under the perspective of analytical chemistry will be discussed in this vendor seminar.

1) Early Neolithic wine of Georgia in the South Caucasus; https://doi.org/10.1073/pnas.1714728114
2) Revealing invisible brews: A new approach to the chemical identification of ancient beer https://doi.org/10.1016/j.jas.2018.05.010
3) Friedrich Accum, Treatise on Adulteration of Food, London 1820

SFC-MS: A viable alternative to LC-MS in food safety analysis

Philipp Jochems and Gesa Schad

Shimadzu Europa GmbH, Duisburg, Germany

Chromatographers have for decades been interested in the technique of supercritical fluid chromatography (SFC) primarily due to the rapid separations and complementary chromatographic selectivity that this technique offers as well as its high "green credentials" compared to LC. Progress in embedding SFC into routine use has been retarded by the lack of reliable SFC instrumentation. However, instrument manufacturers have recently started to invest in the development of equipment that is advanced and reliable enough to meet the demanding expectations of routine analytical laboratories. SFC instrumentation in terms of ease of use and performing method development is similar to (U)HPLC equipment, reducing the barrier for user acceptance. The inherent properties of SFC, high speed of analysis (fast mass transfer), alternate selectivity compared to LC-MS and significant cost savings on solvent disposal relative to HPLC, enables separation scientists to consider alternative approaches in achieving robust, reliable target compound detection. The

elution power of SFC can be adjusted by the addition of a polar co-solvent, creating a pathway for relatively simple method development. Post-column addition of a make-up solvent can be used to further optimize ionization efficiency, therefore MS sensitivity, without dilution effect or degradation of chromatographic performance. The approaches used in optimizing SFC-MS for detecting a panel of pesticides in routine food safety testing will be discussed, highlighting the need to consider ion source dynamics, column selection and the influence of mobile phase composition on selectivity and sensitivity. The optimized SFC-MS results are compared to an optimized LC-MS assay used in routine testing. SFC-MS was found to often deliver higher sensitivity compared to LC-MS, reduced matrix effects in a range of food products and greater selectivity for the analysis of a highly polar panel of pesticides.

November 6, 2019 (13:30–14:15)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

VENDOR SEMINAR:

Real-Time Profiling of Food and Beverages Using Direct MS and Chemometrics

Real-Time profiling of food and beverages using direct MS and chemometrics

Jessica Prenni

Colorado State University, USA

Currently there is no globally harmonised definition for "food fraud" however, there are initiatives coordinating action against fraudulent practices in the food supply chain such as the EU Food Fraud Network [1]. Food and beverages of higher commercial value are frequently subject to fraudulent practice.

Mass spectrometry has traditionally been one of the 'last resorts' for food authenticity analysis. While gas chromatography-MS (GC-MS), GC isotope ratio-MS (GC IR-MS), and liquid chromatography-MS (LC-MS) are often used for authenticity testing, MS methods (including these) are generally considered to be slow, expensive and not amenable for routine application, mostly due to laborious sample preparation procedures. The advent of ambient ionization mass spectrometric methods remove most of the constraints associated with sample preparation and also opened new opportunities for direct-MS and point-of-control monitoring. Since direct MS methods require minimal or no sample preparation, the use of internal standards (or even external calibrators) is not always possible, resulting in the lack of quantitative information provided by these methods. Nevertheless, the spectral profiles are highly characteristic of the type, origin, age, etc. of the sample, which makes these approaches excellent for rapid profiling analysis. In these cases the MS spectral information is used as a 'fingerprint' for the identification of critical attributes associated with both the genetic origin and environmental exposure of the sample.

Proof-of-principle applications employing appropriate direct MS techniques including Direct Analysis in Real Time (DART), Rapid Evaporative Ionisation Mass Spectrometry (REIMS) and Atmospheric Solids Analysis Probe (ASAP) coupled with multivariant statistical analysis have been developed addressing various food authenticity, quality and composition testing requirements. For example, detection of undeclared ingredients in processed foods and establishing authenticity of various products, e.g. Protected Designation of Origin (PDO) status dairy products, quality indicators in meats, farming production methods, geographical origin of pistachio nuts and botanical origin of monofloral honey.

[1] https://ec.europa.eu/food/safety/food-fraud/ffn_en

November 6, 2019 (13:30–14:15)

VENDOR SEMINAR:

Advanced Methods to Ensure the Quality of Foods using Mass Spectrometry

Using Bruker HRMS-techniques for the development of novel methods for food authenticity and fraud investigation

Nikolaos S. Thomaidis, Sofia K. Drakopoulou , Anastasia S. Kritikou, Dimitrios E. Damalas, Marilena Dasenaki

National and Kapodistrian University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis Zografou, 15771 Athens, Greece
**E-mail:* ntho@chem.uoa.gr

During the last decade, food authenticity studies have been found on the frontline of scientific research. The development in the field of food science aimed to achieve high nutritious, superior quality and safe food. Thus, efforts are being made, not only to reassure that the product meets the quality standards, but also to highlight the qualifications of each product that declare its uniqueness. More specifically, special attention has been paid to the assurance of variety and geographical origin (Protected Designation of Origin) of the products. Serving this purpose, recent advances in mass spectrometry have led to the development of novel methods, applicable in food chemistry and technology. In our laboratory with strong cooperation with Bruker, high resolution mass spectrometric (HRMS) methods have been being developed and applied for the detection and substantiation of food authenticity. For that purpose novel methods and workflows have been developed for the identification of target, suspect and unknown compounds in an extensive variety of food matrices. Integrated screening workflows, based on both LC/GC-QToF-MS technologies, provided excellent analytical performance allowing the determination of a wide range of compounds in food matrices like olive oil, honey, wine, juice, milk and dairy products. Furthermore, a novel methodology utilizing Trapped Ion Mobility Spectrometry (TIMS) combined with LC-HRMS has recently been introduced and applied for the first time in olive oil, in order to separate and identify isomers that could be used as potential authenticity markers (variety discrimination). For complex assessment of food authenticity studies, like cheese fraud, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-ToF-MS) presented to be a fundamental high-throughput analytical technique. Advances in MALDI technology have led to the development of novel omics-based methods, heading on maximum collection of information on sample composition. Recently, highly automated proteomics- and lipidomics-based workflows have been developed for rapid detection of PDO dairy products adulteration and possible contamination, exploiting the total protein/lipid profile. Overall, novel MS-techniques in their entirety, proved to be powerful analytical tools, highly-applicable to food authenticity studies, with remarkable possibilities and breakthrough achievements.

Keywords: Food authenticity, LC/GC-QToF-MS, LC-TIMS-HRMS, MALDI-ToF-MS

Mass spectrometric solutions for accurate screening and quantitation of chemical residues in food extracts

Carsten Baessmann

Bruker Daltonik GmbH, Bremen, Germany

The use of pesticides to reduce crop damage and increase horticultural productivity has been implemented on a global scale for many decades. Due to their toxicity, the potential migration of these chemicals into the human food and water supply chains presents considerable health concerns for the population at large. Therefore, the maximum residue levels (MRL) permitted for pesticides in food and feedstuffs are strictly controlled by local and international regulatory bodies. One of the most important aspects in reducing pesticide exposure is to monitor their levels in food extracts. However, with increasing demands for lower detection thresholds to cover hundreds of pesticides originating from numerous sample types, accurate and reliable pesticide screening is a critical and complex analytical task. To meet these challenging demands, new UHPLC-QTOF and GC-APCI-QTOF based solutions have been developed and these, including software enhancements will be presented.

Fast and comprehensive full scan accurate mass screening and quantitation became an excellent tool in food control when the presence or absence of hundreds of pesticides, veterinary drugs, mycotoxins or dioxins must be proved in a short time frame. Additional to the high number of targets being screened for, the technique takes advantage of unknown evaluation and retrospective analysis. The new TargetScreener HR 4.0 application kit is based on the Bruker impact II QTOF. A central part of the solution is the new TASQ 2.1 Screening & Quantitation Software for rapid data processing, including ready methods for multi-target screening. Central to minimizing false positives or negatives is the high quality, robust TargetScreener databases with more than 3000 entries relevant for food safety, environmental protection, and toxicology screening and research. Depending on the compound classes, food extracts can be separated with the Bruker Elute UHPLC connected to the QTOF or with GC-APCI-QTOF, if e.g. pesticides or dioxins are more amenable to GC/MS.

November 6, 2019 (14:45–15:30)

ThermoFisher SCIENTIFIC

VENDOR SEMINAR:

Recent Developments in the Determination of Trace Element Contaminants, and Residues of Polar Pesticides in Food

Fast and Comprehensive Analysis of Elemental Contaminants in Food using ICP-OES and ICP-MS

Matthew Cassap

Thermo Fisher Scientific, Hemel Hempstead, UK
Corresponding author – E-mail: matthew.cassap@thermofisher.com

Elemental Contaminants such as arsenic, cadmium, mercury or lead can enter the food chain via a series of pathways including, but not limited to, industrial pollution or environmental contamination. Analytical techniques to determine the concentration of these contaminants in food samples need to be versatile, sensitive and fast. Inductively coupled plasmas coupled to optical emission spectroscopy (ICP-OES) or mass spectrometry (ICP-MS) satisfies all of the aforementioned needs.

Whilst ICP-OES provides outstanding robustness for a variety of sample matrices and the ability to detect both traces as well as major elements in one run, ICP-MS enables the determination of elemental contaminants in the ultra-trace range. For some analytes, such as arsenic for example, different chemical forms (or species) can be found in different sample types. Therefore, the total concentration may not be enough to estimate potential hazards and speciation analysis is required. Different chromatographic techniques such as ion chromatography (IC) can be hyphenated easily to element selective detectors ICP-MS. In this presentation, comprehensive and fast solutions for the analysis of trace elements in food samples are highlighted, including the use of ICP-OES and interference free detection at low levels using ICP-MS.

Recent developments in integrated workflows for the multi-residue analysis of polar anionic pesticides and metabolites

Richard J Fussell [1], Fausto Pigozzo[2], Qilei Guo[3] and Yingchen Li[3]

[1]Thermo Fisher Scientific, Hemel Hempstead, UK
[2]Thermo Fisher Scientific Milan, Italy,
[3]Thermo Fisher Scientific Beijing China
Corresponding author – E-mail: richard.fussell@thermofisher.com

Laboratories are constantly challenged to analyse more classes of pesticides at lower concentrations in more different commodities and with the expectation that residues will not go undetected. One of the most challenging groups of pesticides is the polar anionic pesticides, such as glyphosate, perchlorate, chlorate and the like, which often occur as residues in food, but are not always included in pesticide monitoring programs. This presentation will provide the latest information on the

development of the Thermo Scientific™ Anionic Pesticides Explorer: a new validated and integrated IC-MS/MS based workflow for the robust, sensitive and reliable routine determination of polar anionic pesticides and metabolites at low µg/kg levels in a single run. The workflow uses a modified QuPPe extraction with solid phase extraction clean-up. Results for wheat leek and baby-food matrices are compliant with SANTE guidelines, and EU MRLs. Quantification limits are ≤10ug/kg with % RSDs typically <10 %. Recovery data obtained with and without internal standards will be presented.

November 6, 2019 (14:45–15:30)

Agilent
Trusted Answers

VENDOR SEMINAR:

Latest Developments in LC- Q/TOF MS for Food Safety Testing and Authenticity Profiling

Recent developments and examples in both targeted and non-targeted strategies to assess food with LC- Q/TOF MS

John Lee

Global Food Market Manager, Agilent Technologies, Cheadle, UK

In food safety assessment, covering all threats from inappropriate use of Pesticides or Vet Drugs requires 100's of compounds to be analyzed, but quantitative assessment can be costly and time consuming. Q/TOF technology offers the chance to quantify what is likely but also to screen for many other possibilities from the same data file. Building such a concept whilst ensuring that data review can be efficient and reliable is key and both hardware and software innovation is required. In food authenticity assessment, the challenge is even greater because there are 1000's of compounds endogenous to food commodities and any of them are potential markers of in an authenticity study. Once again data review needs to be both efficient and reliable. This talk will explore the new tools being developed at Agilent to address these challenges.

Specific case studies looking at authenticity assessment with LC- Q/TOF MS

Olivier Chevallier

School of Biological Sciences, Queen's University Belfast, UK

At our Institute for Global Food Security we are delighted to be part of a hugely important project looking at Rice Fraud mitigation strategies for industry and government across the world. Agilent Hi resolution mass spectrometry is one of our key tools in this project, since it's use with metabolomics data processing is enabling us to identify different signatures from the rice, we analyze. The QTOF's high data rate also enables the rapid 2-minute methods we sometimes employ.

This talk will explore rice and some other commodities current being studied at our institute.

November 6, 2019 (14:45–15:30)

VENDOR SEMINAR:

Plant Alkaloids and Mycotoxins Analyses for Routine Labs: New LCMS/MS Methodologies Presented by SCIEX Customers

Challenges and implementation of LC-MS method for multiple plant alkaloids analysis

Zbynek Dzuman

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

Various groups of toxic plant alkaloids may enter human food chain. For control of the substances, reliable methods are needed. In this study, effective method for the detection of 56 pyrrolizidine, tropane and quinolizidine alkaloids utilizing ultra-high performance liquid chromatography and tandem mass spectrometry (QTRAP 6500+ System, SCIEX) has been developed.

Validation of LC-MS/MS based methods for the simultaneous quantification of several hundreds of analytes: Do we need new guidelines?

Michael Sulyok

Institute of Bioanalytics and Agro-Metabolomics, Department of Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna, Austria

In the recent years, LC-MS based methods covering hundreds of analytes have been successfully developed in the field of mycotoxins, veterinary drugs and pesticides. However, the analytical burden involved in validation of such methods is huge, even if a "user-friendly" guideline like SANTE 11813/2017 is used. Based on the results we obtained on the validation of 550 mycotoxins and other secondary metabolites, this presentation aims to discuss the usefulness of current guidelines for multi-target analysis. In particular, we think that both absolute and relative matrix effects seem to be insufficiently addressed, whereas investigations close to the levels of LOD/LOQ (thus requiring manual checks / interventions as considers peak integration) may be significantly reduced.

November 6, 2019 (14:45–15:30)

VENDOR SEMINAR:

Pushing the Boundaries of Separation & Sensitivity in Complex Food Analysis Using Comprehensive GCxGC & TOF-MS

Dioxin measurements in food and feed beyond MRL regulation using GC×GC-TOFMS

J. F. Focant

CART/Organic and Biological Analytical Chemistry Group, MolSys, University of Liège, 4000, Belgium, e-mail: JF.Focant@uliege.be

GC×GC-ToFMS/FID: a journey beyond the MOSH & MOAH hump in food determination

Giorgia Purcaro

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

Application of two-dimensional gas chromatography with mass spectrometric detection for analysis of pesticide residues in foodstuffs

Radim Stepan[1], Petr Cuhra[1], Martin Kubik[1], Jana Hajslova[2], Michal Stupak[2]

[1]Czech Agriculture and Food Inspection Authority (CAFIA), Za Opravnou 300/6, Prague, Czech Republic
[2] University of Chemistry and Technology, Prague, Technicka 5, Prague, Czech Republic

The analysis of important regulated and health significant substances such as MOSH/MOAH, Dioxins and Pesticides in foods and feedstuffs can often be challenging due to the presence of complex sample matrix interferences and the low levels of detection required.

Here, we present how the use of the latest LECO technology in comprehensive multi-dimensional gas chromatography (GCxGC) and Time-of-Flight Mass Spectrometry (TOF-MS) solves these challenges with far increased separation power and detection sensitivity.

November 7, 2019 (13:30–14:15)

VENDOR SEMINAR:

Recent Advances in Aroma Profiling by GC×GC-TOF MS

Unleash the Trap and Improve Sensitivity in GC-MS Analysis of VOCs and SVOCs

Aaron Parker

SepSolve Analytical, UK

Historically, a wide variety of sampling methods have been used to extract volatiles from food and beverages, with a key driver being the need to improve upon inefficient solvent extraction methods.

This presentation will showcase how the new Centri® platform can generate useful insights into the aroma profiles of food and beverages, by allowing automated pre-concentration of VOCs and SVOCs from gases, liquids and solids.

Using real world examples, we will demonstrate Centri's unique, high-performance operating modes including SPME-trap with enrichment and high capacity sorptive extraction (HiSorb) which allow significant improvements in profiling applications.

Also, we show how Centri greatly improves efficiency for high-throughput laboratories by allowing unattended sequential analysis of multiple sample types using different injection modes.

Pushing the boundaries of hyphenation: Aroma profiling by GC×GC-TOF MS/FID/SCD

Laura McGregor

SepSolve Analytical, UK

Aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. The enhanced separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) is now frequently used to tackle this challenge.

Here, we apply a cryogen-free, multi-hyphenated GC×GC system to obtain comprehensive aroma profiles. The use of parallel detection by three different techniques ensures that three complementary datasets are obtained from a single run:

- Flame ionisation detection (FID) for robust quantitation of high-loading species
- Time-of-flight mass spectrometry (TOF MS) for highly-sensitive, confident identification of aroma-active species
- Sulfur chemiluminescence detection (SCD) for highly specific detection of sulfur odour taints.

We will show the result of using this setup is confident but affordable aroma profiling with fully automated workflows and novel data processing.

Everything's peachy: Correlating sensory evaluation of fruit quality with analysis by TD-GC×GC-TOF MS

Natasha Spadafora

University of Calabria, Italy

The fruit quality (FRUITY) project aims to provide new predictive technologies and a better understanding of physiological changes in fruit for objective quality assessment of fruity quality during post-harvest storage, to allow improved sensorial and final 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 the industry with diagnostic kits for the evaluation of fruit quality during post-harvest storage.

In this presentation, we will focus on the VOC profiles from peach cultivars in an attempt to identify predictive molecular markers of fruit quality. The VOC profiles at the time of harvest and after storage at low temperature will be compared and correlated with results from sensory evaluation.

November 7, 2019 (13:30–14:15)

VENDOR SEMINAR:

Compact Analytical Devices for Rapid Screening of Chemical Contaminants: Affidia Presents New Innovative High-Tech Companies

Compact analytical devices for rapid screening of chemical contaminants: Affidia presents new innovative high-tech companies

Maurizio Paleologo

CEO, Affidia Srl Benefit Corporation, Trieste, Italy
e-mail: paleologo@affidia.tech

Affidia

Affidia is a new company. We are building a competence center in food diagnostics, independent from any kit/device/instrument manufacturer. We want to inform the community of quality managers of the food and feed industries, as well as laboratory managers, about all is needed to control food contaminants, avoid frauds, improve the quality of food and feed. We will continuously listen to the market needs and watch the evolution of products and services. We can be useful to method providers as well, reporting end-user opinions and communicating in a clear way advantages of new products.

We are launching a web portal ("FoodTestCompass") where there will be commercially available test kits by any reliable producer. We are starting with "rapid methods" (LFDs), then we will move online ELISA and PCR kits, as well as key materials for sample preparation,

Referencematerials etc. Now who is interested to rapid methods need to visit 5 to 10 web sites where specifications are shown in different ways and it is hard to make a comparison in a reasonable time. By FoodTestCompass the process will be easier and faster. We will offer to the end-user the possibility to share their experiences, find validation reports, sometimes get our expert evaluation too.

As independent experts, we will watch whether and how the results of the research institutions are exploited by the industries. Sometimes governmental bodies sponsor with million euro works that seems to us not so innovative, sometimes they really are lifting new disruptive enterprises. After this screening we can then help some young scientists and entrepreneurs that are in early pre-market stage to get some visibility.

In this seminar we will introduce two start-ups, both really innovative and promising. They are developing new screening methods and devices that could make control on-site simpler than now.

BIOsens MYCO

Dr. Andrii Karpiuk, BIOsens, Kyiv, Ukraine, will present BIOsens MYCO – an all-in-one device that perform automatically extraction and clean-up of grain samples that have to be tested for

mycotoxins. By mean of disposable part, who use the device does not need to manage methanol or making accurate volume dispensing. At the end of the process the sample preparation cartridge can be disposed and replaced by a clean one. The purified extract, limited volume, can be then analyzed by a fluorimeter or other type of mycotoxins sensors.

Right now, company works on upgrading sensor for testing 7 mycotoxins (Fumonisins B1/B2, Aflatoxins B1/B2/G1/G2, Ochratoxin A, T-2 and HT-2, Zearalenone, Deoxynivalenol) simultaneously and looking for R&D partners.

a@sens.bio / www.sens.bio

Inspecto Solutions

Dr. Lior Eligal, CTO at Inspecto Ltd. from Israel, will present a portable, analytical device that offers both automated sample preparation and analysis for food producers. The Raman SERS based innovation produces results within approximately 30 minutes and can be used by any non-skilled personnel. The model is based on a device with disposable capsules per scan. Inspecto's first application will be for acrylamide detection in various matrices.

info@inspecto.io / www.inspecto.io

The world of start-up companies is characterized by small teams, limited resources and, when the project looks very promising, early acquisitions too. We hope to be able to introduce another interesting enterprise, otherwise we will overlook the scenario of similar initiative we are in touch with.

We want to continue this scouting for innovation as well to let the community aware of failures of past initiatives. Please follow us at affidia.tech, read our magazine (Affidia, The Journal of Food Diagnostics) and when you need to choose a kit please visit FoodTestCompass.com

November 7, 2019 (13:30–14:15)

VENDOR SEMINAR:

The Evolution of Reference Materials

The evolution of reference materials

Dan Biggerstaff

LGC Limited, United Kingdom

The origins of LGC can be traced back to 1842 when the Laboratory of the Board of Excise was founded in the City of London to regulate the adulteration of tobacco, which was prohibited under the Pure Tobacco Act. LGC is the UK National Measurement Laboratory and Designated Institute for chemical and bio-measurement and have been home to the UK Government Chemist for more than 100 years. In both these capacities and a continually diversifying range of interests, LGC has been uniquely positioned to play an influential and observational role in the development of analytical measurement science.

As analytical techniques were developed over the course of the 20th Century to dramatically improve measurement capabilities, this drove the initial need for well characterised neat chemicals to be used as references in testing.

The advancement of techniques, instrumentation and methods soon enabled laboratories to more effectively separate mixtures and identify individual components. Initially analyses were limited to a handful of chemicals per technique so it was not difficult for the chemist to make or purchase the needed chemicals and produce their own standards. As the scope of the methods grew to larger lists, single component solutions emerged as a potentially faster and more economical option for chemists.

As regulatory methods were developed with target lists, multi-component solutions were developed to further streamline the measurement process. A significant step forward, multi-component solutions presented a world of possibilities to meet analytical needs, no matter how complex or specific. However, catalogued multi-component solutions are just the beginning.

Measurement science continues to evolve at pace, and the 21st Century has seen the advancement of custom-made solutions to meet the increasingly complex requirements of analytical testing across many sectors and industries. Presenting an opportunity to deliver bespoke reference standards, custom solutions represent a complex science, but one that enables efficiency and accuracy in even the largest and most complex analytical techniques.

Each format of reference material has advantages for scientists, and an exploration of these advantages and the continual evolution of reference materials will be presented.

November 7, 2019 (13:30–14:15)

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

VENDOR SEMINAR:

A Comprehensive Toolkit for Pesticide Residues, from Highly Polar Pesticides to Multiresidue Analysis

A comprehensive toolkit for pesticide residues, from highly polar pesticides to multiresidue analysis

Euan Ross

Waters Corporation, United Kingdom

The use of pesticides on crops to control pests during the production, storage and transport is common practice to protect crops. Food producers utilise various pesticide combinations to increase the production yields from each crop, improve quality and shelf life for various commodities. This practice has benefited consumers globally, giving them access to a wide range of foods all year which have been grown domestically or in another country.

To ensure that consumers are not placed at risk by the use of pesticides, maximum residue levels (MRLs) have been established by countries based on good agricultural practice and effects on human health, alongside regulations on marketing and usage of these chemicals.

In 2017 the European Union member states analysed in total 88,247 samples for pesticide residues, of which 95.9% fell within the current legal limits for pesticide usage. Of the 88,247 samples tested 41.8% of samples had one or more pesticide residues which was measured below or equal to the MRLs. Out of 11,158 samples measured in the 12 selected food commodities for the 2017 EUCP, 179 (1.6%) samples contained residue concentrations exceeding the legally permitted MRLs.

The analysis of pesticide residues remains a critical analysis for food contaminant laboratories. Due to the physiochemical properties of the various pesticides as well as the different commodities required to be tested, this analysis also still presents many challenges to laboratory workflows, from sample processing through to data analysis and interpretation.

A modern food contaminant laboratory needs to have an advanced analytical toolkit at its disposal to meet the growing demands of increasing multiresidue suites as well as challenging class specific analysis such as highly polar pesticides.

Join us at our talk where we will discuss a comprehensive toolkit for pesticide residue analysis. We will provide an update on new technologies designed to meet the challenges and regulations for this analysis. Find out about the various software functions designed to provide benefits to your analytical workflows as well as hearing about our latest highly polar pesticide solution, designed to bring this analysis into the routine.

November 7, 2019 (14:45–15:30)

VENDOR SEMINAR:

Using Cutting-Edge Mass Spectrometry Technologies to Address New Food Safety Challenges

EU compliant routine quantitation of dioxin and dioxin-like compounds by GC-MS/MS with advanced electron ionisation source

Adam Ladak[1], Jane Cooper[2], Richard Law[2], Alexander Schächtele[3], Tim Anderson[1] and Cristian Cojocariu[2]

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Regulatory changes in Europe in 2014 allowed GC-MS/MS to be used for confirmatory analysis and control of maximum levels (MLs) and action levels (ALs) of dioxins and dioxin-like PCBs in food and feed samples. In this study, the performance of a Thermo ScientificTM TSQTM 9000 GC-MS/MS system equipped with an Advanced Electron Ionization (AEI) source was evaluated for the routine analysis of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in solvent standards and food/feedstuff samples. Chromatographic separation was performed using a TG-Dioxin capillary GC column. Acquisition, processing, and reporting of the data were performed using Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software. Excellent agreement between the measured TEQ values and the supplied
Referencevalues from the EURL was obtained. A custom LOQ standard was also analysed at regular intervals throughout all PCDD/F sequences, in order to demonstrate the sensitivity required to maintain LOQs compliant with 1/5th maximum levels. Ion ratio tolerances were maintained at ±15% and RF deviation of less than 30% from the calibration average. System stability was tested by analysing dioxin samples continuously for two weeks.

Recent developments in the analysis of pesticides and contaminants in food using LC- and GC-Orbitrap Technology

Richard J Fussell[1], Charles Yang[2]

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[2]Thermo Fisher Scientific. San Jose, CA, USA
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This presentation will provide an update on the use of High Resolution Accurate Mass (HRAM) Orbitrap™ mass spectrometers for the targeted and screening analysis of GC, and LC, amenable pesticides and contaminants in food. The UHPLC-HRAM system offers a high degree of selectivity

and sensitivity in full-scan acquisition, with a choice of MS2 fragmentation options for identification, while the Exactive™ GC-Orbitrap™- GC-MS system uses electron ionisation with a capability for selected ion monitoring (SIM), if required. The high resolution and unrivalled mass accuracy of the Orbitrap technology reduces the risk of false detects and false negative results, and thus provides ultimate confidence in obtaining comprehensive and accurate results, even for complex samples.

Preliminary results of the analysis of 250 pesticides using an Orbitrap ID-X™ Tribrid™ Mass Spectrometer utilising Acquire-X, for automated generation of a background exclusion list will also be discussed. Exclusion of matrix components results in more accurate library matching and improved detection, quantification and identification of pesticides at lower levels compared to data dependent acquisition. This approach should be applicable to many food safety applications.

November 7, 2019 (14:45–15:30)

VENDOR SEMINAR:

Migration Screening of Raw and Food Contact Materials Using Intuvo GC MS

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

Dieter Franke, Nina Kranz

Siegwerk Druckfarben AG & Co. KGaA, Siegburg, Germany

Food Contact Materials (FCM) are an essential part of our lives. There is a great interest in the improvement of these materials and chemical substances which are being used for advancing their technological characteristics to the benefit of the consumers. Printing inks are usually applied on the non-food contact side of an FCM, however migration of ink film ingredients may happen though the food contact layer. Therefore, the one objective of our work at Siegwerk Druckfarben AG & Co. KGaA, a company with 180- year history in tailored ink technology and service, focuses on ink solutions for packaging and systematic processes for product safety, especially for packaging material for nutrition, pharma and hygienic applications. The starting point of new innovations entering ink formulations, is the raw material introduction process with a checklist for both approval and exclusion criteria (e.g., carcinogenics, mutagenics, reprotoxics, toxics..etc.), defined purity standards and a full understanding on existing impurities, compliance with chemical registration and full understanding of chemical composition for food packaging. In addition to information of possible suppliers, our own investigations into the purity of the raw material is undertaken, direct on the raw material and with in-house migration screenings. After the initial introduction of the materials to the manufacturing process, routine quality controls of the raw materials and products are applied to asses and ensure consistent quality, overall efficiency and safety.

In this presentation, the workflow for and the Intuvo GC/MS-FID optimization work will be presented along with results of migration samples using the coupled Intuvo GC/MS-FID system.

November 7, 2019 (14:45–15:30)

VENDOR SEMINAR:

QualiT™ - a New Quality Control Toolbox for Mycotoxin and Allergen Analysis

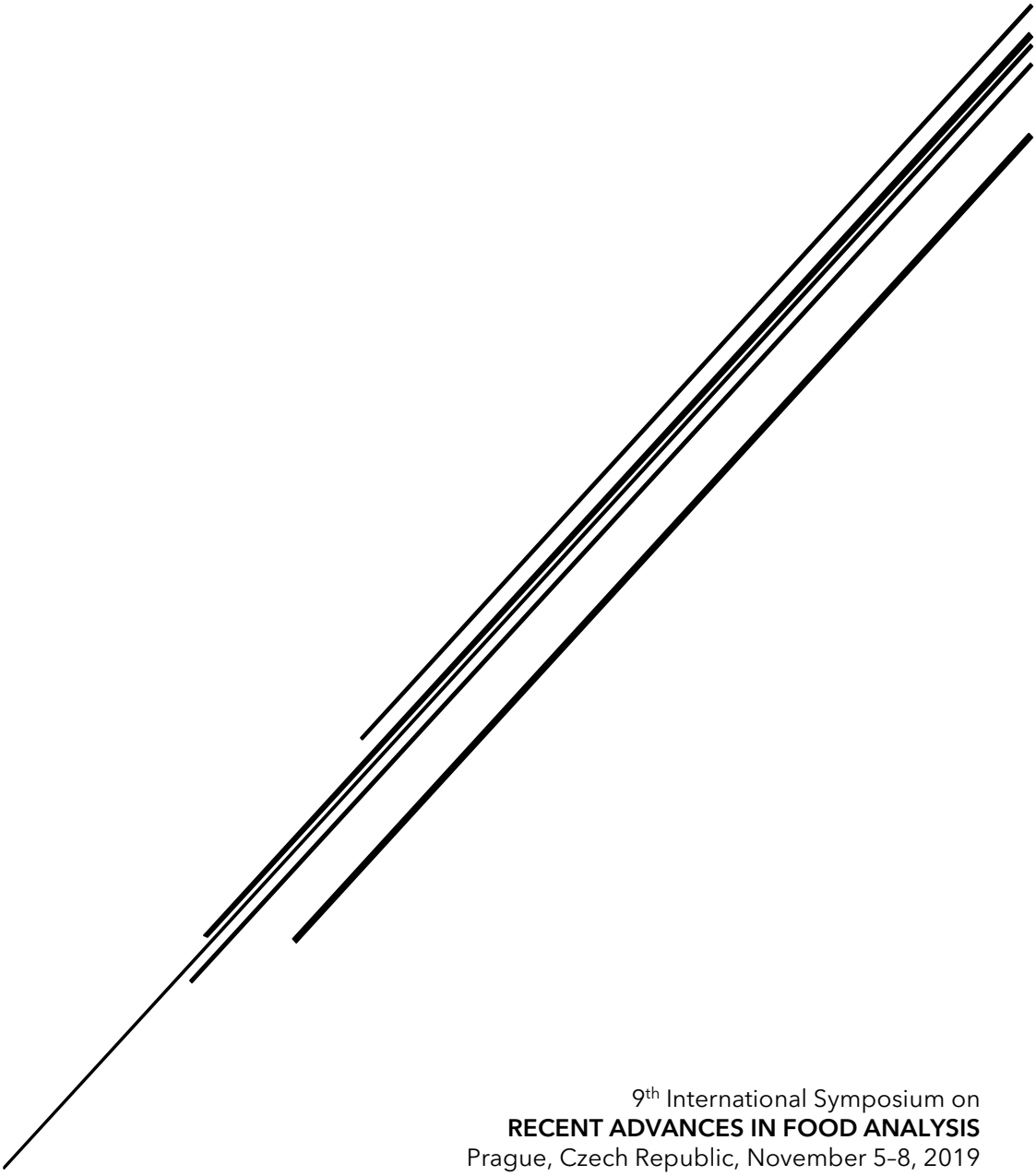
QualiT™ - a new quality control toolbox for mycotoxin and allergen analysis

Christine M. Gutschelhofer & Ronald Niemeijer

R-Biopharm AG, Germany

QualiT™ is a toolbox developed by Trilogy Analytical Laboratory (Washington, MO, USA) for quality control in mycotoxin analysis and - now introducing – also allergen analysis. QualiT™ offers (certified) Referencematerials, both as pure material and as well as naturally contaminated materials, quality control materials and analytical standards. Besides that Trilogy offers additional useful tools for sample preparation and sample clean-up and knowledge database, collecting 20 years of experience as an (ISO 17025 accredited) food testing lab, specialized in mycotoxin, allergen and biogenic amines testing.

LECTURES



LECTURES

L1

PREDICTING THE FUTURE IN FOOD ANALYSIS - USING CRYSTAL BALLS OR FACTS?

Michael Rychlik*[1]

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The last years have seen an tremendous development all fields within food analysis and the generic field of foodomics. In particular for food metabolomics, the number of identified and quantified metabolites has dramatically increased. This is due, on the one hand, to major improvements in the sensitivity and resolution of equipment used, and on the other hand, to increased coverage of compounds in metabolite-specific databases. However, is it possble to predict the developments in this field for the next years or decades? This presentation will attempt to foresee the future of food metabolomics in the mid-term and long term based on current data and recent applications in the field of targeted and non-targeted metabolomics. In particular, mycotoxin and vitamin research applying LC-QQQ, LC-QToF and FT-ICR-MS will serve as examples.

Keywords: *foodomics, high resolution mass spectrometry, metabolomics, metabolite databases*

LECTURES

L2

WHAT ROLE CAN ANALYSIS PLAY IN FIGHTING THE NEXT BIG FOOD INTEGRITY CHALLENGE?

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The challenges to the integrity of the world's food supply system are mounting. The most pressing issue is climate change and how this will impact the availability and safety of the food needed for the world's growing population. We also have the massive issue of supply chain (indeed supply network) complexities. These networks made the food we consume more vulnerable to both accidental and deliberate contamination. This in turn leads to greater possibilities of citizens consuming unsafe and potentially adulterated food. Scandals relating to these issues seem to be increasing in number and scale globally. These in turn can lead to loss of consumer trust, potentially large food recalls leading to economic loss at company level of even country level.
While the risks of food contamination appear to be increasing the means to predict and detect such issues are also increasing in terms of ability a sophtication. How techniques based on risk modelling can attempt to predict potential incidents prior to their happening are now being explored and the tools now referred to as 'predictive analytics' are being developed. One such tool developed, as part of a European Institute of Innovation and Technology (EIT-Food) project (Food Fortress) will be presented.
Also the progress in the development and implementation of non-targeted screening for food contamination and adulteration both in the labortaory and field will be presented and how the combination of'predictive analytics and field based testing can help support the integrity of our global food supply system.

Keywords: *food contamination, adulteration, monitoring, innovations*

Acknowledgement: *Funding from EIT-food and the H2020 programme*

LECTURES

L3

CURRENT CHALLENGES IN THE ANALYSIS OF CANNABIS AND PRODUCTS THEREOF

Jana Hajslova*[1], **Frantisek Benes**[1], **Marie Fenclova**[1], **Petra Peukertova**[1], **Tereza Bacinova**[1], **Kamila Bechynska**[1], **Michal Stupak**[1], **Jana Pulkrabova**[1]

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In recent decade, the interest in phytochemicals occurring in *Cannabis* - based products has been exponentially growing. Besides of quantification of major cannabinoids for which analytical standards are available (not only THC, THCA, CBD and CBDA bot also CBDV, CBG, CBGA, CBN, CBC, CBL.), characterization of entire, enormously complex cocktail of other bioactive phytochemicals contained in various edibles, extracts and products thereof, is a challenging task. In the first part of this presentation, alternative mass spectrometry (MS) based methodologies applicable for accurate analysis of targeted cannabinoids in various types of matrices are critically assessed with a special focus on discussion of benefits offered by supercritical fluid chromatography coupled with high resolution mass spectrometry (SFC-HRMS) specifically in case of analysis of ´CBD oils´ and other matrices with high lipids content. The presentation also addresses troubleshooting issues encountered when matrices with fairly differing concentration of targeted phytocannabinoids (several orders of magnitude) are to be analysed as all analytes quantification in a single run is rather complicated. Similar problem has to be overcome when analysing terpenes contained in *Cannabis* essential oil by GC-MS. The second part of presentation is concerned with UHPLC-HRMS/MS metabolomic profiling of other interesting metabolites occurring in *Cannabis* plants including minor phytocannabinoids, polyphenols and terpenoids associated with various bioactivities (over 300 compounds known from various sources to in Cannabis are screened). The changes induced by elevated temperatures are documented. The established library of metabolomic fingerprints is introduced, big data handling bottlenecks discussed. Special attention is paid to a Quality Assurance / Quality Control (QA/QC) measures in cannabinoids analysis, troubleshooting experiences are presented.

Keywords: *cannabis, high resolution mass spectrometry (HRMS) based methods, target analysis, metabolomic profiling*

Acknowledgement: *This project was supported by Technological Agency of the Czech Republic and Center of Competence BIOCIRTECH (TN010000048).*

LECTURES

L4

HOW DO THE RECENT ANALYTICAL TECHNOLOGIES EXTEND THE KNOWLEDGE OF THE HUMAN EXPOSOME

Bruno Le Bizec*[1], **Jean-Philippe Antignac**[1], **Ronan Cariou**[1], **German Cano-Sancho**[1], **Gaud Dervilly**[1]

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Long-term management of human health requires a global understanding of the environmental influences on it. Christopher Paul Wild (IARC) first proposed the concept of exposome in 2005, as it becomes apparent that alongside the genetic diseases, extends a vast field of increasingly extensive pathologies of environmental and social origin, such as obesity for example. The exposome encompasses lifelong environmental exposures (including lifestyle factors) from the prenatal period. This new vision draws the attention of decision-makers to the need for comprehensive exposure data of high quality to ultimately investigate the causes of certain pathologies in humans and therefore to manage more effectively the situations. Diet is a significant source of human exposure to chemicals, whether it is chemical residues of controlled substances or environmental contaminants. The characterization of a wide spectrum of chemical substances to which we are exposed clashes with the ignorance we have about the largest number of them. This requires the development of new analytical strategies to highlight and characterize substances that we do not know yet. Non-targeted approaches must be implemented; they are most often based on high or very high-resolution mass spectrometry and must be supported by bioinformatics tools embedding algorithms capable of fishing for compounds not yet described. These analytical strategies must be able to tackle substances already described but not necessarily sought in the field concerned; we will then speak of searches for "known unknown" by NTS (Non Target Screening) approaches. These substances may have never been described because being the consequence of a degradation process or a metabolism reaction; we will speak then about "unknown unknown". 'Omics or more largely, accurate mass profiling is then a possible seeking approach. Analytical strategies are then radically different on their principle. These strategies will be illustrated by examples taken in Environmental Science (sentinel matrices), in the Food Industry (animal of production) and in Health Science where a particular focus will be given to human biomonitoring. Biomarkers of exposure and effect, considered all together, may give understanding of the link between chemical exposure and some human pathologies.

Keywords: *human exposome, non-target screening, omics, accurate mass profiling, chemical exposure*

LECTURES

L5

A NOVEL INTEGRATIVE STRATEGY TO PREVENT COLORECTAL CANCER WITHIN THE DIET-HOST-MICROBIOTA TRIANGLE: FROM ORGANOIDS TO HUMAN IN VIVO REALITY

Josep Rubert*[1], **Andrea Lunardi**[1]

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Colorectal cancer (CRC) is one of the most common cancers in the western world. Several hundreds of thousands of people are diagnosed annually with CRC and over half of the patients die or have comorbidities. Research has suggested that dietary patterns, dysbiosis, and gut microbial metabolites may play a pivotal role, leading to increasing interest among scientists. However, despite the fact that gut microbial metabolites play a crucial role in many biological cases, adequate tools for deciphering the relationship between diet-microbiome-host are not yet available. TRIANGLE aims to provide new insight into the mechanisms by which gut microbial metabolites may prevent CRC. The first objective is targeted at designing *in vitro* models mimicking human organogenesis and tumorigenesis to evaluate the role of gut microbial metabolites. Human intestinal organoids capture most, if not all, of the cellular diversity present in the native intestinal tissue, mirroring structural alterations, mutational signatures and gene expression between patient tissues and 3D intestinal organoids. The second objective is to identify gut microbial metabolites that can act as cancer-preventive agents. If gut microbial metabolites are commercially available, such as short-chain fatty acids, are purchased. Otherwise, using a food model, a gastrointestinal tract model able to simulate the digestion and colonic fermentation releases these metabolites. Lastly, intestinal organoid responses to gut microbial metabolites are studied combining metabolomics analysis and live-cell imaging. Preliminary results have provided valuable new insights into the mechanisms by which nutrient-gene interaction influences colon stem cell niche and CRC, and in the future, this concept will open up new possibilities for CRC understanding and prevention.

Keywords: *human intestinal organoids, gut microbial metabolites, metabolomics, live cell imaging, metabolism*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 794417. I am grateful to Prof. Quattrone, Prof. Mattivi, Prof. Kim B. Jensen, Dr. Pazienza and Dr. Binda for the help provided and support.*

LECTURES

L6

THE PROTECTIVE AND ADVERSE EFFECTS OF Ω-3 POLYUNSATURATED FATTY ACIDS IN THE CONTEXT OF PARENTERAL NUTRITION DEMONSTRATED BY OMICS STRATEGY

Vit Kosek*[1], **Kamila Bechynska**[1], **Marie Heczkova**[2], **Nikola Daskova**[2], **Monika Cahova**[2], **Jana Hajslova**[a]

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Parenteral nutrition (PN) is a life-saving treatment for patients with short-bowel syndrome. It however poses significant adverse effect such as deterioration of liver function which progresses with the time of the treatment. To improve this effect, ω-3 polyunsaturated fatty acids (ω-3 PUFA), obtained mainly from fish oil, are introduced into the lipidic portion of PN as a hepatoprotective component. In contrast, the number of double bonds in fatty acyl chains makes them more susceptible to peroxidation which in turn may increase oxidative stress in patients receiving it. The aim of two performed studies was to assess the effect of parenteral nutrition on the liver lipidome of a rat model (short term effect) and on plasma and erythrocyte lipidomes of human patients dependent on PN (long term effect). The methods used involved "classic" markers of inflammation and oxidative stress, in addition lipidomics and proteomics were employed for better insight into processes ocurring on the molecular level. In the rat model ω-3 PUFA decrease the inflammation but do not seem to increase oxidative stress. In humans both "classic" and lipidomic markers showed that ω-3 PUFA in PN increase oxidative stress significantly as demonstrated by decreased levels of plasmalogens, endogenous antioxidants. The decrease of inflammation was demonstrated by lower levels of both traditional markers as well as of fatty acyl esters of hydroxylated fatty acids. These new data could be beneficial when designing of a new generation of PN lipid emulsions.

Keywords: *parenteral nutrition, omics, lipidomics, rat model*

Acknowledgement: *This study was supported by the Ministry of Health of the Czech Republic, grants no. 15–28745A AZV CR. 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)*

LECTURES

L7

PAIRING ATOMIC SPECTROSCOPY WITH MULTIPLEXED IMMUNOASSAYS FOR RAPID AND PORTABLE ANTIGEN DETECTION

Carmen Gondhalekar[1], **Bartek Rajwa**[1], **Euiwon Bae**[1], **Valery Patsekin**[1], **Jennifer Sturgis**[1], **Iyll-Joon Doh**[1], **Prasoon Diwakar**[2], **J Paul Robinson***[1]

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Laser-induced breakdown spectroscopy (LIBS) is a technique with huge opportunities in food analysis. We are targeting portability, food identification/characterization/authentication, and integration with other detection modalities. Our initial bench-based LIBS system has been broadly applied to characterization of cheeses, detection of phosphates in pesticides, and analysis of paper immuno-assays (PIs). The system is being optimized for its primary focus, which is the analysis of PIs for food contaminant detection. *Escherichia coli* is only one of the many targets of interest, and later studies will apply LIBS to detecting a wider variety of microbial/ molecular food contaminants and chemicals such as pesticides. PIs are highly successful as a platform for concentrating and labeling antigens in a portable, fast and easy-to-use format. We are pairing PIs with a portable LIBS system whose design is based on the optimized design of our benchtop model. We pursue LIBS as a food analysis technology because its application in food science is not prolific, though its success in other disciplines suggest that it has significant potential.
LIBS is an element characterization technique that has reached many fields, primarily metal and soil analysis, but also archaeology, forensics, microbiology and food analysis. Reasons for its success include its portability, sampling and analytical speed, multi-element detection, compatibility with solids, liquids and gasses, minimal sample prep, and decreasing cost. In food science, LIBS studies can be categorized as chemical detection, bio-contaminant detection, and food authentication. Examples of published data include studies on mineral composition and identification of foods, molds, pollens, yeast, protein, bacteria, pesticides, heavy-metals and fertilizers in soil, food or water.
We are pairing LIBS with heavy metal bio-labels and PI sample-prep platforms to create a portable and rapid food contamination detection system. 22 heavy metals are known to be used as antigen labels in immunoassays. We selected 5 of these labels (Eu, Dy, Pr, Gd, Nd) and determined which yielded the lowest limit of detection (LOD), and which are most appropriate for LIBS multiplexed antigen detection. Parameters for optimizing LOD were sampling area, delay time between laser pulse and data collection, laser energy, and wavelength region. We then apply these parameters to gram negative bacteria detection on PIs and compare the LOD to conventional PI analysis techniques. To evaluate our system's potential for multi-analyte detection on a single paper assay, we use LIBS to sample mixtures of metal labels to identify the presence of each metal simultaneously. Our results indicate that LIBS can be paired with PIs for single and multiplexed contaminant detection.
LIBS is a technology not very familiar to the biological community but offers a lot of opportunities. Our research finds promise in the use of LIBS for food contamination detection.

Acknowledgement: *This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Agreement No. 59-8072-6-001 and the National Science Foundation (DGE-1333468). We'd like to give special thanks to the Laser Technologies Group at Lawrence Berkeley National Laboratories for providing guidance in LIBS system design.*

LECTURES

L8

MULTI-RESIDUE SCREENING AND QUANTITATION FROM MULTIPLE FRUIT MATRICES VIA AUTOMATED COATED BLADE SPRAY

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Increased produce consumption and globalization requires increased pesticide residue monitoring by regulatory agencies. One avenue (of the many) to reduce the economic and bureaucratic load of the analysis of an increasing number of imported produce samples is to investigate faster and less expensive analytical methods. One such method of interest is Coated Blade Spray (CBS). The CBS device consists of a sword-shaped stainless-steel blade coated with a polymeric coating for compound of interest isolation and enrichment via SPME-like processes. The device is used as an electrospray ionization (ESI) source following the application of desorption solution. Leveraging the high-speed transition monitoring capabilities of modern instrumentation, using CBS for fast, multiresidue pesticide monitoring is made possible. As pesticide multiresidue analysis is of great importance to the security of the food supply of many nations, the suitability of CBS to improve the speed and cost per analysis was explored in fruit matrices. An automated CBS methodology is proposed for the screening and quantification of pesticides (+150) from apple, blueberry, strawberry, and grape matrices. CBS devices were coated with in-house manufactured hydrophilic-lipophilic balance (HLB) particles which facilitated the solid-phase microextraction (SPME) of compounds of interest. Extraction of samples (cryoground) was completed for 15 minutes using a high-throughput 96-well holder, from 96-1 mL samples simultaneously, followed by a 10 second rinsing step in water. Signal acquisition was complete using an in-house manufactured 12 blade CBS autosampler coupled to a triple quadrupole MS (TSQ Quantiva) with a spray time of 15 seconds/sample. During development, dilution of sample, buffer addition, desorption solution optimization, and internal standard selection were explored to yield final conditions proposing the best compromise for extraction/desorption/ionization for all compounds of interest. It was found that high dilution levels (1 part matrix:9 parts water) yielded the best signal-to-noise for mid-range logP (2 - 5) compounds, whereas the opposite was true for low logP (< 1) compounds (such as neonicotinoids). As a compromise, a high dilution level was chosen for all matrices, as most compounds with low MRLs (sub 50 ppb) have mid-range logP values. Optimum conditions determined, coupled with signal correction via deuterated analogues as internal standards, yielded quantification capabilities (%RSD < 20 %,80 % < accuracy < 120%) for over 75 % of compounds under study. This included low MRL compounds such as carbofuran, propham, and pyrimethanil where limits of quantitation were reached at 2.5 ppb, 1 ppb, and 1 ppb, respectively. Additionally, the duality of CBS was demonstrated via the validation of direct-to-MS figures of merit with CBS-LC-MS/MS; where the extracted sample was desorbed and run using conventional LC-MS/MS.

Keywords: *direct-to-MS, coated blade spray, solid-phase microextraction, fruits*

Acknowledgement: *We are grateful to the Natural Science and Engineering Research Council (NSERC) of Canada for their financial support through an Industrial Research Chair program. We would like to thank Restek Corporation collaborators Dave Bell and German Augusto Gomez-Rios, for providing funding, consumables, technical discussion and encouragement during the project. We would also like to thank Thermo Fisher Scientific collaborators for their technical expertise as well as for lending the triple quadrupole mass spectrometer that was used in this work (TSQ Quantiva). Finally, we would like to thank Varoon Singh and Mohammad Huq for synthesizing the sorbent particles used in this work.*

LECTURES

L9

DEVELOPMENT AND OPTIMIZATION OF MINIATURIZED DEVICES FOR DNA ANALYSIS OF FOOD SAMPLES

Joana Carvalho*[1], **Andrey Ipatov**[1], **Lorena Diéguez**[1], **João Gaspar**[1], **Alejandro Garrido-Maestu**[1], **Sarah Azinheiro**[1], **J. Rafaela Guerreiro**[1], **Monisha Elumalai**[1], **Jorge Barros-Velázquez**[2], **Marta Prado**[1]

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Maintaining food quality and safety along the food supply chain is a challenging problem of international concern. Consumers worldwide demand reassurance about the safety and authenticity of their food. DNA-based technologies can be applied not only to food authenticity and traceability but also to the detection of genetically modified organisms, allergenic ingredients, and foodborne pathogens. However, there is still a need for fast and sensitive *in situ* DNA analysis, allowing reliable results through the food supply chain. Miniaturized devices for DNA analysis have several advantages, such as requiring smaller sample volumes, allowing the use of smaller quantities of reagents, reducing costs, and improving system performance by being faster and more sensitive. At INL, our group combines DNA-based methods with micro and nanotechnologies to create portable, miniaturized, and automated devices to perform accurate and sensitive DNA analysis of complex food samples. These devices have been developed by focusing on each step of DNA analysis separately, enabling better protocol optimization and providing more flexibility on the techniques implemented for the analysis.
For the first step of DNA analysis, extraction and purification, microscale solid phased extraction (μSPE) is one of the most attractive methods for microchips, concentrating the DNA even when minute amounts are present (e.g. olive oil, wine) by putting in contact a higher initial volume with the solid phase and recovering DNA in a lower volume during elution. At INL, a disposable miniaturized device based on μSPE has been developed, containing a chamber with micropillars functionalized for pH-induced DNA capture and release. The optimized protocol has shown high DNA recovery yields and its application to complex food samples is currently being evaluated. For the second step of DNA analysis, amplification, PCR is the most widely adopted method. However, alternative isothermal amplification methods, such as LAMP and RPA, have been developed. These methods have several advantages, such as being performed at constant temperature, higher tolerance to the presence of inhibitors, reduced reaction time and, in the case of LAMP, the possibility of naked-eye detection. In addition, the equipment and personnel requirements are greatly reduced, making it suitable for miniaturization. At INL, we also have been developing miniaturized devices for isothermal DNA amplification, showing good performance and achieving promising results.

Keywords: *DNA analysis, miniaturized DNA purification, miniaturized DNA amplification, food quality and safety*

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LECTURES

L10

AROMA PROFILING OF BREWING HOPS BY ION MOBILITY SPECTROMETRY AND MODERN SIGNAL PROCESSING

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Due to low detection limits, high selectivity and its rugged design, gas chromatography ion mobility spectrometry (GC - IMS) gains increasing attention for the analysis of volatile organic compounds (VOCs) in fields of breath analysis, food quality and process control [1]. Depending on mass, charge and structure related collision cross section (CCS) ions are separated along the drift tube at ambient pressure under the influence of an electrical field. Ion formation in IMS has already been well investigated, comprising the concentration dependent occurrence of protonated monomers and proton-bound dimers via proton transfer mechanisms. Headspace GC-IMS (HS-GC-IMS) was applied for analyzing the complex aroma profiles of different hop cultivars, consisting of a variety of different VOCs, such as aldehydes, ketones, terpenes and terpenoids [2]. The resulting two dimensional aroma fingerprints of the investigated hop species were classified according to similarities in their VOC profile. The aim of this approach was to group typically used hop cultivars from all over the world with similar aroma profiles in order to facilitate substitution with regard to the partially limited availability. Additionally, specific substance classes could be distinguished according to the characteristic drift times of their ions. Interestingly, some ions of terpenes and terpenoids featured similar drift behavior indicating a complex gas phase ion chemistry occurring in the ionization process. With the help of the temperature-ramped, high-resolving HS-GC-IMS setup used in this study together with modern signal processing and data analysis strategies, the characteristic ion patterns of hop aroma profiles obtained are extensively described for the first time. Furthermore, clustering according to their aroma profiles was accomplished. This indicates that HS-GC-IMS could serve as easy to handle and cost-effective profiling technique for hop aroma analysis.

Keywords: *aroma profiling, classification, data analysis, ion mobility spectrometry, brewing hops*

LECTURES

L11

KEY CHALLENGES IN ANALYTICAL AUTHENTICATION

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In the past, many test methods for the analytical authentication of food and feed have been developed and applied at various control points in respective supply chains - from quality checks within the business operators to spot checks by official surveillance.
However, in particular for modern data-rich approaches, several bottle necks remain that limit their broad harmonized application. Within this context, a variety of activities are currently launched to improve analytical authentication in routine applications, e.g. the formation of the new CEN technical committee "Food Authenticity". In addition to the validation and standardization of specific test methods, terminology and the development of validation concepts for non-targeted approaches are important topics for the committee and beyond.
Depending on the type of the methodology, the most prominent limitations are the use and acceptance of Referencedata, agreed interpretation and in some cases also the scientific prerequisites for sharing and jointly using data among different facilities. The key challenges linked to
Referencedata in analytical authentication from BfR's point of view will be discussed in terms of establishment, exchangeability, accessibility, sharing and interpretation. Therein not only scientific aspects play a role but also administrative hurdles between different data owners need to be overcome. Exemplary approaches for the joint usage of FT-IR and NMR spectroscopic data from different instruments, e.g. for the authentication of wine, will be presented.

Keywords: *authentication, data, non-targeted analysis*

LECTURES

L12

KEY FACTORS CONTROLLING STABLE ISOTOPE SIGNATURES OF PLANT-BASED FOODS

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Stable isotope ratio analysis is a powerful and widely applied analytical tool in plant and food science. It relies on the fact that most elements of the periodic table exist as two or more stable isotopes, only differing in their number of neutrons. Stable isotope ratio analysis predominantly focuses on the biogenic elements: hydrogen (H), carbon (C), Oxygen (O), nitrogen (N) and sulfur (S) [1]. In plants, these elements constitute more than 95% of dry matter and their total content is highly conserved for H, C and O while N and S contents differ depending on growth conditions [2]. In contrast, ratios between heavy versus light isotopes of these five elements ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{34}S/^{32}S$) can vary significantly as a result of isotopic fractionation during various physical, chemical or biological processes. These unique isotopic signatures are utilized in a variety of applications. In plant science, hydrogen and oxygen isotopes are *e.g.* used to trace water sources, photosynthesis is studied by carbon isotopes, while nitrogen and sulfur isotopes can be used to determine nutrient availability and utilization [1]. In food science, one of the most popular applications of stable isotope ratio analysis is authenticity testing [3]. It largely relies on the same fractionation factors and isotopic signatures utilized in fundamental plant science. However, only few studies have focused on unravelling key factors controlling stable isotope signatures of plant-based foods. Thus, major knowledge gaps still exist, and mechanistic studies are required to expand the use of stable isotope ratio analysis in food science – *e.g.* for authenticity testing of organically grown plants [4]. At the conference, an overview of climatic, edaphic and biological factors controlling stable isotope signatures of plant-based foods will be given, and common pitfalls in data interpretation will be discussed. Emerging analytical approaches, such as compound-specific stable isotope ratio analysis, and its suitability for authenticity testing of high-value plant products will be demonstrated and discussed.

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Keywords: *plants, food authentication, stable isotopes*

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LECTURES

L13

PARADIGM SHIFT IN NON-TARGET SCEENING WITH GC-MS(/MS): FOOD ANALYSIS USING SOFT IONISATION, STATISTICAL WORKFLOWS AND COMPOUND DATABASES

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Today, a broad range of analytical techniques, such as gas, liquid and supercritical fluid chromatography (GC, LC and SFC) in coupling with mass spectrometry (MS) are available to answer several questions regarding presence and quantity of organic molecules in food. With increasing interest in analytical testing for food authenticity, food contamination (e.g. by pesticides), food fraud, or the complexity several analytical tasks increase strongly. Therefore, advanced analytical approaches are utilized and combined with powerful workflows using non-target screening strategies. This allows to track new, unknown, unexpected or unintentionally added substances from raw materials to final products. Requirements for that are robust separation techniques, efficient non-destroying compound ionization, specific compound fragmentation and accurate mass detection.
For the first time a novel GC-MS(/MS) setup using a cold-plasma ionization source (DBDI) to couple GC with tandem high resolution mass spectrometric detection (MS/HRMS) is presented using LC-MS instruments (and including statistical tools in hardware development and data evaluation). In contrast to electron ionization (EI), DBDI produces molecular ions which can directly be detected and fragmented afterwards in (tandem) LC mass spectrometers. This strategy allows subsequently to use the full power of data analysis in foodomics and non-target screening as known so far only from LC-MS/HRMS and SFC-MS/HRMS.
Optimization of the utilized setup for the separation of a broad range of compounds was performed with 'GC separation modelling tool'. Further, for efficient ionization and molecular MS detection 'Design-of-Experiment approaches' were applied. Finally, for MS data evaluation a workflow was observed including data processing, data handling and molecule identification using novel compound databases (like DuftSTOFF-IDENT at the FOR-IDENT platform) and statistical strategies. As a result, complex tasks, such as food authenticity or the presence of non-intentionally added substances can be addressed more easily and will exemplarily be shown in this presentation.
A first prototype of GCxGC-DBDI-MS/HRMS including robust data evaluation workflows for food analytics will be presented as a new generation of non-target screening strategies. The setup combines robust GC separations with advanced ionization and powerful MS detection, allowing to conduct comprehensive pesticide screening, foodomics and non-target screening, i.e. a paradigm shift in analyzing volatile compounds in food matrices.

Keywords: *GC-MS/MS, soft ionisation, HRMS, non-target screening, DoE*

LECTURES

L14

GC-IRMS TECHNIQUE SNIFFS OUT AROMA FRAUDS

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Flavour is an important quality trait of food and beverages and is determined by taste and aroma, but the growing demand for natural aromas and increasing raw material costs is raising prices and lowering quality of food products. One solution is to replace natural flavourings with cheaper, more readily available synthetic flavourings, and adulteration of products for economic gain is a concomitant phenomenon in the production of aromas. Gas chromatography-combustion/pyrolysis-isotope ratio mass spectrometry (GC-C/P-IRMS) for the analysis of volatile compounds, sampled using solid phase microextraction (SPME), is an appropriate tool for authenticity assessment of aromas because it allows differentiation between synthetic and natural produced aroma compounds. The demanding work involved in GC-C/P-IRMS and lack of knowledge about how SPME parameters affect stable isotope measurements have meant that SPME-GC-IRMS has so far typically been used for the determination of isotopic values of single (or few) aroma compounds. This study examined the suitability of the method for the determination of $\delta^{13}C$ and $\delta^{2}H$ values of more than 15 aroma compounds within the same run. It also investigated how SPME and analysis conditions affect the relative abundance of isotopic values resulting in possible isotopic fractionation and, in this regard, outlines the process of data normalisation and method validation necessary to obtain meaningful data for use in authenticity studies. The results show that by using an optimised SPME method with the appropriate processing, we can obtain highly reproducible $\delta^{13}C$ and $\delta^{2}H$ values without fractionation for different volatile compounds. Verification of commercial samples also requires building databases of authentic flavour compounds with well-defined origins. In this study, stable isotope databases for apple, strawberry, vanillin, and truffle aroma compounds were established and comprised 50 apple samples, and 18 strawberry laboratory produced recovery aroma samples of natural origin, 173 samples of 10 different truffle species and 50 authentic samples from vanilla pods. Pure synthetically derived characteristic aroma compounds and nature-identical vanillin samples were also characterised. Additional types of fruits were tested, and the apple and strawberry database was expanded to include the analysis of raw fruit and fruit juices. For most of the selected aroma compounds, good discrimination was obtained between the natural and synthetic authentic range of isotope values. Finally, commercial samples were tested and revealed possible falsification for several fruit aroma compounds. All the products labelled as "natural vanilla flavour" contained synthetic vanillin. Also, fresh truffle samples from the market are suspected of being flavoured with synthetically derived truffle aroma. As these results indicate, significant doubt exists about the authenticity of flavoured products on the market, and extensive testing is necessary.

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LECTURES

L15

HOW CLIMATE CHANGE CAN HELP FIGHTING FRAUDULENT DECLARATION OF CEREAL GRAINS

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According to a report by the German Meteorological Service, the year 2018 was one of the driest and warmest since weather records began in 1881. As a result, farmers had to cope with heavy losses in both crop yield and crop quality throughout the country. Such extreme weather events lead to drastic market fluctuations, as they create shortages in high-quality grain supply while simultaneously producing an excess of lower quality grains. To maintain quality parameters like protein content and bulk density, traders are often forced to mix batches from several harvest years, which must be declared. At the same time, more and more customers insist on buying cereal grains from a single harvest year and pay less for mixed batches. Combined with the competitiveness of the market, the risk of mislabelling increases, especially since no method for determination of harvest year is established yet.

Isotope-ratio mass spectrometry (IRMS) could be a suitable approach as plant-based products reflect characteristics of their environment and physiology through the stable isotope ratios of the light elements ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$), hence also inter-annual changes in e.g. temperature, amount of precipitation and air humidity. IRMS is already a well-established approach in wine authenticity testing, in which it helps verifying the vintage year stated on the label. To test the feasibility of this method for commercial crops from Germany, we analysed 406 cereal grain samples (barley: 219, spelt: 187) from the harvest years 2016-2018 for their carbon, nitrogen and oxygen stable isotope data as well as their element concentrations. In addition, we applied near-infrared spectroscopy (NIRS), a technique with various advantages like affordability, rapid measurements, little to no sample preparation and potential to be used online.

Special attention was paid during sample selection and data evaluation in order to consider and minimize the variability introduced by factors such as genotype or region. Isolated methods showed clear trends for discrimination between harvest years, especially for the year 2018. Separation of the groups was significantly improved when using combined data sets and multivariate statistical approaches with high correct classification rates. Carbon and oxygen natural isotope abundance were the most important variables included into the model.

We are convinced that due to the increased frequency of extreme weather events, this multi-method approach turns out in the future as a valuable and urgently required tool in fighting fraudulent declaration of cereal harvest year.

Keywords: *stable isotopes, NIRS, cereal grains, harvest year, climate change*

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LECTURES

L16

TACKLING RICE FRAUD: AN INDIAN MODEL

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The demand for high quality Basmati rice has increased significantly in the last decade and therefore not surprising that we find Basmati rice mixed with various proportions of lower quality varieties on the world markets. Consequently, this is an issue for Basmati rice exporters. In order to help address this issue, a two tiered system using rapid portable screening and confirmatory techniques, was developed. A handheld NIR was used to scan Indian samples (n=1399). The data was exported to SIMCA 15.0.2 for chemometric model building. Chemometrics were applied to the spectral data to produce calibration models and prediction statistics. A multiclass OPLS-DA model comparing the high quality 1121 variety with its potential adulterants (1509 and Sugandha) correctly predicted the correct variety nearly 100% of the time. When moving to a 2 class model (1121 vs adulterants), the OPLS-DA model correctly predicts 100% of the time. Similarly, this was repeated for the high quality Taraori basmati variety and its potential adulterants (Duplicate Basmati, Sharbati and Shabnam). The multiclass OPLSA-DA model showed good separation among the 4 varieties. Again, the model correctly predicted the variety nearly 100% of the time. In the 2 class OPLS-DA model, there appears to be some overlap visually between Taraori and adulterants. However, when the model is tested with independent samples, it predicts correctly 100% of the time. To compliment this data, a Head space gas chromatography mass spectrometry (HS-GC-MS) strategy for analysing untargeted volatile organic compounds (VOCs) profile to distinguish between Indian rice samples of different classification was employed as the confirmatory tier in this system. The 7 rice varieties from India were analysed using PDMS/DVB/Carboxane SPME fiber. Correlations among the measured flavour characteristics and sensory attributes evaluated by applying unsupervised Principal component analysis (PCA), Hierarchical clustering analysis (HCA) and supervised Partial least square discriminant analysis (PLS-DA). The VOC profile fingerprints identified classification (R2= 0.975, Q2= 0.952, Accuracy = 0.857) between various varieties of Indian Basmati rice samples. In the investigated samples, models correctly classified more than 97% of the samples. The major advantage of this system is that a large number of samples can be screened in-field using the handheld NIR and non-conforming samples can be sent for confirmatory analysis, cutting down on both time and expense. This study suggests that using a two tiered system of a rapid screening method alongside a confirmatory method may be appropriate to classify Indian Basmati rice varieties and thus help further protect the supply chain.

Keywords: *fraud, rice, handheld NIR, HS-GC-MS, chemometric modelling*

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LECTURES

L17

SMART MICRO-SENSORING: MICRO-ELISA AS PERFORMING OFF LINE/AT LINE TOOL FOR CONTAMINANTS DETECTION IN FOODS

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Food safety is a top-flight issue. Being able to accurately assess the presence of contaminants, residues, pathogens, allergens in foods is crucial, and there's a constant research for new performing analytical methods. Both mycotoxins and food allergens can be easily directly detected by immunochemistry-based methods (ELISA) , as well as by chromatographic or mass-based analytical approaches. Similarly, mycotoxins can be detected by antibodies-based ELISA kits and HPLC-MS methods. Moreover, the interest towards "smart" analytical approaches based on the use of micro- and nano-sensors is continuously growing worldwide, particularly permitting their use at line and on line (sometimes in line).
In this communication the performances of a new device based on a microfluidic disposable card (LOC, Micro-ELISA) and a small portable correlated instrument are shown. The LOC can give quantitative analysis in a few minutes with high sensitivity using as ligand both antibodies as well as aptamers. Some examples of applications - obtained from "Food Digital Monitoring Project", a Regione Piemonte/EU funded Project under the European Funds for Regional Research measure – POR-FESR are showed, critically discussing either advantage and critical points.
The method has been tested for aflatoxins (M1 and B1), ochratoxin, lysozyme as example of food allergen, and gluten, in different food matrices. Sensitivity and robustness of this approach was assessed, comparing the results with the standard ELISA detection. Finally, the Micro-ELISA method was experimentally coupled with a novel semi-automatic extractor for gluten, evaluating its usefulness as well as the potential reduction of the time of the analysis.
All these findings confirm the usefulness of this analytical approach, leading to an easy detection of contaminants and opening new perspectives in "at line" monitoring of contamination.

Keywords: *rapid methods, Micro-ELISA, at line analysis, food contaminants*

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LECTURES

L18

ANALYTICAL ASSESSMENT OF THE IMPACT OF ALTERNATIVE PROCESSING TECHNOLOGIES ON METABOLOME OF SEA BUCKTHORN ´SUPERFRUIT´

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Sea buckthorn (*Hippophae rhamnoides*) is a cold-resistant shrub, which produces yellow-red berries with a tough skin covering pulp and small hard seed. This small fruit has gained global attention for their nutritional benefits, which can be related to bioactive compounds, such as polyphenols or carotenoids. Several studies have associated sea buckthorn with beneficial effects on human health. These valuable effects have made sea buckthorn a desirable product for medicinal, food and cosmetic purposes.
Over the years, consumers demand for fresh and natural tasting products with a longer shelf life has increased. For this reason, new mild preservation technologies such as Pulsed Electric Field (PEF), Ohmic Heating (OH) or High-Pressure Processing (HPP) were developed in order to achieve gentle, non-thermal pasteurization of various raw materials. In comparison with conventional heating processes, mild technologies allow the final product to retain flavor, texture and nutritional value. These techniques are suitable mainly for liquid foods such as fruit juices, purees, oils, etc.
In our study, two varieties of sea buckthorn (Botanica and Leicora) were used to prepare fruit syrup. Berries were gently crushed and juiced to prepare fruit must, in the next step 0.5-0.7 kg of sucrose per 1 L of the must was added. This product was then treated by PEF, OH, HPP and conventional heating and stored for 8 weeks in the refrigerator, in order to find differences caused by preservation technology or storage time. Metabolomic fingerprints of methanolic extracts of fruit syrups were obtained using ultra-high performance liquid chromatography coupled to tandem high resolution mass spectrometry (U-HPLC-HRMS/MS). The chromatographic system was equipped with a reversed phase column and a quadrupole-time-of-flight TripleTOF 6600 mass spectrometer from Sciex was used as a detector. Subsequently, chemometric evaluation was performed to assess the differences between the samples. After data pretreatment and processing, the PCA (Principal Component Analysis) revealed significant differences between metabolome fingerprints of samples. Clear clustering, which was more pronounced for data obtained in a negative ionization mode, was found for different types of treatment. In parallel, accurate *m/z* value of molecular ion, isotopic profiles and characteristic fragments (MS/MS spectra) were employed for tentative marker identification using various software packages and online libraries. Among the compounds most impacted by processing were flavonoids, phospholipids, and free fatty acids. During the storage, not too significant changes occurred.

Keywords: *sea buckthorn, metabolomic fingerprinting, U-HPLC-HRMS/MS, mild preservation technologies*

Acknowledgement: *Project "Volumetric preservation technologies for food quality improvement by retention of sensitive and mitigation of neoformed compounds (volTECH)", supported by CORNET (Collective Research Networking) and funded in the Czech Republic within the national Operational program Enterprise and Innovations for Competitiveness, support programme Cooperation - Clusters (CZ.01.1.02/0.0/0.0/16_079/0008876). 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 by the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT- 43760/2015).*

LECTURES

L19

A TOP-DOWN COMPUTER-DRIVEN WORKFLOW TO IDENTIFY ANGIOTENSIN I CONVERTING ENZYME INHIBITORY PEPTIDES - A BREAKTHROUGH IN THE LARGE SCALE IDENTIFICATION OF ACTIVE SEQUENCES

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Hypertension is a global health problem affecting up to 20 % of adults and carrying a high-risk factor for arteriosclerosis, stroke and myocardial infarction. Among the processes underlying hypertension, angiotensin-I-converting enzyme (ACE) plays a prominent role in the regulation of blood pressure converting angiotensin II to the vasoconstrictor angiotensin I and degrading the vasodilator bradykinin. On this basis, ACE is an ideal target to inhibit for the sake of treating hypertension. Indeed, many synthetic inhibitors have been developed but their use in pharmacological therapies may cause side effects. On the other hand, the identification of "natural" inhibitors is getting interest to either treat or prevent mild hypertension. As an example, the discovery of food-grade compounds and food constituents is of particular relevance to design functional foods to tackle/prevent mild hypertension or to support pharmacological treatments. Bioactive peptides are among the most promising food components with ACE inhibitory activity though the identification of active sequences is still a major challenge. Indeed, the identification of inhibitory peptides usually requires the digestion of specific sources of interest followed by: i) *in vitro* test on ACE-inhibitory activity of the mixtures generated; ii) sequence elucidation of the peptides released. In these bottom-up approaches the sequences identified inherently depend on the source under investigation. Also, the approach is biased by the number of peptides that can be practically tested and most of the active sequences identified in the mix are likely to get uncharacterized due to technical limitations.
The work-flow proposed here relies on a computer-driven approach to support the large-scale and source-independent identification of ACE inhibitory peptides. The computational model relies on virtual screening and molecular dynamics to detail the molecular basis of inhibition. The model was validated on peptides from soy and dry-cured ham (it successfully identified ACE inhibitory peptides in agreement with experimental data) and it was then used to screen a combinatoric library of tripeptides (3-combination of 20 amino-acids with no repetitions resulting in 6840 peptides). The output was compared to some of the gold benchmark databases of bioactive peptides (e.g. BIOPEP-UWM and BioPepDB). Almost all the ACE inhibitory tripeptides recorded in the
Referencedatabases were in the top ranked list of calculated peptides (independently identified) strongly supporting the approach reliability. Finally, a set of peptides not yet characterized underwent experimental trials leading to the identification of novel sequences with anti-hypertensive potential. Such an approach may allow a straightforward, top-down and source-independent identification of ACE inhibitory sequences that may serve to define a sequences database of
Referencefor the large-scale identification of ACE-inhibitory peptides in food.

Keywords: *bioactive peptides, in silico screening, ACE-inhibitory peptides, large-scale screening, angiotensin I converting enzyme*

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LECTURES

L20

COMPREHENSIVE INSIGHT INTO COMPOSITION AND BIOACTIVITY OF MILK THISTLE-BASED FOOD SUPPLEMENTS

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Milk thistle (*Silybum marianum*) is a medicinal plant, extract of which displays antioxidant properties and thus is widely used as a basis of various liver-protective food supplements. Its antioxidant potency is commonly considered to be associated with silymarin, mixture of bioactive flavonoids / flavonolignans (including mainly silybin A and B, isosilybin A and B, silydianin, silychristin, isosilychristin and taxifolin). No attention has been until now paid to other natural components present in milk thistle that are co-extracted together with silymarin, no focus has been paid to them in various bioactivity studies. The aim of this study was to investigate 26 milk thistle-based food by an array of antioxidant activity assays and correlate the results not only with the determined silymarin flavonoid / flavonolignans content but also with other natural antioxidants detected in the samples by U-HPLC-HRMS/MS screening. At the same time, natural milk thistle extract and a model mixture of pure flavonoid / flavonolignans mimicking the natural extract composition as a control samples were involved in measurements. Three traditionally used biochemical assays (ABTS, ORAC and DPPH) and also the cellular antioxidant test CAA reflecting the bioavailability and bioaccessibility of the tested compounds were utilized. Significant differences both in the silymarin composition and the antioxidant capacity among the various food supplements were observed. Unlike the DPPH, the results of ABTS and ORAC significantly correlated with the determined content of silymarin flavonoid / flavonolignans. The responses in CAA were considerably lower, and, as expected, many samples having the ability to reduce radicals in biochemical assays failed to do so in CAA. For some of the low-silymarin samples, unexpectedly high antioxidant capacity was observed; moreover, the natural milk thistle extract exhibited significantly higher responses in all of the antioxidant activity assays compared to the pure flavonoid / flavonolignans mixture. Such results support the hypothesis on the presence of other natural antioxidants. Using the U-HPLC-HRMS/MS method, tens of non-silymarin bioactive compounds belonging to various chemical classes were detected in the milk thistle extract and food supplements samples. Comparing their relative content with the observed antioxidant capacity, especially the results of CAA correlated significantly more with the sums of detected non-silymarin simple phenolics and total phenolics (sum of simple phenolics and flavonoids) than with the silymarin flavonoid / flavonolignans content. This work clearly demonstrates the need for detailed chemical characterization of milk thistle-based preparations that are used in bioactivity tests and / or clinical studies and also for the proper choice of the bioactivity assays.

Keywords: *silymarin, Silybum marianum, antioxidant activity, U-HPLC-HRMS/MS, food supplements*

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LECTURES

L21

IDENTIFICATION OF TROPOMYOSIN AS THE MOST RELEVANT ALLERGEN IN EDIBLE INSECTS

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In the last years, edible insects have been explored as a promising alternative source of proteins in order to overcome the future food demands connected to growing world population. Since 2018, insects have been definitely declared to belong to the category of Novel Foods, making necessary deep investigations to assess their safety (Regulation EU 2015/2283). The presence of allergens represents one of the main hazards connected to their consumption, also due to the potential cross-reactivity with Arthropoda pan-allergens.
In the present work, the allergenicity assessment of two different insect species, *Hermetia illucens* and *Alphitobius diaperinus* larvae, was performed. First, a complete shotgun proteomic characterization by high resolution mass spectrometry on a LTQ-Orbitrap instrument was carried out, setting the basis for an *in silico* allergenicity evaluation by bioinformatic tool, using Allermatch™ webtool. The relevant potential allergens identified by similarity to known allergens, belonged to two distinct classes, associated to distinct animal groups: crustaceans, very relevant for food allergies, and other Arthropoda, already known as being responsible for inhalatory or stinging allergies (e.g. mites, midge). Among the identified potential allergens, shotgun proteomics techniques outlined tropomyosin as one of the most abundant protein in insect extracts. Denaturing polyacrylamide gel electrophoresis (SDS–PAGE) was used to separate proteins, while pan-allergens were identified with both Western blotting and Immunoblotting assays. Western blotting analysis with Tropomyosin I antibodies (isolated from rabbit) outlined a positivity in both insects, demonstrating that there is cross-reactivity between insect tropomyosin and other tropomyosins, with a clear potential to trigger an immunological reaction. IgE-immunoblotting with sera from crustaceans-allergic patient (n=10) also confirmed the presence of a cross-reactivity with insect proteins.
The immunoassays were carried out also on protein hydrolysates obtained from both insects by treatment with Protease from *Bacillus licheniformis* (1%, 60°C, pH 7.5). We explored this biotechnology tool, widely used in the production of hypoallergenic food, on insect biomass in order to reduce their allergenic property for a safer use in the food sector.

Keywords: *edible insects, tropomyosin, enzymatic hydrolysis, allergenicity assessment, Novel Food*

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L22

GOING WITH THE FLOW: THE DEVELOPMENT OF SMARTPHONE-BASED LATERAL/FLOW-THROUGH IMMUNOASSAYS FOR THE HIGH-SPEED DETECTION OF FOOD ALLERGENS.

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Smartphone diagnostics aim to bridge the gap between the laboratory and the general public by engaging consumers with their own health and dietary needs; promoting citizen science. Currently, consumers are largely distanced from the analytical procedure and rely of third parties for results. Food allergies represent a significant worldwide health concern and consumers should be able to analyse their foods, independent of the analytical laboratory, for allergen presence. Owing to the need for a scientific background, traditional laboratory-based detection methods are generally unsuitable for the consumer. Smartphones make excellent detection systems because of their familiarity, cameras, embedded flash functions, portability, connectivity, and affordability. Many consumer-oriented food allergen tests are based on lateral flow immunoassay (LFIA). Despite being classified as rapid tests, LFIAs take around 10-20 minutes to complete. In order to overcome these extended assay times, the immuno-reagents being applied in the assays should first be properly characterised. By using surface plasmon resonance (SPR) it is possible to screen even un-purified antibodies for their affinities, association speeds and cross-reactivity's and towards the targeted allergen antibodies. Thanks to the SPR screening process, it was possible to select an extremely sensitive and rapid antibody sandwich pair against hazelnut allergen. When applying this antibody pair in carbon-nanoparticle labelled LFIA, the resulting assay exhibited un-paralleled speed, producing a positive result within 30 seconds. In addition to the rapidity, the test also can detect hazelnut allergen at trace levels of 0.5 ppm in a real life matrix, making it more sensitive than current commercially available hazelnut LFIAs. The assays were semi-quantified using two freely downloadable applications from google play which allow color value measurements in RGB and LAB color models. By taking the L values of the LAB measurements, there is a clear relationship between a lower L value corresponding to a higher amount of hazelnut being present in the food. Despite its inherent advantages, LFIA falls short when using for high-speed multiplex analysis. The literature shows that when including multiple analytes on an LFIA, the upstream analyte can be adversely affected by the downstream analyte and the reliance on capillary forces can prolong the assay time. This can be limiting for the allergic consumer, especially when considering many allergies co-exist, and without a multiplex procedure, users would have to perform tests in parallel. By converting to flow-through assay formats, assay times can be reduced as capillary forces no longer limit the rate. In this talk, the journey from singleplex lateral flow immunoassay to multiplex flow through immunoassay will be discussed, with a focus on how smartphones can be applied for quick and simple semi-quantification of allergens in a real life matrix.

Keywords: *carbon nanoparticles, lateral flow immunoassay, flow-through immunoassay, food allergen, smartphone*

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LECTURES

L23

GLUTEN ANALYSIS IN PROCESSED FOODSTUFFS BY A MULTIALLERGEN AND GRAIN-SPECIFIC UHPLC-MS/MS METHOD

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Celiac disease (CD), a complex long-term autoimmune disorder is observed in genetically susceptible individuals in response to exposure to dietary gluten (1-3). CD and gluten intolerance are estimated to affect approximately 1 and 5% of the world's population, respectively (4).
Gluten, the main cause of these important public health issues, is defined as "the protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and which is insoluble in water and 0.5 M sodium chloride solution" (5–6). Gluten is not an individual protein but rather a generic name given to a complex mixture of seed storage proteins.
As a consequence, to protect gluten-sensitive consumers, the development of reliable analytical methods allowing the detection of gluten in various food products is needed.
Currently, ELISA is probably the most widespread used methodology. The method based on the R5 antibody has received type I status in Codex Alimentarius for the quantification of gluten (6–7). However, ELISA method suffers from some limitations, especially concerning quantification of nonwheat gluten (7). Therefore, the development of another complementary methodology such as Liquid Chromatography – tandem Mass Spectrometry (UHPLC-MS/MS) is considered to be essential. Furthermore, this method could also be used for the simultaneous detection of gluten with other allergens (8-10), which will constitute a great additional benefit for producers of "free-from" food products and/or having a management policy integrated for several allergies and/or intolerances.
A multiallergen and grain-specific UHPLC-MS/MS method allowing the identification and the discrimination of gluten from seven cereals, simultaneously with the detection and identification of 10 allergens in only one analysis, is described here (11). This method can be used for the analysis of a broad range of foodstuff matrices containing wheat and/or its derivatives, including cereals, flours, heat-treated and foodstuffs, but also more complex samples having undergone fermentation processes (such as beers) (11).

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Keywords: *multiallergen, grain-specific, UHPLC-MS/MS method, celiac disease, gluten intolerance*

LECTURES

L24

COMPARISON OF MASS SPECTROMETRY, ELISA AND PCR FOR THE DETECTION OF ALLERGENS IN PROCESSED FOOD

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Food allergies form a growing health problem around the world. Since no cure is available for allergic individuals, complete avoidance of the culprit food is essential. European legislation therefore requires the labelling of 14 ingredients that have the potential to cause an allergenic reaction when used as an ingredient (regulation 1169/2011/UE). However, trace amounts of allergens can enter food products by cross contamination, using shared equipment for processing of multiple ingredients or suboptimal cleaning of such equipment. For protection of patients, accurate and sensitive detection methods of trace amounts of allergens are needed. One such method is liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), which is gaining popularity in the field due to its high specificity and sensitivity, robustness towards food processing and the possibility of multiplexing. This technique is based on the detection of proteotypic peptides, peptides specific for the ingredient of interest, generated by tryptic digestion of the proteins extracted from food samples. In this project, we developed a multi-allergen detection method for milk, egg, hazelnut and peanut that is robust towards food processing. This combined MRM method using ultra high performance liquid chromatography coupled to tandem mass spectrometry was tested on in-house produced test material containing the four allergens at different ppm (mg total proteins per kg material) levels. Detection limits were established for each allergen. Current detection methods applied in routine analysis comprise the protein based ELISA technique and DNA based qPCR. To test the performance of our MS based detection method, we used the in-house produced test material for analysis with commercial ELISA and PCR kits. In this study, we compared the performance, accuracy and detection limits of these different methods, as well as their robustness towards food processing.

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LECTURES

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DEVELOPMENT OF A NEW ISOTOPICALLY LABELLED INTERNAL STANDARD FOR ALLERGEN QUANTIFICATION BY MASS SPECTROMETRY

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During the last decade, several mass spectrometry-based methods were developed to detect allergens in various food matrices. The next step is the development and validation of accurate quantitative methods. Studies based on risk and safety assessments using clinical challenge trials in food-allergic humans are paving the way for a legislation on allergen thresholds. Combining these thresholds with quantitative methods should guarantee correct food labelling and will help allergic patients to protect themselves. For reasons including sample preparation and ion suppression, a compound cannot be accurately quantified unless using internal standard. Here, an isotopically labelled form of an analyte-of-interest is added to the sample. Quantification is achieved by comparing the signals of such internal standards (for which the amount is known) to those of the analytes. The choice of the internal standard is crucial for method performance. In the case of allergen analysis by mass spectrometry, the analytes are peptides obtained following proteolysis of allergen proteins. Isotopically labelled internal standards can therefore be peptides, proteins or so-called concatemers that are intermediate solutions, being chimeric proteins composed of peptides from different allergen proteins. Thus far, concatemers have not yet been used for food allergen quantification. Although isotopically labelled synthetic peptides compensate for variability of mass spectrometry readouts, they fail to do so for other sample preparation steps (e.g., protein extraction and enzymatic digestion). Isotopically labelled proteins are ideal as they can compensate for matrix effects and for the different sample preparation steps. However, such proteins are very expensive for routine analyses as multiple proteins are required for multiple allergen analysis. Isotopically labelled concatemers are thus good compromises as they compensates for all sample preparation steps and contain peptides from different allergen proteins in a single construct. Typically, a DNA sequence coding for selected peptides is designed and cloned into a bacterial expression plasmid. Transformed bacteria are then cultured in a ^{15}N isotope-labelled growth media and express the concatemer. Using high resolution mass spectrometry, peptide biomarkers that are robust to food process were identified for four allergens (egg, milk, peanut and hazelnut), which were then used to develop a quantitative mass spectrometry-based method and a ^{15}N isotope-labelled concatemer. Using processed food products incurred with the four allergens, the performance of this quantitative strategy was compared to the performance achieved by using isotopically labelled peptides or proteins for one protein (beta-lactoglobulin, the major whey protein of cow's milk).

Keywords: *allergens, mass spectrometry, internal standard, stable isotope dilution, isotopically labelled concatemer*

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LECTURES

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INTEGRATED METHODS TO REDUCE, CONTROL AND DETECT MYCOTOXINS ALONG THE FOOD CHAIN

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The total costs of losses due to mycotoxin contamination, such as reduced yields, food and feed losses, increased costs for inspection and analyses, and others, may easily reach billions of Euros annually[1]. Moreover, extreme weather events challenge the global mycotoxin landscape, requiring forecasting and detection methods to adapt to this new and changing landscape. Within MyToolBox (www.mytoolbox.eu) we have been pursuing not only a field-to-fork approach but also considering safe use options of contaminated batches, such as the efficient production of biofuels through the successful application of recombinant enzymes. The successful detoxification has been verified by the determination of the hydrolysed mycotoxin degradation products by LC-MS/MS. In the area of innovative silo monitoring we have utilized novel sensor technology combined with improved algorithms, with CO_2 as an early-warning parameter to forecast zearalenone and aflatoxin contamination in grains.
Our research into the effects of baking on mycotoxin levels provides better understanding of process factors used in mycotoxin risk assessment. We have successfully elucidated the fate of the most prevalent *Fusarium* mycotoxin Deoxynivalenol during baking of crackers, biscuits and bread, which were produced from fortified dough and processed under pilot plant conditions. Untargeted stable isotope assisted liquid chromatography high resolution mass spectrometry was used to determine all extractable degradation products and targeted LC – tandem mass spectrometry based was used to quantify the degradation products isoDON (up to 3.9%), norDON B (up to 0.9%) and norDON C (up to 1.2%). The involvement of leading institutions from China has led to a sustainable cooperation in mycotoxin research between the EU and China and especially in the area of novel and effective prediction tools and post-harvest decision support systems employed in large grain silos.

Mitchell N, Bowers E, Hurburgh C, Wu F (2016). Potential economic losses to the US corn industry from aflatoxin contamination. Food Additives and Contaminants: Part A 33(3):540-550

Keywords: *MyToolbox, LC-MS/MS, mycotoxins*

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LECTURES

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MASS SPECTROMETRY IMAGING AS A TOOL TO VISUALIZE THE PLANT METABOLOME CHANGES IN RESPONSE TO MYCOTOXIN ACCUMULATION

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Mass spectrometry-based metabolomics has been applied to understand the molecular interaction between plant and pathogens, essential to develop possible strategies to counteract mycotoxin accumulation. Nevertheless, this approach results in a loss of the spatial information of the metabolites due to the extraction process. In this regard, mass spectrometry imaging (MSI) has become a powerful tool capable of achieving the spatial distributions and chemical specificity, useful to assign the metabolites' functional role.
Here, we aimed to visualize the distribution of metabolites involved in the plant response after mycotoxin administration using an *in-vitro* plant model. To address this challenge, transversal cross-sections were obtained from wheat roots, stems, leaves and were analyzed using atmospheric-pressure (AP)-scanning microprobe matrix-assisted laser desorption/ionization MSI ion source (AP-SMALDI5 AF, TransMIT GmbH, Giessen, Germany) coupled to a Q-Exactive HF orbital trapping mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany).
The data obtained were processed using Mirion (TransMIT GmbH, Giessen, Germany) and LipostarMSI (Molecular Horizon srl, Italy), novel comprehensive software to assist MSI-based untargeted metabolomics. Segmentation and region of interests (ROI) were created to perform multivariate statistical analysis, both unsupervised and supervised techniques.
The loading plots highlighted several lipids (i.e. galactolipids, diacylglycerols) and metabolites (i.e. hydroxycinnamic acids) differentially accumulated and distributed in mycotoxin-traded samples vs control samples.
Our results demonstrated the analytical potential of the innovative high-throughput technique for gaining insight into the plant resistance mechanism against mycotoxin accumulation. MSI holds unique potential for untargeted detection and spatial localization of metabolites from intact plant samples without the need for extraction or extensive sample preparation.

Keywords: *mycotoxins, mass spectrometry imaging, metabolomics, in-vitro plant model, spatial localization*

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SCRATCHING ON THE EDGE: DEVELOPMENT OF A QUANTITATIVE MULTI-TARGET LC-MS/MS METHOD FOR THE DETERMINATION OF >1,400 PESTICIDES, VETERINARY DRUGS, FUNGAL METABOLITES AND PLANT TOXINS IN FOOD AND FEED

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Influencing factors such as climate, storage conditions or food processing enable the occurrence of a variety of contaminants like fungal metabolites or plant toxins in the food chain. In addition, the impact of environmental chemical pollution has steadily increased within the last decades by the excessive use of pesticides and pharmaceuticals. These so-called emerging contaminants become a new environmental problem, since there is still a lack of knowledge about long-term risks for non-target organisms. Consequently, there is a growing need for robust, reliable and comprehensive analytical methods, which allow a sensitive, selective and rapid determination of such naturally and anthropogenic pollutants in environmental samples.
In this work, a liquid chromatography-electrospray ionization tandem mass spectrometric method was developed, to allow a simultaneous quantification of about 700 fungal metabolites, 500 pesticides, 150 veterinary drugs and 50 plant toxins. The aim of this work is a demonstration of the tandem mass spectrometry limitations in relation to the high number of analytes that can be detected within a single chromatographic run. In order to increase the method performance in terms of repeatability and reproducibility, critical parameters such as Dwell time and cycle time were optimized. Further focus is on the assumption that significant differences in signal suppression and enhancement (SSE) as well as in the extraction efficiency (RE) within different lots of a sample type is substantially influencing the method performance. In order to perform a complete characterization and assignment of corresponding matrix effects, artificial complex model matrices were prepared and the related numerical values for signal suppression/enhancement were modelled from data derived from the individual single feed ingredients.

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

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L29

THE OCCURRENCE OF EMERGING MARINE TOXINS IN SHELLFISH FROM THE NETHERLANDS

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The emergence of marine and freshwater toxins in geographical areas where they have never been reported before is a concern of considerable impact on (sea)food contamination, and consequently, on public health. In the last decade the European Food Safety Authority (EFSA) prepared a number of opinions on both already EU regulated toxins such as diarrhetic shellfish poisoning (DSP) toxins, as well as EU unregulated toxins such as cyclic imines, palytoxins, brevetoxins and tetrodotoxins. The latter is currently only regulated for shellfish within the Netherlands, all others are not regulated yet. Most of the known marine toxin classes are now covered in EFSA opinions and one of the most frequent mentioned conclusion in these opinions is the need for methods and the lack of occurrence data. Therefore we developed various analytical methodologies and performed a number of surveys.
After positive findings in a survey of tetrodotoxins (TTX) in shellfish harvested in the Netherlands it became clear that these more tropical related toxins also might occur in temperate regions. Currently, for TTX a liquid chromatography tandem mass spectrometric (LC-MS/MS) method, a national monitoring program and legislation (44µg TTX/kg) are established. In the last 4 years, annually TTX has been observed between half June and July with a maximum concentration of 250µg TTX/kg in June 2016. For brevetoxins (PbTXs) a LC-MS/MS method has been developed and subsequently an one year survey was organized. The conclusion of the survey was that no detectable concentrations of PbTXs were observed in shellfish from the Dutch production areas. For both palytoxins (PlTX) and cyclic imines (CIs) sensitive LC-MS/MS methods have been developed. Palytoxin is a large molecule, mw 2680 Da, which is difficult to analyse at low µg/kg concentrations in shellfish. We developed an LC-MS/MS method based on so called lithium cationization, with this method we're capable of detecting low µg/kg concentrations of PlTX in shellfish (LOD 8µg/kg in mussel). The CIs are a class of "toxins" consisting out of spirolides (SPXs), gymnodimines and pinnatoxins, which are all known to occur also in more temperate areas. During the course of 2019 a survey is organised for these "toxins". With the presence of some SPX producing algae (*Alexandrium Ostenfeldii*) in close proximity to shellfish production areas it is assumable these SPXs will be detected at low concentrations in shellfish.
In conclusion the reason for the occurrence of these emerging toxins might be related to climate change and the increased availability of nutrients have been considered as the key factors in the expansion of all of these toxins into new areas. However, this could also be due to more intense biological invasions, improved detection methods, or perhaps even an increased scientific interest in these emerging toxins.

Keywords: *marine biotoxins, cyclic imines, tetrodotoxins, brevetoxins, emerging toxins*

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LECTURES

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MULTI-OMICS APPROACH FOR UNDERSTANDING THE BIOTRANSFORMATION OF MYCOTOXINS IN MICROPROPAGATED DURUM WHEAT PLANTLETS

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It is well-known that plants may employ different detoxification mechanisms to cope with the adverse effect of xenobiotics, among them mycotoxins. Multiple enzymatic pathways may lead in the formation of modified mycotoxins that, once entered the food and feed production chain, may significantly contribute to the overall toxic load related to mycotoxin exposure. Therefore, understanding the plant metabolism of mycotoxins and thus the toxicological role of resulting modified forms, is becoming increasingly important for risk assessment. On the other side, investigating the biotransformation exerted by the plants towards mycotoxins may shed a light on the biological interaction between plant and fungi. While an increasing number of studies have been performed on deoxynivalenol and major *Fusarium* mycotoxins in soft wheat and barley, less is known about the biotransformation occurring in durum wheat and maize involving other mycotoxins.
In this context, *in vitro* techniques represent a consolidated approach to investigate the metabolic fate of mycotoxins enabling the characterization of phase I and II biotransformation products. For this purpose, an in vitro model was set up to elucidate the uptake and metabolic fate of mycotoxins in durum wheat, using five wheat varieties with different level of resistance. In addition to plants, leaves and roots experiments were independently set up to study the organ- and tissue-biotransformation dependency.
Tissues and growing media have been analysed by HR-LC/MS to return the full profile of metabolites produced in plants. A large spectrum of phase I and phase II biotransformation metabolites were depicted, some of them never reported before. When possible *in silico*/in vitro approaches have been used for a preliminary evaluation of their toxicological relevance.
In addition, fully untargeted metabolomics/lipidomics and proteomics were used to elucidate the plant response to mycotoxin administration. A preliminary attempt to connect pathways originated from metabolomics and proteomics profiles has been performed as well.
Although still preliminary, the approach presented herein has demonstrated its potential to address the challenge of mycotoxin biotransformation in plants.

Keywords: *metabolomics, HR-LCMS, modified mycotoxins, cereals*

LECTURES

L31

MULTI-ALKALOID METHOD FOR EFFECTIVE FOOD SAFETY CONTROL

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Alkaloids are a wide group of toxic secondary metabolites occurring naturally in a variety of plant species. Their production is a part of defense mechanism against pests and herbivores. In some cases, plants containing these toxins appear as weeds in food crops. Accidental contamination of the main crop by seeds or leaves may occur at harvest. Because of this, low levels of alkaloids can be detected in cereals, herbal and other products including feeds. Based on a health risk assessment by EFSA, maximum limits have been set for some tropane alkaloids. For other compounds, occurrence data are being collected to estimate dietary exposure. Under these conditions, high throughput analytical method for determination of multiple alkaloid groups in a single run is a challenging task. However, many representatives of the major groups of these toxins such as tropane and pyrrolizidine alkaloids, are structurally similar compounds including a number of isomers. On this account, instrumental methods employed for their detection suffer of poor separation of individual analytes what may lead to biased results. In this study, we focused on the development of analytical method for 56 pyrrolizidine, tropane and quinolizidine alkaloids. Solid-liquid extraction (SLE) with aqueous methanol acidified with formic acid was used for analytes extraction. For instrumental analysis, ultra-high performance liquid chromatography coupled with tandem mass spectrometry (U-HPLC–MS/MS) employing QTRAP 6500+ system (Sciex) was utilized. To achieve a good chromatographic resolution, small particle size reverse phase column Luna Omega C18 (150 x 2.1 mm, 1.6 µm; Phenomenex) was used. Nevertheless, for the most problematic groups of co-eluting isomers, (i) pyrrolizidine alkaloids indicine, echinatine, intermedine, lycopsamine and (ii) their N-oxides, complete separation could only be achieved after additional separation on a hydrophilic interaction liquid chromatography (HILIC) column. The developed method was validated and used for analysis of tens of naturally contaminated materials (cereals, herbal teas, and infusions).

Keywords: *pyrrolizidine alkaloids, tropane alkaloids, separation, ultra-high performance liquid chromatography, 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.*

LECTURES

L32

ISOMER-SPECIFIC ANALYSIS OF PYRROLIZIDINE ALKALOIDS: CHALLENGES, INVESTIGATIONS AND SOLUTIONS

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Pyrrolizidine alkaloids are plant toxins, which may contaminate food such as herbs, tea or honey. Many pyrrolizidine alkaloids are isomeric to one another, being either structural isomers or stereoisomers. These isomers share some or even all MS/MS transitions; however, the relative intensities of the product ions may vary significantly, complicating quantification and impeding confirmation by MS/MS transition ratios. Thus, chromatographic separation is essential to differentiate between the isomers and allow sound quantification. However, with the ever-growing number of analytes the simultaneous separation of the various isomers becomes more and more challenging.
In-depth investigations concerning the chromatographic and mass spectrometric behaviour (separation on different columns, fragmentation patterns and ionisation efficiencies) of more than 40 pyrrolizidine alkaloids were carried out to provide essential input for developing a method allowing the specific qualitative and quantitative analysis of as many pyrrolizidine alkaloid isomers as possible.
The majority of studied isomers could finally be chromatographically resolved on a C18 column within less than twenty minutes, with additional lycopsamine isomers being baseline separated on a phenylhexyl column. For the remaining co-eluting isomers it was confirmed that both fragmentation patterns and ionisation efficiencies are comparable, thus allowing for a summary assessment without making an error concerning the quantitative results.
Finally, data from various food products from the market will be presented, highlighting which isomers frequently occur in practice and thus fully justify analytical efforts for their specific analysis.

Keywords: *pyrrolizidine alkaloids, isomers, separation, fragmentation, LC-MS/MS*

LECTURES

L33

DEVELOPMENT OF A COMPREHENSIVE MASS SPECTRAL DATABASE FOR PYRROLIZIDINE ALKALOIDS USING UHPLC COUPLED TO Q-EXACTIVE (ORBITRAP) MASS SPECTROMETRY

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Pyrrolizidine alkaloids (PAs) are one of the most important groups of natural toxins due to their wide distribution and high risk of unintentional consumption via contaminated food products. More than 6000 plant species, constituting 3% of all flowering plants, produce these secondary metabolites. They occur in an exceptionally wide structural diversity and more than 600 alkaloids have already been identified. In recent years PAs are gaining increased attention, mainly due to their toxic properties, that can negatively affect the health of humans and animals. Many PAs possess hepatotoxic, genotoxic, cytotoxic, tumorigenic or neurotoxic potential. The presence of PAs has been revealed in feeds and many food products, including grains, honey, eggs, milk, teas, herbal teas and plant food supplements.
For the determination of PAs in feed and food commodities, mostly LC-MS/MS is used. However, the technique poses some limitations as only PAs included in the method can be determined. Due to a limited number of available standards, many PAs, not included in the method, can remain undetected. As a result, contamination levels in samples are often underestimated. Liquid chromatography full scan high resolution mass spectrometry (LC-HRMS) does not have this limitation as it offers non-targeted analysis. Q-Exactive (Orbitrap) MS offers a great potential for rapid screening of samples on the presence of a wide range of plant toxins. However, to obtain a reliable identification of compounds, a database containing comprehensive information on the compounds of interest is required. The aim of this study was the development of a broad database on PAs containing detailed spectral information such as name, accurate mass, elementary composition, retention time and accurate masses of the characteristic fragments.
In the past years, a large set of plant samples has been compiled, consisting of samples collected in the field and samples obtained from seed suppliers and plant nurseries. The collection covers most of the major genera of PA-producing plant species. Over 60 PAs standards and extracts from around 200 plant species were available for analysis. The data were collected using the LC-HRMS non-targeted method applying full scan and data independent acquisition events.
Analysis of the standards allowed the identification of characteristic fragmentation patterns of monoesters, diesters, macrocyclic diesters, corresponding N-oxides, otonecine and platynecine type PAs. Identification of the characteristic fragments and determination of their accurate masses allowed the development of a screening method enabling easy and effective detection of compounds potentially belonging to the class of PAs in plant extracts. As a result, a large number of PAs has been tentatively identified and included in the database with detailed spectral information. A set of contaminated food and feed samples was screened against the database to test the applicability of the method.

Keywords: *pyrrolizidine alkaloids, database, non-targeted analysis, UHPLC-HRMS*

Acknowledgement: *Funded by KNOW (Leading National Research Center) Scientific Consortium "Healthy Animal - Safe Food", decision of Ministry of Science and Higher Education No. 05-1/KNOW2/2015.*

LECTURES

L34

REGULATORY SCIENCE: AN UPDATED CONCEPT FOR PROFICIENCY TESTING OF OFFICIAL CONTROL LABORATORIES

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The new Regulation (EU) 2017/625 on official controls for food and feed places very clear competence requirements on official control laboratories in the Member States. In this frame EU ReferenceLaboratories (EURLs) have the task to organise regular proficiency tests (PTs) for National ReferenceLaboratories (NRLs) and, if necessary, other official laboratories and have to ensure an appropriate follow-up of the results.
During the last 12-15 years proficiency testing has been established as a widely accepted and applied instrument for analytical quality assurance. There are international standards, in particular ISO/IEC 17043, and guidance documents which describe good practice and recommendations for PT organisers and participants. However, their application in a regulatory context such as the competence assessment of official control laboratories and the complexity of the analytical tasks in the area of food & feed product monitoring require the further development of PT concepts and specifications.
Therefore, this presentation will outline the most recent approaches of the EURLs hosted by the European Commission's Joint Research Centre (JRC) in the food & feed area for the design and execution of proficiency tests. They include the selection and characterisation of PT materials mimicking closer complex marketed products, the establishment of
Referencevalues as main benchmark for the assessment of PT results, the systematic consideration of measurement uncertainties and a comprehensive evaluation and follow-up of the PT outcomes.
Recent examples of PTs performed in the areas of genetically modified food & feed, feed additives, food contact materials and emerging food contaminants such as fipronil will be used to illustrate the achieved progress.

Keywords: *regulatory science, proficiency testing, official controls, food analysis, feed analysis*

LECTURES

L35

ACHIEVEMENTS AND CHALLENGES OF THE EURL FOR HALOGENATED PERSISTENT ORGANIC POLLUTANTS IN FEED AND FOOD

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Commission Regulation 2018/192 widened the scope of activities of the EU Reference laboratories for contaminants. Given the growing importance of chlorinated persistent contaminants other than PCBs and dioxins, brominated persistent contaminants and fluorinated persistent contaminants for the safety of feed and food, the tasks of the former "EU Reference Laboratory for Dioxins and PCBs in Feed and Food" was extended to halogenated persistent organic pollutants (POPs) in feed and food in general.
As a result, four groups are currently important for the network of the EURL with National Reference Laboratories (NRLs): (i) PCDD/Fs and PCBs, (ii) brominated flame retardants (in particular PBDEs and HBCDDs), (iii) perfluoroalkylated substances (PFAS) and (iv) chlorinated paraffins (CPs).
The proficiency tests were expanded to cover also these substances.
In November 2018 EFSA published a comprehensive review on dioxins and dioxin-like PCBs. A new tolerable weekly intake (TWI) of 2 pg/kg bw/week was derived which is 7-times lower than the TWI of 2001. Therefore, options and limitations to lower legal limits from an analytical point of view and options for changes of analytical requirements were evaluated.
In December 2018 EFSA published an opinion on risks to human health related to the presence of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in food. In comparison to the Health Based Guidance Values of EFSA of 2008, the new TWI for PFOS (13 ng/kg bw/week) is about a factor of 80 lower, for PFOA (6 ng/kg bw/week) about a factor of 1750. The analytical aspects and consequences are addressed.
A demanding task is also the analysis of short and medium chain chlorinated paraffins in feed and food. Part of these activities is the performance of international interlaboratory studies for a harmonized analytical approach.

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) and experts is gratefully acknowledged, including several core-working groups formed on specific tasks.*

LECTURES

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RESULTS OF EURL PROFICIENCY TESTS AND INTERLABORATORY STUDIES ON THE DETERMINATION OF VARIOUS HALOGENATED PERSISTENT ORGANIC POLLUTANTS IN FEED AND FOOD

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In 2018, the scope of the activities of the European Union (EU) Reference laboratory for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food (formerly EURL for Dioxins and PCBs in Feed and Food) was extended to other chlorinated, brominated and fluorinated persistent contaminants. As a consequence, also the number of covered groups of substances in EURL proficiency tests and interlaboratory studies increased considerably.
Since 2018 the EURL organized eight proficiency tests and interlaboratory studies on the determination of chlorinated, brominated and/or fluorinated persistent contaminants in feed and food. For some new analytes (in particular PBDEs and CPs) studies were already performed before 2018:
- PCDD/Fs, PCBs, PBDEs and HBCDDs in cod liver/fish oil (2014), liver of cattle (2017), soybean meal and beef (2018), grass and egg yolk powder (2019, ongoing)
- Dioxin-like compounds in egg oil by bioanalytical screening methods (2018)
- Perfluoroalkyl substances (PFCAs and PFSAs) in wheat flour (2019)
- Chlorinated paraffins (CPs) in coconut fat (2017), lard (2018), CP standards and pork (2019, ongoing)

For these PTs and studies naturally contaminated samples, e.g. from contamination incidents, were used as far as possible. If necessary, the test samples were additionally fortified with further relevant analytes of interest.
Between five and 91 participants reported results for PCDD/Fs, PCBs, PBDEs, HBCDDs, PFASs and CPs in the studies with highest participation rates for PCDD/Fs, PCBs, PBDEs and PFASs. In addition participating laboratories were asked to report the applied methods for sample preparation and quantification, in order to obtain possible further information on deviation of analytical results.
In general, participants' results showed a good agreement between the laboratories - not only for PCDD/Fs, PCBs, for which methods are well established and analytical criteria have been defined in EU regulations for a long time, but also for PBDEs, HBCDDs, PFASs and CPs. For most evaluated matrix/analyte combinations more than two thirds of participants' results were within the acceptable range for satisfactory performance of ± 2 z-scores. However, certain analytical aspects, whether matrix or analyte specific, show still room for improvement of analytical methods for better comparability of analytical results. For some new parameters, in particular CPs, improvement could be observed for the latest PT compared to the first one, for others, e.g. PBDEs, the performance level was constantly high from the beginning.
The EURL for Halogenated POPs in Feed and Food is currently working on the harmonization of analytical approaches and the establishment of certain analytical criteria for brominated flame retardants, perfluoroalkyl substances and chlorinated paraffins.

Keywords: *Proficiency test, Dioxins and PCBs, Brominated flame retardants, PFAS, Chlorinated paraffins*

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) and experts is gratefully acknowledged, including several core working groups formed on specific tasks.*

LECTURES

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NON-CONSERVATIVE ANALYTICAL METHODOLOGIES. THE HALLMARK OF THE EURL NETWORK FOR PESTICIDE RESIDUES

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The longstanding approach to incorporate and evolve both new and well-established analytical methods has been a constant ethos for European official laboratories, working under the EURL umbrella. The benefits of such an approach have been successful in increasing the scope of analysis, decreasing the analytical throughputs and generally reporting more reliable results.
Examples of this method evolution can be seen in the Acetone and Ethyl acetate-based extraction procedures, now commonly referred to as the miniDutch and SWEET methods, respectively. Likewise, the introduction of the modular QuEChERs approach has exemplified this overall performance improvement.
The introduction of improved mass spectrometric platforms, like high sensitive triple quadrupole, has increased sensitivity by 5-10 times and facilitated a reduction in sample injection amounts for each analysis thus avoiding interferences or matrix effects.
High-resolution Mass Spectrometry has had a significant impact in the area of pesticide residue analysis, the EURLs have partially consolidated this method, implementing it into the laboratory regular activities, allowing them a clear vision as to how they can attain still better results.
More recently, well-known chromatographic systems, such as ion chromatography and supercritical fluid, have been considered relevant for the routine control of pesticides. These are being evaluated and are set to offer new possibilities not discovered until now.
During this presentation, we will present examples of these new activities, some of them consolidated in EU routine laboratories, and will look forward to see what the near future may hold.

Keywords: *pesticides, extraction methods, mass spectrometry, high resolution, chromatography*

LECTURES

L38

FAO/IAEA FOOD AUTHENTICITY RESEARCH – SOME RESULTS IN THE FIELD AND FUTURE DIRECTIONS

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Over the past decade, the Food and Environmental Protection Laboratory (FEPL) of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture has been involved in assisting FAO and IAEA Member States in response to increased demands to improve their capabilities to deal with issues such as food fraud and food authenticity. Research has been carried out in the Joint Division's laboratories and through coordinated research with laboratories in Member States on analytical methods for food authentication, with the goal of developing methods that can be incorporated into food control systems to strengthen food safety and security and protect consumers and industry from health risks and fraudulent practices.

A number of analytical approaches have been developed under this research strand at IAEA and in collaboration with other projects focusing on various aspects of food integrity, for example those funded by the EU. Until recently, however, it has been somewhat difficult to translate the results of the research into actual applications in the food supply chain and to visualise their impact. In addition to capacity building in terms of the development of analytical methods, quality control materials and human resource expertise for authenticity testing, the work of FEPL in this field has recently shown significant impact in several countries. These impacts include the integration of nuclear, isotopic and related methodology into food control systems, food quality schemes and regulatory standards; bringing food testing capabilities closer to the field with rapid and simple screening methods; forging of important links between research and industry with respect to food authenticity and traceability of geographical origin; building awareness of the issues related to food fraud and the role of nuclear techniques in controlling the problems, and gathering 'baseline' data on food fraud and its control to facilitate targeted research and capacity building. Some examples will be elaborated in this presentation. An overview will also be given of current and future coordinated research focusing on the implementation of nuclear techniques for the authentication of foods with high-value labelling claims.

Keywords: *food authenticity, analytical methods, food control systems, impacts*

LECTURES

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FOOD ANALYSIS FOR FOOD INTEGRITY FOR FOOD INDUSTRY

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European foods are under constant threat of frauds & adulterations and the consumer does expect to buy products of which safety, quality and authenticity are assured: these parallel aspects represent a big challenge for the industry. For this reason, the demand of both rapid and confirmatory methods able to detect different frauds increased in these years and several scientific publications are focused on this topic. The academic and the production worlds are still not largely connected: several methods presented in literature are not applied (or even not known) in the plants, also because some "academic suggestions" are not applicable in the routine analyses. These two entities should continue to talk, in order to develop more sophisticated but also robust techniques that could merge the scientific novelty with the reliable application in the production plants.
The industrial partnership and collaboration in the recently completed European funded FoodIntegrity (FI) project have permitted to fully understand the opportunities that this multi-faceted initiative have presented to food businesses, contributing also to compare the perspective of food chain vulnerabilities vs current analytical methods and technologies.
Among practical instruments that help food industries into assuring the integrity of their products, FI provided online resources such as FI Knowledge Base & FI Network (simplifying, the first one to find the right analytical strategies to use; the second to suggest which organizations/authorities in different countries have the right expertise for performing them). Whether there is the need to defend the quality of food products against frauds by legal means, stakeholders can rely on FI scientific opinions that can be used as a reliable point of reference. A selection/evaluation of relevant chemical/molecular-biology markers, which can be connected to specific quality/authenticity aspects of both raw materials/ingredients and corresponding finished products has been created. Testing and validation of: (i) rapid screening high-throughput technologies & multivariate approaches, (ii) profiling/fingerprinting/ targeted or non-targeted fundamental methods have been executed.
A set of industrial guidelines, which describe the procedures that any company can adopt to prevent and counteract frauds through the whole supply chain, has been concretized. Dissemination activities and training to researchers and industrial operators has been put in place from a cross-disciplinary point of view which brings together analytical chemistry, quality management, marketing, consumer and economic science.
Protecting the authenticity is more than a legal obligation. It is about offering a guarantee of safety, quality, health and taste; preserving the trade, tradition and food culture of a company, giving to the correspondent food products more economic and ethical value and therefore strengthening the trust that consumers place in food industry itself.

Keywords: *food analysis, food integrity, food industry*

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LECTURES

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A REALISTIC AND FAST METHOD FOR EFFICACY ASSESSMENT OF NATURAL ANTI-OXIDANTS IN THE INHIBITION OF LIPID OXIDATION IN DRY FOODS

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As known for centuries, drying is a highly efficient method for preserving nutritional quality and flavor of vegetables, spices and herbs. It is for this reason that dried soups and sauces are important products for the preparation of nutritious meals in the kitchen. Many of these dry powdered products contain low levels of fat, for a better flavor experience, a better mouthfeel, or to avoid dusting in the factory or the kitchen. For health reasons these fats are high in unsaturated lipids. Unfortunately, unsaturated fats are susceptible to lipid oxidation and the addition of natural antioxidants such as e.g. rosemary extracts etc. is needed. To assess the efficacy of natural oxidation inhibition strategies, fast, yet realistic and objective methods are needed to study oxidation inhibition. Here we propose a model system for dry food that can be used to test anti-oxidants under realistic conditions. This also includes a very fast chromatographic measurement method to study the degree of oxidation with- and without added antioxidant.
Oxidized lipids have a characteristic rancid smell for which the human nose is very sensitive. Although this rancid smell can be caused by many compounds, for the most widely used lipids hexanal is one of the main causative molecules. Hexanal development over time usually follows an exponential curve. In the incubation period hexanal levels are low, whereas after an onset point they increase rapidly. The extent to which this onset point is delayed is a measure for the efficacy of an antioxidant. Here we propose a model system consisting of salt and sunflower oil as a mimic of dry powdered soups or sauces. A very fast GC-MS method is described that allows measuring hexanal levels in the model system within 2 minutes. The application of the new strategy to assess lipid-oxidation deceleration factors of novel, natural anti-oxidants is demonstrated.

Keywords: *lipid oxidation, off-flavour, anti-oxidants, fast GC-MS*

LECTURES

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A TRAPPING APPROACH TO PREVENT THE FORMATION OF MONOCHLOROPROPANEDIOLS IN VEGETABLE OILS

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Monochloropropanediol fatty acid esters (MCPDEs) are a family of process contaminants found in most refined edible oils.
This study presents the first results on depleting certain chlorine carrying substances from vegetable oils without the use of any solvent in order to mitigate MCPDEs. The concept is based on separating the chlorine carriers from the oil by using trapping agents (e.g. monoacylglycerols) that can be easily separated from the oil. The process starts by mixing and homogenizing crude vegetable oils with the trapping agent and subsequently separating the trapping agent from the oil bulk via crystallization. The approach is demonstrated on a solvent extracted crude sunflower oil, industrially produced crude soybean and corn oils. The depletion of chlorine carriers in the crude oils and its beneficial effect on the MCPDE content in the heat treated samples is measured by LC-MS and by AOCS Official Methods Cd 29a,b,c-13.
The depletion efficacy of the monitored chlorine carriers was estimated to be in the 60-95% range. Both the melting point and polarity of the trapping agents affected their depletion efficacy. Trapping agents with higher melting point and high polarity, such as monostearin were more effective in comparison to high melting point but less polar agents such as palm stearin or agents rich in polar but low melting point monolinolein/ monoolein. The benefit of depleting the chlorine carrier on the subsequent MPCDE levels in heat treated oil was in the range of 60–90 % reduction depending on the type of the studied oil.
These lab-scale results suggest that early, solvent free removal of potential source of chlorine from the vegetable oils is possible and this has a beneficial effect on the ultimately formed MCPDE content of the oils.

LECTURES

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ANALYSIS OF THE COMPOSITION AND HEAT DAMAGE OF MILK-DERIVED WHEY PROTEIN INGREDIENTS USING QUANTITATIVE PROTEOME ANALYSIS (QPA)

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Bovine milk and dairy products have been part of the human diet for thousands of years and are of fundamental physiological importance for human nutrition. Especially the whey proteins are known for their high nutritional and biological value, their good digestibility, sensory characteristics and therefore are commonly used in specialized nutrition, e.g. sport and dietary supplements. Depending on the raw material and the production process, commercial ingredients, such as Whey Protein Concentrates (WPC) and Whey Protein Isolates (WPI), differ in composition, physical properties and functionality. The protein composition and the processing-induced structural and conformational changes of dairy ingredients are important parameters for product development because they determine the technological functionality of the ingredient and the physical, sensorial and nutritional properties of the final product.
The aim of the present project was to develop a quantitative proteome analysis (QPA) to analyze in parallel the protein composition and heat damage of whey protein ingredients derived from milk. With this approach, it was possible to achieve simultaneous quantification of the two major whey proteins α-lactalbumin and β-lactoglobulin, as well as the three caseins α_{S1}-casein, α_{S2}-casein and β-casein. The whey products were specifically hydrolyzed by the endoprotease Glu-C, and proteotypic peptides for each protein were quantified by microLC-ESI-MS/MS in sMRM mode. Marker peptides were selected using several selection criteria including selectivity and signal intensity. Absolute quantification can be achieved by stable isotope labeled (SIL) peptides.
Apart from the protein composition, heat damage of proteins is a second important parameter influencing the quality of whey products, since the industrial processing can cause a broad variety of non-enzymatic post-translational protein modifications (nePTMs). Therefore, it was our second aim to investigate the effect of the applied heat treatment on the major whey protein β-lactoglobulin in the dairy ingredients by a targeted nePTM profiling using microLC-ESI-MS/MS. Thus, the sMRM method was extended to include nePTM-modified peptides with 15 different heat induced nePTMs located at 18 binding sites. The modifications include early and advanced Maillard reaction-, oxidation-, condensation- and hydrolysis products. Relative quantification was achieved using a stable internal
Referencepeptide.
The advantage of quantitative proteome analysis is that the method can be easily extended to include other protein components and various protein modifications. This allows the comprehensive quality analysis of milk products in one run.

Keywords: *milk proteins, quantitative proteome analysis (QPA), PTM profiling, mass spectrometry, milk-derived whey protein ingredients*

LECTURES

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INVESTIGATION OF THE IMPACT OF PULSED ELECTRIC FIELD (PEF) ON BIOACTIVE COMPOUNDS IN CARROT

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Thermal treatment represents a traditional way to primarily extend the shelf life of food (elimination of microbiological spoilage, avoiding undesirable enzymatic reactions) but also to make food more sensory attractive and, in some cases, digestibility is improved. However, due to high thermal loads, some sensitive bioactive compounds of high-nutritional value, such as vitamins, flavonoids, carotenoids etc., can be lost. To avoid these negative impacts, Pulsed Electric Field (PEF), a mild non-thermal processing technology, might be employed as an alternative to conventional processing. PEF is based on application of high electrical pulses that cause irreversible disintegration of cell membranes thus killing microorganisms. In addition, under such conditions, disruption of plant cells also occurs and results in a release of their content. In case of carrot (*Daucus carota*), both softening of its texture and an increase of juice yield can be expected.
As limited knowledge is available on the impact of PEF treatment on bioactive compounds in carrot, non-target screening of aqueous methanolic extracts was conducted applying ultra-high performance liquid chromatography coupled to high-resolution mass spectrometry (U-HPLC-HRMS/MS). Metabolomic fingerprints were obtained also of fresh and conventionally processed carrot; the data were processed by multivariate statistics. To visualise possible changes of bioactive compounds induced by PEF, profiles of phenolic compounds were extracted from primary data using an established database. Similarly, carotenoids were recorded using HPLC-DAD. The suitability of employed analytical strategies for novel food technologies assessment will be discussed.

Keywords: *PEF, carrot, polyphenolic compounds and carotenoids, ultra-high performance liquid chromatography, high resolution tandem mass spectrometry*

Acknowledgement: *Project "Volumetric preservation technologies for food quality improvement by retention of sensitive and mitigation of neoformed compounds (volTECH)", supported by CORNET (Collective Research Networking) and funded in the Czech Republic within the national Operational program Enterprise and Innovations for Competitiveness, support programme Cooperation - Clusters (CZ.01.1.02/0.0/0.0/16_079/0008876). Special acknowledgement goes to Vitave Tech, s.r.o., Modeltice, Czech Republic for providing the PEF equipment.*

LECTURES

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FOOD SAFETY CONTROL SYSTEM IN CHINA: PAST, PRESENT AND FUTURE

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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 evolution in China national food safety control system and nationwide progresses in 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 risk management 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. 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.

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

Acknowledgement: *The National Key R&D Program of China under Grant Agreement No 2017YFC1601502 and EU Horizon 2020 Research and Innovation Programme under Grant Agreement No 727864 EU-China-Safe*

LECTURES

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H2020 EU-CHINA SAFE PROJECT PROGRESS: DELIVERING AN EFFECTIVE, RESILIENT AND SUSTAINABLE EU-CHINA FOOD SAFETY PARTNERSHIP

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EU-China-Safe will mobilise resources in Europe and China to develop a cohesive partnership that will deliver a shared vision for food safety and authenticity and work towards "mutual recognition". Comprising 15 participants from the EU and 18 from China, EU-China-Safe contains key research organisations, Government and industry needed to develop and jointly implement major advances in improving food safety and combating food fraud in the two trading blocks.
EU-China-Safe will build the core components needed for a joint EU-China food safety control system comprising: control management, food legislation, food inspection, food control laboratories, and food safety and quality information, education and communication. The project will develop an EU-China Joint Laboratory Network that will achieve and demonstrate equivalency of results, and will develop a state of the art virtual laboratory, with interchangeable staff from two continents, that will be used as a "showcase" to communicate and demonstrate best practice. Innovative traceability tools will strengthen the most vulnerable supply chains. New or improved detection capabilities for chemical/microbiological hazards and food fraud will be implemented in a harmonised way across the EU-China network. Trade barriers caused by food safety and fraud issues will be analysed and recommendations of how to predict and prevent future events disseminated. The project will focus on the most commonly reported foods linked to chemical and microbiological contamination and fraud (infant formula, processed meat, fruits, vegetables, wine, honey, spices). Substantial knowledge transfers and training actions will build high-level and long-term collaboration, synergies and trust between a wide range of EU and China actors.
These advances, in addition to a wider range of confidence building measures towards food safety, authenticity and transparency, will address consumer expectations and facilitate an expansion of EU- China trade.

Keywords: *EU-China-Safe, food safety, authenticity, transparency, EU-China joint laboratory network*

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LECTURES

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DESIGN AND IMPLEMENTATION OF FOOD COLD CHAIN TRACEABILITY SYSTEM BASED ON BLOCKCHAIN AND RFID

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Food cold chain traceability is a supply chain with strict temperature control. The food cold chain is a combination of multiple industries and departments. The cold chain information comes from food production and processing enterprises, logistics and distribution enterprises, and supermarket retailers. Food needs a unique physical identification at the source, and the information of each enterprise in the food cold chain is written to the physical identification to achieve seamless docking and anti-counterfeiting, to guarantee that the traceability system information is credible and traceable. Based on blockchain and RFID-related technologies, this paper discusses the functions and features of the food cold chain traceability system and proposes technical design and implementation methods.

Keywords: *food safety, blockchain, RFID, cold chain, traceability*

LECTURES

L47

LC-MS TOOLS IN THE CAMPAIGN AGAINST FOOD FRAUD IN INFANT FORMULA

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Constantly growing of globalization and expansion of international food supply chains have raised unprecedented challenges in the new millennium. The melamine scandal exploded in 2008, was a catastrophe to Chinese local dairy industry which aroused public awareness toward food integrity. The compound itself is rich in nitrogen, thus became a perfect candidate to cheat existing GB testing standard at the time for protein in infant formula via mono Kjeldahl method. At the meantime, new terms of adulterations were found by replacing whey proteins with cheap bovine milk caseins and plant sourced (Soy proteins as example)/hydrolyzed protein (Not for special clinical use) which might carry potential risks. Although a series of well-developed method specifically targeting melamine was established even at Ultra-trace levels, it's vulnerability against other N-rich and melamine-like compounds have placed every effort in jeopardy once again. Being one of state-of-the-art analytical tools, LC-MS made it possible for scientist to discover chemicals and proteins in food both qualitatively and quantitatively based on non-targeted screening/fingerprinting and targeted proteomic analysis. Our research has combined rapid LC-HRMS N-rich screening database with paralleled tryptic peptide measure by introducing stable isotope labeled signature peptide to minimize matrix effect of AUQA assay during ionization. In 2017, it has become as part of H2020 EU-China-Safe intergovernmental research program in implementation of developing food safety and authenticity network. Within the H2020 framework, we are able to share our experience with partners in E.U. and U.S. and the ultimate goal is to provide a harmonized all in one LC-MS solution for milk fraud discrimination andeventually a mutual recognized global standard to be applied in the whole supply chain.

Keywords: *infant formula, authenticity, LC-MS, targeted protein quantification, stable isotope dilution*

Acknowledgement: *National Key R&D Program of China 2017YFE0110800 and EU H2020 EU-China-Safe 727864*

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CREATING A MULTI-PARTNER EU-CHINA VIRTUAL LABORATORY FOR FOOD CONTROL AND INCIDENT RESPONSE

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The nature of current food production and distribution means that many food incidents are not confined to local geographical regions, but can become major international or global incidents. This has been the case in particular with dioxins and PCBs where several major incidents have occurred (Hoogenboom et al 2015)[1].
The EU funded EU-China-Safe project has a primary objective of cooperation and harmonization of food control between Europe and China. The development of a virtual laboratory, RL2020 is part of this project. RL2020 is a network of laboratories in Europe and China involved in the testing of food and feed, similar in purpose to the European Union Reference Laboratory network The ambition is to give scientists in both regions real time access to data generated in order to enable shared effort in both method development and validation, and also in analysis for food control via a web-based platform.
Analysis of dioxins using GC-MS/MS was chosen as the first example to assess the feasibility of RL2020. Data files are currently shared and stored using a secure area of the EU-China-Safe project website; http://www.euchinasafe.eu/ .
The next stages of the project will be to expand the number of participants, to develop a similar approach for other food chemicals, and to test the versatility for data generated using different instrumentation and software.
RL2020 has the potential to support global food control. Examples where it may be used include
- technology transfer
- training
- validation
- certification of reference materials etc
- analysis of samples for export, reducing times that products are held in customs
- incident and crisis management
- improvement of trust and mutual recognition

All of these applications have the potential to (1) improve food safety (2) reduce costs, and (3) improve response time.

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Keywords: *EU-China-Safe, virtual laboratory, food control, incidence response*

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LECTURES

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APPLICATION OF NEXT GENERATION SEQUENCING TECHNOLOGY IN FOOD AUTHENTICITY

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The assessment of food authenticity is a critical issue that has gained much interest internationally. The reliable identification of the species is of paramount importance for food authenticity. However, most of the existing methods are inappropriate for the identification of multiple species in unknown food products, especially highly processed products. Therefore, it is necessary to develop a high-throughput, high-accuracy and untargeted system for identifying the species of food products. This presentation provides a next generation sequencing (NGS) approach to identify animal species in mixed food products. Next generation sequencing of a short segment of the 16S ribosomal RNA (16S rRNA) mitochondrial gene was performed fo the authenticity of food products containing multiple species. By mixing different kinds of animal species according to different proportions, the mixed samples were prepared and the sequencing library was constructed. The sensitivity, accuracy and quantitative ability of NGS in species identification of mixed samples were evaluated. Although the relative abundance of reads obtained from each species could not make a quantitative assessment of the original species composition, this method still has the potential to the determination of high and low contents of animal species in mixed products. Subsequently, as an initial test, we performed a market survey to identify animal species in commercial food products using the developed NGS approach. Overall results demonstrated that the NGS approach could simultaneously identify all major species and impurity species and has high potential possible for application in routine analysis in the near future.
We are also testing the applicability of NGS to authenticate variously foodstuffs, such as spices, fish products, and mushroom. The latest results on the application of NGS for species identification in various food products will also be presented.

Keywords: *food authenticity, DNA metabarcoding, next generation sequencing (NGS), species identification, food mislabeling*

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LECTURES

L50

INTAKE OF DIOXINS AND DIOXIN-LIKE COMPOUNDS IN CHINA: OCCURRENCE AND TEMPORAL TREND

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Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental pollutants. The human exposure to PCDD/Fs and dl-PCBs as well as potential health risk is still a matter of great concern in the world. On 20 November 2018, the European Food Safety Authority (EFSA) published the Authority's first comprehensive review of the risks to human and animal health from dioxins and PCBs in food and feed. The panel set a new tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs in food of 2 pg/kg bw/week, which lead to a large exceedance of TWI in EU.

In China, dietary intakes of PCDD/Fs and dl-PCBs for general population were estimated from 3th, 4th and 5th Chinese total diet studies, in which a decline of the average dietary intake was observed from 2000 to 2011. The latest dietary intake of PCDD/Fs and dl-PCBs is estimated from 6th Chinese TDS conducted from 2017-2019. The national average intake and intakes in most of provinces keep the decline trend. However, a considerable intakes of PCDD/Fs and dl-PCBs from 6th Chinese TDS are still higher than the new TWI from EFSA.

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LECTURES

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EMERGING OF MONOPHASIC SALMONELLA ENTERICA *SEROTYPE TYPHIMURIUM* IN CHINA

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Background: Monophasic *Salmonella enterica* serotype Typhimurium is a worldwide emerging pathogen causing numerous food-borne outbreaks. While there is a good understanding of their genomic landscape in Europe and United States, such investigation has not yet been performed extensively for those originating from China.
Materials/methods: Whole genome sequencing was applied for multiple comparisons of 113 isolates from diarrhea patients in China, 2007 to 2017, and published data of 669 isolates from Japan, United States and European countries, were collected to assess their genetic heterogeneity. Resistance genes and mutations were identified and the carriage of resistance and virulence associated genes were then compared between isolates from different countries. The high quality single nucleotide polymorphisms were obtained and then the emerging time of the Chinese strains were estimated.
Results: Phylogenetic analysis revealed that multiple lineages of *S.* might be transmitted to China from Europe by the imported breeding pigs along with their original *S.* Typhimurium ancestors. The carriage rates of 14 resistance genes in Chinese isolates were extremely high, including *arr-3* (53.10%), *aadA* (53.09%), *aac(6')Ib-cr* (52.21%), bla_{OXA-1} (42.48%), etc, which were much higher than those in isolates from other countries (all less than 10%). The Chinese strains formed two main clades: C1(n=47) and C2 (n=43). C1 was predominantly intercontinental from Europe to China around 1995 (95% CI: 1991-2000) and C2 was around 2009 (95% CI: 2007-2012) . More resistance genes and mutations were accumulated in C1 than C2, most of which were harbored by multiple drug resistant plasmids highly similar with those in resistance bacteria isolated from China originated from the backbone of IncHI2 plasmids. These results indicated dramatically genetic variation for local adaptations of Chinese isolates by horizontal gene transfer.
Conclusions: The ancestor of this set of *S.* isolates occurred around 2007 and currently circulating in China are likely to be part of an emerging multidrug resistant clade first reported in Europe. It is likely that these events were facilitated by animal movement (e.g. breeding pigs). These findings will inform policy on action that is crucial to reduce further spread of *Salmonella* and other (emerging) Salmonella strains globally.

Keywords: *monophasic salmonella enterica serotype typhimurium emergence, emergence, whole genome sequence, antibiotic resistance*

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INTERNAL AND DIETARY EXPOSURE ASSESSMENT TO ZEARALENONE IN A TYPICAL AREA OF CHINA

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Zearalenone is a widespread mycotoxin with high estrogenic activity, which contaminates grains and also occurs in cereal products. This study aimed to characterize the exposure of ZEN in a Chinese population in Anhui province during harvest season. 199 healthy volunteers with age ranged from 4 to 80 years old participated in this study. Two approaches of exposure assessment were applied. One is the dietary approach based on 24 hour duplicate diet of staple food samples. From the participants staple food samples were collected and subsequently analyzed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). With the consumption data obtained by the food frequency questionnaire in this study, measured concentrations of food samples were used to estimate dietary intakes for individuals and for the population. The other approach is the human biomonitoring (HBM) method. As biomarkers of exposure, ZEN and its metabolites in urine samples were analyzed using HPLC-MS/MS before and after enzymatic hydrolysis. Therefore both their concentrations of free and conjugated form were obtained, also the human metabolism pattern of ZEN among populations were investigated. We will discuss the detailed results of exposure assessment to ZEN via these two approaches in this presentation, including the estimated intakes of the cohort comparing to the EFSA's TDI and their age- and gender-related differences. The relationship between the internal and the external exposure will also be discussed.

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LECTURES

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RESEARCH SCOPE OF HIGHLY SENSITIVE IMMUNE-DETECTION OF AFLATOXIN IN PEANUTS

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Aflatoxin is the most toxic and the most potent carcinogen in humans. Food and feed contamination by aflatoxins are a significant food safety issue in the developing and developed countries because of lack of detection, monitoring and regulating measures to safe guard the food supply. The surveillance system for monitoring of aflatoxins in peanuts would allow better risk assessment of aflatoxin occurrence and better decisions to be taken at earlier stages in the supply chain. But, the establishment of the system must base on the fast and highly sensitive detection of aflatoxins. On the one side, the research group has the rich experience for the development of detection strategies, and has successfully developed fast detection techniques against varied mycotoxins and residue pesticides in the food based on the conventional antibody, which could guarantee the successful development of detection technologies based on conventional monoclonal antibody. On the other side, the group has also performed antigen preparation, immunization and selection of specific Nbs, as well as the characterization of their biochemical properties. The utilization of Nbs for the quantitative detection of Aflatoxin will extend the research scope of the lab for the qualitative and quantitative technologies of the mycotoxin detection in peanuts.

Keywords: *peanut, aflatoxin, immune-detection, monoclonal antibody, nanobody*

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LECTURES

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ANALYSIS HAZARDS IN FOOD: FROM ONE-BY-ONE DETERMINATION TO CLASS-BY CLASS SCREENING AND FINALLY TO CHEMOMETRICS-BASED DISCRIMINATION

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The risky chemical substances in food include pesticide residues, veterinary drug residues, mycotoxins, persistent organic pollutants and illegal additives. At present, there are a lot of reports about the detection methods of the above-mentioned hazards. However, in order to escape the routine detection, the abuse of structural analogues with similar property to risky substances has become a trend, while the discovery and detection of unknown structural analogues is still a huge challenge in food safety science and technology due to the diversity of similar species.
In recent years, we used the mass spectrometry with soft ionization technique to reveal fragmentation mechanism of 12 different structural chemical hazards such as phenylethanolamine. It is found that compounds with the same skeleton structure have the same fragmentation pathway. Inspired from this discovery, exploitation method based on "mass spectrometry fragmentation markers" has been developed, which can solve the problem of difficult detection with new structural derivatives. And thus achieved the detection of harmful substances from one-by-one determination to class-by class screening.
Although the technologies mentioned above solve the problem of screening for new structural derivatives, they cannot screening the potential risks caused by non-standard food processing. In the process of food production, processing, transportation and storage, non-standard operation can cause food safety problems and produce harmful substances. The detection of these trace harmful substances is tantamount to finding a needle in a haystack. In this work, a holographic discrimination technology was developed for screening potential risks in hot-processed milk. By simulating the non-standard processing of raw milk, using multi-dimensional and multi-mode "holographic" analysis techniques such as chromatography, mass spectrometry, spectroscopy, combing with statistical methods such as chemometrics, five high-risk markers of advanced glycation end products were screened out and a safety identification model for hot-processed milk was constructed, which can realize the "holographic" discrimination of risk milk. Thus, a technological breakthrough of food safety can be realized from one-by-one determination to class-by-class screening and finally to chemometrics-based discrimination.

Keywords: *food analysis, determination, screening, discrimination*

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LECTURES

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MULTI-PLUG FILTRATION CLEAN-UP (M-PFC) METHOD AND AUTOMATED DEVICE FOR ANALYSIS OF PESTICIDE AND VETERINARY DRUG RESIDUES

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A novel design of rapid multi-plug filtration clean-up (m-PFC) method was developed for analysis of pesticide and veterinary drug residues in various matrices of agricultural products. Based on QuEChERS extraction, liquid-liquid partition and salting-out procedure, the supernatant was cleaned up by m-PFC columns packed with various sorbents. Multi-walled carbon nano-tubes (MWCNTs) were used as an alternative material in pesticide residue analysis for its adsorption specificity. It intended to adsorb the interfering substances in the matrix, rather than the analytes. Using m-PFC columns was shown to be a more practical way to perform the d-SPE cleanup. The m-PFC method was very rapid, which took just one minute to perform without any solvent evaporation, vortex or centrifugation. Moreover, m-PFC increased the contact time and surface area between extracts and sorbents, which provided lower RSDs and LOQs than d-SPE. Because of the better cleanup performance, m-PFC could lower the matrix effects. Recently, an automated m-PFC cleanup device was developed. The cleanup process was helpful to reduce the workload in sample preparation. In automated m-PFC cleanup method, the parameters of m-PFC cycles, volume and speed could be optimized separately to obtain the best recovery and cleanup performance. Automated m-PFC methods based on QuEChERS method were widely used in both relatively simple and complex matrices. It was labor-saving and easy to be operated. The automation of the m-PFC method could significantly improve method accuracy and robustness. Applications of this method on various tea samples, vegetables, fruits and animal stuffs will be discussed in the presentation.

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LECTURES

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RAPID FOOD ANALYSIS BY AMBIENT MASS SPECTROMETRY

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Ambient mass spectrometry involves the direct sampling and ionization of analytes from samples under ambient conditions and requires minimal or no sample pretreatment. While the ionization process in mass-spectrometric analyses was traditionally performed in vacuo inside the mass spectrometer, the advent of differentially pumped interfaces and atmospheric pressure ionization (API) methods allowed the ionization step to be performed at atmospheric pressure, greatly simplifying sample introduction. Food and related products are complex matrix and its rapid analysis has been challenging. In this talking, we have introduced some applications for food safety analysis, such as by different ionization sources (paper spray, DART or DCBI etc) and different food products (beverage or food packaging material) or chemical containments (food additives).
For example, because thirdhand smoke (THS) components have properties of remaining, remitting and reaction on surfaces, in-situ analysis of the components on different surfaces has been significant and challenging for the THS researches. in-situ DCBI-MS/MS quantitative analysis of typical THS environmental markers on different surfaces such as fruit, clothing, glass, and toy etc. was developed. It was also applied to direct detection of THS on finger without any body damages. In addition, formation of tobacco-specific nitrosamines (TSNAs) such as NNA, NNK, and NNN, was in-situ characterized by DCBI-MS/MS successfully. A PS-MS method was applied for the rapid in situ screening and simultaneous quantitative analysis of bisphenol A and its analogues, i.e., bisphenol S, bisphenol F, and bisphenol AF, in food packaging products. The calibration curves of bisphenols in the range of 1−100 μg/mL were linear. The correlation coefficients were higher than 0.998. The samples were analyzed by PS-MS in situ for rapid screening without a traditional sample pretreatment procedure. The analytical time of the PS-MS method was less than 1 min. In comparison with conventional HPLC-MS/MS, it was demonstrated that PS-MS was a more effective high-throughput screening and quantitative analysis method.
A high temperature desorption (HTD) direct analysis in real time−high-resolution mass spectrometric (DART-HRMS) method was developed for the rapid analysis of four banned cationic dyes. Rhodamine B is used to dye foods, while malachite green, crystal violet, and methylene blue are added to fishponds as antimicrobials. A simple induced phase separation extraction was used to pretreat samples. The DART-HRMS method employed two temperature steps, i.e., 200 °C for drying, purification, and enrichment of sample solution and 500 °C for thermal desorption and ionization of analytes. The calibration curves of dyes in the range of 50−2000 ng/mL were linear using deuterated malachite green as an internal standard. The LODs vary for all analytes between 0.1 and 30 ppb depending on the matrix and experimental conditions.

Keywords: *ambient mass spectrometry, rapid analysis, DART, DCBI, paper spray*

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LECTURES

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FILLING THE KNOWLEDGE GAPS TO MANAGE THE CHALLENGES RELATED TO MICROPLASTICS IN THE ENVIRONMENT AND FOOD

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The benefits of plastics in society are unquestionable. However, there is an urgent need to better manage their value chain. The European Strategy for Plastics adopted in 2018 stressed the need to tackle the challenges related to plastics, especially with a focus on the end of life of plastic products. In addition to plastic litter, tiny plastic fragments, the so-called microplastics have become a major concern as they have been detected mainly in the marine environment, but can also be present in freshwater, soil and air, and – based on today's knowledge - to a limited extent in food products. It has become increasingly apparent that a broad and systemic approach is required to achieve sustainable actions and solutions along the entire supply chain. It needs to be stressed that, although the number of research projects is increasing, there is still a lack of suitable and validated analytical methods for detection and quantification of microplastics, as well as a lack of hazard and fate data which would allow for their risk assessment.
This presentation will present the outcome of recent expert discussions and literature reviews and will propose a way forward to tackle the challenges.

Keywords: *microplastics, Food supply chain, Analytical methods, Risk assessment*

LECTURES

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DRUGS IN OUR VEGETABLES? UPTAKE AND METABOLIZATION OF EMERGING CONTAMINANTS BY PLANTS UPON IRRIGATION WITH RECLAIMED WATER

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Pharmaceuticals are widely used in human and in veterinary medicine and prescriptions are increasing continuously. As a negative side-effect of this development pharmaceutically active ingredients (AI´s) can be found in the aquatic system nearly everywhere. Although contaminated municipal wastewater is treated in wastewater treatment plants (WTP), due to their stability many of these substances are unaffected by the treatment process – so WTP-effluents can still be contaminated with AI´s in the ng L^{-1} to the µg L^{-1} range [1]. Population growth requires an increase in the production of food and feed - climate change results in increasing water scarcity – so waters from WTPs are more and more used for irrigation in agriculture. Thereby plants can come into direct contact with the AI´s, leading to uptake, translocation and metabolization of the drug, a fact particularly of concern in the case of edible plants [1].
We investigated the uptake of three classes of widely used pharmaceuticals - non-steroidal anti-inflammatory drugs [2], tricyclic antidepressants [3] and statins [4] by garden cress, onion, lettuce, pea, radish, and maize. A special focus was set on the transformation of the AI´s by the plant and the identification of metabolites either already formed in the aquatic environment and taken up by the plant or formed within the plant organism. Plants were cultivated in Petri dishes in the presence of drug containing water. After harvesting plants were extracted and analyzed by a RP-HPLC and high-resolution mass spectrometry (HR-MS). For most of the drugs studied, a series of metabolites was identified. Phase one metabolites formed by hydroxylation or oxidation of the parent drug were found together with a series of phase two metabolites (conjugates with glucose, different amino acids and malonic acid). From QTOF MS^2 experiments, specific fragment ions were selected for each metabolite for establishing a highly sensitive multiple reaction monitoring (MRM) method on a QqQ MS instrument allowing estimating the formation of metabolites also in plants treated with drugs at environmentally relevant concentrations. Also the distribution of the parent drugs and the metabolites in different plant parts was investigated as well as the formation kinetics of the metabolites.

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

LECTURES

L59

CAN MINING FOR DEVIANT SIGNALS IN HRMS FULL-SCAN DATA REVEAL TOMORROW'S FOOD CONTAMINANTS?

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Many current methods, referred to as "non-target" screening methods for contaminants, can detect as many compounds as *e.g.* 400 in water [1], 600 in food [2], or over 1600 for forensic screening [3]. They are based on TOF or Orbitrap technology (HRMS) and the detection is thereby indeed non-targeted.
However, the data evaluation is still of a more targeted approach. It relies on predefined compound lists or databases, but although these may contain thousands of contaminants, the methodology will inevitably still miss all other possible contaminants.
There are currently over 50 million compounds in ChemSpider, and no database, despite its size, will contain tomorrow's chemicals. Thus, the gap is several orders of magnitude to the number of compounds included in the methods mentioned above. A metabolomics based (true) non-targeted approach might at least partially fill this gap.
This approach makes use of Reference samples and univariate statistics of the HRMS signals in order to detect unique or deviating compounds in the particular sample(s) under investigation. Such differential analysis methods were initially developed at our laboratory in order to be used for investigations in crisis situations, *e.g.* a severe intoxication – cases that normally would require at least ppm levels of toxic compounds. However, obtained detection capabilities at ppb levels suggest the method approach can be used also for contaminants at concentrations well below acute toxic levels.
Main drawback of the approach is the high workload, especially when the chemical identity of the compound behind any obtained MS signal need to be confirmed. Nevertheless, the approach is already in routine use in the surveillance of raw water used for drinking water production (*e.g.* in Germany, [4,5]). Results from tests at our laboratory on juice and milk – bearing the melamine scandal in mind – indicate that the difficulties for more complex drinks and foods may not be greater than for water.

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Keywords: *HRMS, food contaminants, non-target analysis*

LECTURES

L60

THE SEPARATION OF ADVANCED GLYCATION ENDPRODUCTS (AGE) ISOMERS MG-H1, 2 AND 3 AND GH-1, 2 AND 3 IN FOOD MATRICES

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Advanced Glycation Endproducts (AGEs) are Maillard Reaction Products (MRPs) that are generated from amino acids (lysine, ornithine) and reducing sugars during high-heat treatment of foods. Several studies showed that AGEs are present in a wide range of foods, including dairy products, baked and fried products (bakery products, fries and crisps), chocolate products and meats. In recent years, analytical methods focussed primarily on the detection of Nε-(carboxymethyl)lysine (CML), Nε-(1-carboxyethyl)lysine (CEL), using ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Recently, the attention towards Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) and glyoxal-derived hydroimidasolone (G-H1) is increasing. Studies reported on these compounds in foods, but while we investigated these compounds, we noticed that the accuracy of the method may be compromised by co-elution of G-H2 and G-H3 and MG-H2 and 3. As G-H1, 2 and 3 isomers provide same precursor- and fragment ions (of MRM transitions), selectivity by MS/MS alone is insufficient. The same holds for MG-H1, 2 and 3 isomers. Ion mobility may provide the required additional resolution, but we initially focussed on testing several chromatographic separations, using different stationary phases (C18 and HILIC based), and elution parameters (gradient, isocratic), focussing on the separation of these 6 isomers. The elution on a hydrophilic interaction liquid chromatography (HILIC) column using isocratic elution provided the best separation. Near-baseline separation was achieved for the G-H1, G-H2 and G-H3 isomers. MG-H1 was baseline separated from MG-H2 and -3, but the latter 2 isomers were not (yet) completely resolved. Future work will focus on further optimisations.
We have applied this approach to fried potato products and demonstrate that these isomers are present in fried potato products, combined with other AGEs like CML and CEL. Based on our work, we suggest that laboratories to adapt their analytical methods in order to accommodate for the separation of these isomers. This will allow a more accurate reporting of AGEs in various food products and will support risk assessment in the future. Moreover, this enables deeper insights in the occurrence and formation of various MG-Hx and G-Hx isomers, which will support risk assessment on these processing contaminants in the future.

Keywords: *advanced glycation endproducts (AGE), processing contaminants, frying, MG-H1, G-H1*

Acknowledgement: *The Dutch Ministry of Agriculture, Nature and Food Quality is gratefully acknowledged for funding this project (WOT-02-002-004)*

LECTURES

L61

SIMULTANEOUS QUANTIFICATION OF FURAN, 2- & 3-METHYLFURANS AND THREE ADDITIONAL ALKYLFURANS IN VARIOUS FOOD COMMODITIES BY GC/MS

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Furan is a low molecular weight and high volatile chemical known to be present in heat-treated foods [1], particularly in canned or jarred products because of impossibility of losses by vaporization. Furan was classified as possibly carcinogenic for humans by the International Agency for Research on Cancer (group 2B) [2]. Alkylfurans such as 2- and 3-methylfuran, 2-ethylfuran, 2,5-dimethylfuran, and 2-pentylfuran have been found concurrently with furan in different foods and beverages, and they are likely to exhibit also a toxicological potential [3]. The Standing Committee on plants, animals, food and feed of the European Commission, recommends monitoring furan and alkylfurans, although they acknowledge the lack of reliable methods for the determination of 2,5-dimethylfuran, 2-ethylfuran and 2-pentylfuran [3].
We present the development and validation of a reliable analytical methodology for the simultaneous quantification of furan 2- and 3-methylfurans, 2-ethylfuran, 2,5-dimethylfuran, and 2-pentylfuran by gas chromatography and single quadrupole mass spectrometry detection. Two techniques were evaluated for injection: Static Head Space (HS) and Solid Phase Micro-Extraction (SPME). Isotope dilution was the approach applied for quantification. Key points during method development were the selection of a proper column and temperature gradient for separation of isobaric 2,5-diemthylfuran and 2-ethylfuran, and the selection of proper internal standards, especially for 2-pentylfuran. The method has been fully validated using both HS and SPME in i) apple/raspberry based baby food, ii) semolina/vegetables/chicken based baby food, iii) wheat based cereals for infants and iv) roasted and ground coffee. Validated LOQs for baby food and infant cereals was 5 µg/kg, and 200 µg/kg for coffee. Recoveries were in the range 80 – 110 % with RSD lower than 20 %. The performance of the method was further checked in 21 other different matrices belonging to the same commodity groups.

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[2] Furan: IARC Monographs on the evaluation of carcinogenic risks to humans 63 (1995) 393-407.
[3] Summary report of the standing committee on plants, animals, food and feed held in Brussels on 08 February 2019, European Commission, Health and Food Safety Directorate General: sante.ddg2.g.5(2019)1319051.

Keywords: *furan, alkylfurans, process contaminants, head space*

LECTURES

L62

NON-TARGETED ANALYSIS OF THE DEGRADATION OF FOUR PLASTIC-RELATED CONTAMINANTS IN FOOD DURING THERMAL TREATMENT

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Recently, several plastic-related contaminants have been associated with public health concerns. For example, bisphenol A (BPA) and diethylhexyl phthalate (DEHP) have been suggested as critical endocrine disrupters. In the present study, the thermal degradation of four plastic-related contaminants (BPA, bisphenol S (BPS), DEHP and diethylhexyl adipate (DEHA)) was investigated in water (model matrix) and fish muscle (real food). Contaminant residues were extracted from fish muscles using ultrasound assisted solvent extraction. All the samples were analyzed by high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry (HPLC-QTOF-MS). The targeted analysis of the parent compound allowed for the assessment of the degradation percentage. In addition, a non-targeted approach was conducted to identify unknown or unexpected degradation products. Good instrumental linearity (r^2>0.998) and method recoveries (86-120%) were achieved for all the four target compounds. The limit of detection for the test compounds ranged from 0.1 to 0.6 ng/g. DEHA showed the highest degradation percentage during heating, as boiling for 1 hr induced about 80% degradation in both water and fish. The concentration of DEHP was reduced by about 40% and 25% under the same conditions in water and fish, respectively. BPA and BPS did not degrade in water (less than 1% degradation) but degraded in fish matrix (about 35% degradation in fish for both BPA and BPS). Their thermal degradation products were identified and compared between in water and in fish. To the best of our knowledge, this is the first study reporting the thermal degradation of plastic-related contaminants in food using a non-targeted approach. The present results will contribute to refine current food safety risk assessments.

Keywords: *plastic-related contaminants, thermal degradation, bisphenol, phthalate*

Acknowledgement: *CIHR Grants: Endocrine disrupting chemicals: towards responsible replacements*

LECTURES

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THINK LIKE A CRIMINAL: WHO IS VULNERABLE TO FRAUD?

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Why would we think as a criminal as a first step to fraud prevention? It is because once you begin thinking like a criminal, you will be better prepared to stop criminal activity. To put yourself in the shoes of bad guys you do not need to be a bright detective, but just to think like one. Thus, by determining their most likely courses of action when attacking us, we can arm ourselves with that knowledge, and work out plans to address our vulnerabilities. Many who work with criminals, study criminal behaviours and learn to think like criminals. This helps them to do their jobs efficiently. Food fraud takes many shapes. It can occur internally or externally and in any supply chain. The starting point is individual integrity, is a person motivated or has the integrity to resist opportunity? If a person's integrity is compromised, (s)he may give into enticement of opportunity. According to the Routine Activities theory, both a motivated offender and opportunities should avail for a crime to occur, in the absence of guardianship. A fraud vulnerability assessment was developed based on this concept and consists of 50 food fraud vulnerability indicators. Various food supply chains and tiers were assessed for their fraud vulnerabilities and underlying factors. Differences between chains and tiers were identified, but also which aspects are behind high risk actors across chains. This information helps us to understand why people engage in food fraud, and to pinpoint the weakest spots in our food supply chain networks in order to mitigate vulnerabilities and develop control plans effectively.

Keywords: *assessment, crime, fraud, vulnerability*

LECTURES

L64

REVIEW OF ANALYTICAL METHODS, HORIZON SCANNING AND CAPABILITIES FOR FOOD AUTHENTICITY

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Food fraud / authentication of food is no longer an emerging topic but represents a popular and wide ranging arena. Given the numerous types of foodstuffs and the variety of frauds that can be perpetrated, it is not surprising that the field has generated numerous methodologies using a variety of approaches to detect, prevent and deter fraud.
Through the lens of the future UK policy needs, the aim of this presentation is to: illustrate the current landscape of analytical tools and methods routinely used to verify food authenticity; assess their fitness for purpose; and explore how these methods may need to be refocussed or developed to respond to a changing landscape. It will also map the level of capability these tools provide in terms of covering needs to support food law enforcement, both now and in the future.
We assess existing tools available to verify UK protected food status within a global context (including Geographic indications, geographic origin and method of production). We also explore technological and analytical advances for their application in the protected food status area (verifying authenticity), and identify any gaps.
We will also present an overview of advances in data collection, sharing and curation; reference materials/markers, distributed ledger technology; and advances in digital platforms and their potential application to verify food provenance and authenticity to support food law enforcement.

Keywords: *authenticity, fraud*

Acknowledgement: *We thank the UK Department of Environment, Food and Rural affairs for funding.*

LECTURES

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A COMPARISON OF HIGH AND LOW RESOLUTION AMBIENT MASS SPECTROMETRY TECHNIQUES TO CHARACTERISE POULTRY MEAT

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Rapid evaporative ionisation mass spectrometry has established itself in recent years as an innovative technique capable of rapidly identifying different factors relating to food safety, authenticity and quality, primarily in the meat and fisheries industries. REIMS is, however, limited by the need for costly, delicate and bulky high resolution mass spectrometers, which can make at-line testing prohibitively expensive and/or technically challenging.
DART is an alternative ambient mass spectrometry technique which can be used on both high and low resolution mass spectrometers, including the inexpensive, compact and portable Waters QDa single-quadrupole mass spectrometer. DART currently has a wider range of applications, making the significant financial outlay required to purchase and operate a mass spectrometer potentially more viable for some end-users.
In this study, the same set of poultry samples were first analysed on high-resolution mass spectrometers; REIMS analysis was conducted using a Waters G2-XS QTof instrument and DART analysis was conducted using a Thermo Exactive Orbitrap instrument. DART analysis was then conducted using a low-resolution Waters QDa single-quadrupole instrument to assess the limitations that may be encountered when moving from high to low resolution mass spectrometers fitted with the same ion-source.
The presentation will outline the comparison of REIMS and DART mass spectrometry, and will then discuss the relative advantages and disadvantages of using a compact low resolution mass spectrometer for DART-MS analysis.

Keywords: *DART, REIMS, meat fraud, ambient mass spectrometry, near-instantaneous profiling*

Acknowledgement: *Funding provided by Waters Corporation and EU MultiCoop*

LECTURES

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EXPRESS METHOD FOR PROFILING OF STEROLS IN COMPLEX FOOD MATRICES BY IN-SITU DERIVATIZATION IN DART MASS SPECTROMETRY CONDITIONS

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Application of ambient ionization mass spectrometry methods is very promising for food analysis, because such approaches provide express qualitative and, sometimes, quantitative results without or with minimal sample preparation. At the same time, all of these MS methods are based mainly on such 'soft' ionization processes as protonation, cationization and deprotonation. Thus, analytes, which are not capable of these processes, have high detection limits or cannot be analyzed by such methods at all. Furthermore, some compounds readily eliminate leaving groups after ionization. Some of these problems can be overcome by using the chemical modification approaches providing the permanent charge derivatization or yielding the readily ionizable derivatives. The main requirement for probable derivatization methods involved in analysis by ambient ionization mass spectrometry is the application of extremely simple and fast reaction procedures. Herein, we describe the first example of such approach for fast detection of sterols in food matrixes by 'direct analysis in real time' (DART) mass spectrometry. The proposed method is based on the earlier described reaction of alcohols with pyridine directly in DART sample gap yielding fixed-charge derivatives [1].
The approach was tested using sample of cheese, sour cream, farmer cheese and condensed milk acquired at local market. DART mass spectra were registered using standardized voltage and pressure (DART SVP, IonSense Inc., USA) model ion source (3,5 L/min helium flow, temperature 150-450 °C, ion-source grid voltage 350 V) which was coupled to Shimadzu LCMS-8040 tandem triple-quadrupole mass spectrometer (Shimadzu, Japan), operated in positive ion mode (desolvation line temperature 250 °C, heat block temperature 400 °C, interface voltage 4.5 kV,50-800 Da scan range), via a Vapur® interface (IonSense Inc., USA). MS/MS experiments were performed using 20 eV collision energy and argon as collision gas. For the analysis of milk products 100 mg of each sample were extracted by 1mL of hexane and 30 μL of pyridine were added to the separated supernatant. Than 5μL of the resulting solution were deposited by pipette on the cell of a QuickStrip™ Sample Card. The results of DART MS analysis were compared to GC/MS data, aquired using standart approaches for detection of sterols.
The analysis of the obtained data allowed detecting of fraud milk products containing high amounts of plant sterols as a result of vegetable fats addition. All results were proved by GC/MS analysis, but it should be underlined, that the described approach requires much shorter time for analysis.

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Keywords: *mass spectrometry, DART, derivatization, sterols, milk products*

Acknowledgement: *The work supported by 'RUDN University Program 5-100'.*

LECTURES

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SAME SAME BUT DIFFERENT? – INSTRUMENT COMPARISON OF NON-TARGETED 1H-NMR ANALYSIS FOR WINE AUTHENTICATION

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Non-targeted metabolomics has become a powerful tool in various fields of science, e. g. food authentication. The great benefit compared to classical, targeted approaches is the comprehensive characterization of a product. Non-targeted spectroscopic or spectrometric techniques with subsequent multivariate data evaluation enable the verification of the geographical or botanical origin of a food product and the detection of unknown adulterants [1]. Although numerous feasibility studies have demonstrated the great potential of non-targeted analytical approaches for food authentication, there are still limitations currently restricting the application of such methods in routine analysis. The major challenge is the harmonization of non-targeted methods. The comparability of the results, e. g. between instruments or laboratories, is an important prerequisite for the implementation of non-targeted methods in official control and for the creation of joint databases [2].
This presentation focuses on the instrument comparability of non-targeted proton nuclear magnetic resonance (^{1}H-NMR) spectroscopic analysis of wine. In order to differentiate the grape varieties, 201 commercial red and white wines (10 varieties) were analyzed using ^{1}H-NMR spectroscopy. For instrument comparison, the same samples were prepared and measured in two different laboratories following the same protocols and using identically constructed spectrometers of the same vendor. Spectra obtained with the two instruments showed differences, e. g. in the signal intensity and baseline, which also affect the results of unsupervised data analysis and classification models. Several approaches to improve the comparability, like correction with an instrument-specific factor or with Piecewise Direct Standardization (PDS), were applied and will be discussed.

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[2] Riedl, J., et al. Analytica Chimica Acta (2015), 885, 17-32.

Keywords: *non-targeted analysis, harmonization, instrument comparability, 1H-NMR, wine authentication*

LECTURES

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APPLICATION OF MACHINE LEARNING AND LASER-INDUCED BREAKDOWN SPECTROSCOPY FOR CLASSIFICATION OF ALPINE-STYLE CHEESES

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Laser-induced breakdown spectroscopy (LIBS) is an emerging analysis technology employed in geology, archaeology, engineering, and other fields of study demanding multi-elemental characterization of materials. LIBS measurement involves exciting a sample with a focused, high-energy laser beam to generate plasma. The optical emission spectrum produced by the cool-down process of the excited chemical species present in the analyzed specimen is detected using a spectrometer. Minimal sample-preparation requirements make LIBS particularly attractive for field-deployable systems. Recently, several food-science-related applications of LIBS have been reported in the literature, including detection of adulteration in meats and milk, classification of wines and vegetable oils, and analysis of cheese quality. Verification of provenience and analysis of food quality are essential for ensuring consumers' trust. Cheese manufacturing is a significant component of the agricultural economy in the US. Over the last decade, the production of cheese increased in the US from 4.5 million tons in 2008 to almost six in 2018. The cheese imports from the EU have also been steadily growing. Artisanal cheeses and rising demand for farmstead cheese produced using traditional manufacturing processes have driven a significant portion of the growth in the value of the cheese market. However, some industry experts estimate that ca. 20% of hard cheese sold in the US is mislabeled. Mass-produced low-quality cheeses are often misrepresented as genuine goods. Owing to the lack of robust testing platforms and priority of health hazard investigations over food-fraud cases, the mislabeling is rarely prosecuted. The presented report describes the development of a LIBS-based system for automated recognition and classification of alpine-style cheeses (such as Comte, Gruyère, Etivaz, and others) using analytical food-fingerprints technology. The prototype employs customized LIBS device paired a cloud-based machine learning platform performing feature selection, standardization, and reduction followed by sample classification The system uses a pulsed Nd:YAG laser with the energy output set to 30 mJ. The laser beam is expanded using a plano-convex lens and then focused with a double-convex lens onto a sample placed in a 3-D printed chamber. Plasma signal is collected using plano-convex lenses which direct the emission via fiberoptic to two spectrometers covering 200-600 nm wavelength range. Samples of cheese (10×10×3 mm) are placed in the sample chamber and receive five laser pulses across the surface. The pre-processed and standardized spectra were used to train and test multiple classifiers, including elastic-net regressors, SVM, and neural networks. The results demonstrated the feasibility of automated cheese classification and provided a strong argument for the further development of field-deployable, portable LIBS instrument optimized for food authentication tasks.

Keywords: *LIBS, cheese, machine learning, spectroscopy*

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LECTURES

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TOWARDS PORTABLE RAFA

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The historical dominancy of GC- and LC-MS in food analysis is due, at least in part, to legislation dictating that such evidence be provided through these techniques. Sooner or later, though, I would expect novel enabling technologies to be integrated into these regulations; this is why RAFA has always focused both on emerging analytical technologies and inventive food analysis workflows.
RAFA 2019 will showcase major developments in portable, rapid and non-invasive instruments, often referred to as "food scanners," which are expected to facilitate a massive increase in the number of food samples we're able to test. Optical spectroscopy (such as hyperspectral UV scanners covering visible and near-infrared wavelengths with image recognition) is a great example, and is experiencing a resurgence in food studies due to successful instrument miniaturization, the availability of advanced chemometric data handling tools, wireless data communication and 'Big Data' compatibility – in many cases, ordinary smartphones will provide a readout system. What's more, major developments in biochemical assays, such as strip tests, lateral flow devices, and biosensors will be showcased at the meeting, as will advances in portable MS – though these tend to move more slowly in food analysis than other fields.
Within the next decade, food inspectors, farmers, retailers, and even consumers, will be demanding the ability to test food themselves. To this end, I anticipate that rapid, smartphone based technologies with built-in and remote quality assessment features will supersede laborious laboratory practices – delayed action due to analytical limitations will not be acceptable. These events will trigger a paradigm shift: labs will move from dealing with high numbers of compliant samples to fewer, more interesting samples that have been identified as suspicious. Such a shift may even provide an opportunity to pinpoint completely new food contaminants and metabolites.

Keywords: *portable food analysis, biosensor, food scanner, immuno assay, smartphone*

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LECTURES

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FOOD ANALYSIS MADE EASY: THE PHASMAFOOD PROJECT APPROACH

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We are living in an era where public health concerns have been identified as a main issue. Food contamination and spoilage due to microbial activity and other biological and chemical hazards throughout the food chain is one of the most significant threats to food security, being directly linked with health concerns. Indicatively, every year unsafe food causes more than 200 diseases worldwide (ranging from diarrhea to cancers) and 600 million people fall ill after consuming contaminated food, from which almost 420.000 die every year (a third of them young children), resulting in the loss of 33 million healthy life years (disability-adjusted life year - DALYs). Furthermore, the authenticity of food produced with defined quality standards is a key expectation of consumers as well as a key selling point for the agri-food economy.

Consequently, the guarantee of food security and authenticity is of paramount importance not only in Europe, but worldwide. PhasmaFOOD is an EU collaborative R&D project funded by the Horizon 2020 Programme which has been working on delivering a compact multi-sensor optical sensing device for the detection of food safety threats such as food spoilage, adulteration and mycotoxins. PhasmaFOOD's vision has been to improve existing food inspection methods that are time-consuming, non-portable and provide in many cases only retrospective information and thus cannot be used for scientific laboratory analysis, daily consumer usage and in general in scenarios where feedback on the quality of the inspected food needs to be provided in a timely but still trustworthy and reliable manner (e.g. in supply chains). This is achieved by integrating heterogeneous visible (VIS) and near infrared (NIR) spectroscopy technologies which together with the overall developed system (PhasmaFOOD device, mobile phone application, cloud hosted reference databases and analysis algorithms) allow for food inspection that in addition to being time efficient and user friendly, is also non-destructive and can be applied to a variety of food types along all steps in a food supply chain, from the producers all the way to the consumers. The value proposition of PhasmaFOOD to consumers, food scientists or business actors in the food supply chain comes from preventing consumption of contaminated and spoiled food, protection of lawsuits, bad reputation or medical costs, reduction of dependence on the trustworthiness of food providers, avoiding money losses from food wasted in facilities or households. This work presents in more detail the areas (use cases) targeted by PhasmaFOOD and their importance that has been driving the project's technical directions, the design of the developed system together with the features it supports, as well as some promising results from food analysis leveraging the developed hardware and software components; finally, some directions for future work are also presented, as there is always room for improvements and enhancements.

Keywords: *spectroscopy, food analysis, mycotoxins, spoilage, adulteration*

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LECTURES

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TOWARDS PORTABLE, CONNECTED AND HIGH PERFORMANCE SMART SYSTEMS FOR FOOD QUALITY MEASUREMENT

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PhasmaFOOD delivers a miniaturized multi-sensor optical sensing device for the detection of food safety threats such as food spoilage, adulteration and aflatoxins. The system integrates heterogeneous visible and near infrared spectroscopy technologies, a digital camera and is supported by a custom electronics design featuring embedded memory, processing power and communication modules. An extendable software framework delivers fast characterisation of foods, allowing the deployment of smart chemometric algorithms and data fusion strategies.
In this context, WINGS key contribution is the design, implementation and evaluation of the electronic board (PCB) on which the discrete components will be mounted on. These comprise the sensing subsystem that includes the two spectrophotometers and the digital camera, the communication interface modules, the energy supply system and a powerful and fast processor. The latter is required to accommodate the functional requirements of the aforementioned components and most importantly the high-bandwidth communication demand of the on board digital camera. The main electronic board is designed and manufactured from scratch in order to meet the above requirements. Being custom designed, the electronic board requires specific files in order to boot and start loading a kernel and a root file system. Such a low level programming task demands the configuration of a series of boot-related files and the development of a device tree tailored to the functionalities and the components assembled on the electronic board.
The mobile device, which hosts the PhasmaFOOD mobile application, communicates with the sensing device using the Bluetooth wireless protocol. This communication demands a well-tailored and -defined data model in order to accommodate all the different sub-use cases at the mobile application and, also, any test use cases required for the calibration of the sensors.
WINGS is also responsible for the overall project's technical management and leads the activities for the assembly of all PhasmaFOOD system components into an initial system prototype. WINGS integration activities include testing of all basic functions concerning sensor operation, data transfer to a mobile application and recording of test spectra and images.
PhasmaFOOD project aims to improve the existing food inspection methods that are time-consuming, non-portable and cannot be used on-line facilitating scientific laboratory analysis or for daily consumer usage. WINGS vision is to further contribute in the development of innovative and portable sensing systems such as PhasmaFOOD in the domain of food quality. Such smart systems can be utilized throughout the complete span of the food value chain for farm to fork.

Keywords: *smart sensing, integrated photonics, cloud-based analytics, smart electronics integration*

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LECTURES

L73

DETECTION OF ADULTERATION IN SOLID AND LIQUID SAMPLES USING A PORTABLE, NON-INVASIVE SPECTROSCOPIC DEVICE

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The application of fast, inexpensive, non-invasive, and portable spectroscopic devices for screening in food control has increased over the past years to substitute and compliment the current expensive and labour-intensive (reference) laboratory methods. In particular, vibrational spectroscopic devices using ultraviolet, visible, (Fourier-transformation) near-infrared, and raman spectroscopy, and hyperspectral, and multispectral imaging devices have been miniaturised. Furthermore, software applications and databases have been transferred to cloud systems and smartphones, respectively. Currently, the drawback of these miniaturized spectroscopic devices lies in the limitation to be applied in differing food control areas. Therefore, one of the aims of the PhasmaFOOD project was to combine a number of miniaturized optical sensors into one integrated photonics sensor.
The PhasmaFOOD device is based on heterogeneous fluorescence, visible, and near-infrared spectroscopy technologies combined with a RGB miniaturized camera. It is suitable for the measurement of both solid and liquid samples, . In four pilot studies, i.e., skimmed milk powder, olive oil, spirits, and meat, the detection of adulterants in genuine samples was explored. After processing of the spectroscopic data and comparison with a database of genuine
Referencesamples, adulterated samples are identified as 'out' of the genuine class via a combination of 'one-class' and two-class multivariate statistics.
Results of two pilot studies will be presented to show the devices' application range in the field of food fraud. In particular, insights in the processing of spectroscopic data and fusion of data from different sensor types to achieve accurate fraud predictions will be given.

Keywords: *adulteration fraud spectroscopy food sensor-combination*

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LECTURES

L74

PHASMAFOOD SOFTWARE PLATFORM FOR BUILDING REFERENCEDATASETS AND VALIDATING DATA ANALYSIS AND DECISION MAKING CHAINS FOR FOOD SAFETY AND QUALITY ANALYSIS

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PhasmaFOOD project delivers an IoT system for food quality and safety analysis. The solution comprises a miniaturized multi-sensor optical sensing device and software platform. The PhasmaFOOD software platform enables the sensing device to collect new measurements, perform data preprocessing, communicate measurements to the cloud platform for storing and analysis. The platform employs data analysis (DA) procedures and trained machine learning (ML) models to perform analysis of food quality and safety parameters in line with the three project use cases: Mycotoxin detection, Food spoilage and shelf life estimation and Food adulteration. The PhasmaFOOD software platform spans three system layers:

1. embedded software residing on the PhasmaFOOD sensing device;
2. PhasmaFOOD mobile application residing on end user's smartphone; and
3. PhasmaFOOD cloud platform deployed on selected Infrastructure as a Service.

The embedded software is responsible for operating the integrated sensing device, driving sensors and lighting sources, performing measurement procedures, performing data preprocessing and operating a Bluetooth interface with the PhasmaFOOD mobile application.

The PhasmaFOOD mobile application provides the interface between the sensing device, which is the primary source of the sensory data, and the cloud platform, which is the primary sink for all data streams. Moreover, the mobile application interfaces with the end-user enabling them to configure the food analysis process and see the analysis results.

The PhasmaFOOD cloud platform is the focal point of all sensory and contextual data coming from the PhasmaFOOD sensing device, the mobile application and 3rd party data sources. It hosts cloud database for all sensory and contextual datasets necessary for performing food analysis required by the project use cases. It operates data analysis pipelines and rule engine for reactive and proactive decision making. The cloud platform provides the web dashboard and ML playground as the interface for managing the cloud database and decision-making procedures.

The software platform, combined with the PhasmaFOOD sensing device, enables end users to perform quick, on the spot analysis of food quality and safety parameters. It relies on the

Referencedatasets and data analysis/decision making procedures implemented for each project use case. The software platform is conceptualized as a set of software tools for supporting food analysis experts to collect new and enhance existing

Referencedatasets, perform quality assessment of collected measurements, configure and test different data analysis pipelines and data fusion techniques. The goal was to enable users in food analysis laboratories to use the PhasmaFOOD system in line with their well-established measurement protocols. The PhasmaFOOD system is designed to support both food analysis experts with their laboratory needs, and end users with their needs for on the spot food analysis.

Keywords: *IoT, software platform, data analysis, cloud database, decision making*

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LECTURES

L75

DNA DIRECTED IMMOBILIZATION (DDI) FOR THE DEVELOPMENT OF AN ANTIBODY FLUORESCENT MICROARRAY FOR THE DETERMINATION OF THREE FAMILY ANTIBIOTIC RESIDUES IN MILK.

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Misuse of antibiotics in food producing animals enhances the possibility to develop antibiotic resistance and can also lead to the appearance of these compounds in derivate products. New strategies to overcome this issue are required. In this context, the FoodSmartphone project will provide analytical tools to ensure food quality concerning pesticides, veterinary products or allergens, from farm to fork. The first approach is based on fluorescent microarrays which emerge as potential screening tools to determine the presence of multiple analytes at the same time. In this study, we present a multiplexed analytical platform based on hapten-oligonucleotide arrays to detect three families of antibiotics. The use of hapten-oligonucleotide conjugates allows the directed immobilization (DDI) of the signal for each antibiotic family by immobilizing the complementary oligo-probes over the surface of glass slide. After hybridization, the cocktail of primary antibodies is added in the samples and this binding is elucidated by the addition of secondary antibodies labeled to a fluorophore which provide the fluorescent signal. Using this system a group of penicillin, sulfonamides and tylosin can be quantified on buffer and milk samples. The format of this assay will provide the basis for its implementation on a Smartphone readout system.

Keywords: *antibiotic residues, food control, milk, DNA direct immobilization (DDI), microarray*

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LECTURES

L76

TOWARD THE SMARTPHONE-BASED ELECTROCHEMICAL DETECTION OF AFLATOXINS IN FOOD SAMPLES

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Aflatoxins as carcinogenic food contaminants are considered a significant public health risk and when present cannot be eliminated through cooking or food processing. The current Laboratory-based analysis is expensive, not available to the public, and not frequent enough. As an innovative solution, an electrochemical smartphone-based food analyzer can provide simple, rapid, cheap, and on-site food safety testing of aflatoxins available to the farmer, border inspector, and the consumer.
The focus will be on how smartphones could be applied for onsite quantitative detection of aflatoxins in food samples using an electrochemical biosensing platform. The common electrochemical biosensing platforms based on antibody biorecognition elements provide sensitive and selective detection of the analyte. Their main advantage is giving quantitative results using cost-effective and disposable screen-printed electrodes. Also, the miniaturized data acquisition device can be connected by USB/Bluetooth to the smartphone.
An Enzyme-Linked-Immuno-Magnetic-Electrochemical (ELIME) assay has been developed, which is based on indirect competitive immunoassay. The use of magnetic beads facilitates the washing steps and effectively minimizes the matrix effect in real sample analysis. The developed assay provides sensitive detection of aflatoxin B1 with the detection limit of 33 pg/mL in the buffer which is lower than the EU regulatory limit for aflatoxin B1 in corn, 2 µg/Kg corresponding to 50 pg/mL after the extraction. The real sample analysis has been performed using a simple extraction procedure, including filtration and dilution, without any clean-up procedure. The accuracy studies have been assessed with spiked and naturally contaminated corn samples, obtaining recoveries in the range of 70-120%. Thus the described system can be considered as a promising candidate for simple, rapid, cheap, and on-site food screening on a smartphone.
To further minimize the steps necessary for the detection and thus facilitate the integration of the assay in a user-friendly portable system, the use of an aptamer as recognition element will be studied. This detection platform will be based on aptamer conformational change after binding with aflatoxins and provide single step reagent-less detection.

Keywords: *aflatoxins, electrochemical detection, smartphone-based detection, corn*

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LECTURES

L77

A MINIATURIZED SMARTPHONE-BASED METHOD FOR CARBOFURAN SCREENING IN FRUITS AND VEGETABLES BASED ON ACETYLCHOLINESTERASE INHIBITION

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Recently, our group introduced the so-called "enzymatic paper-on-a-chip" (EPOC) method aiming to improve the screening of organophosphate (OP) and carbamate (CM) pesticides [1]. EPOC is a multidisciplinary concept consisted of paper-based acetylcholinesterase (AChE) sensors, 3D-printed prototype devices and a smartphone as the analytical detector. In principle, AChE activity is reduced in the presence of an OP or a CM compound. Moreover, AChE hydrolyzes colorless substrates to color products when is active. Therefore, the color intensity decrease can be related to an inhibitor concentration. To this end, AChE was adsorbed on chromatography paper and two AChE strips, one control strip and one test strip, were used per EPOC device. Integrated sample handling was provided by the 3D-printed prototype featuring silicone tubing injectors able to pump samples and substrates to the strips. This is a very attractive characteristic eliminating the need of pipetting and showing the EPOC device potential for *in-situ* detection. Regarding the colorimetric detection, this was monitored by recording a video of the enzymatic reaction and video data processing was performed by Python development software. To demonstrate the method applicability, carbofuran, a CM with frequent MRL exceedances, was detected in the range of 0.010 to 10 mg Kg^{-1} in apple extracts. Different sample preparation approaches were investigated comparing QuEChERS, an ethyl acetate extraction and a simplified ethanol extraction. In conclusion, further improvement of the EPOC method was achieved and work is underway to improve assay fluidics and color development by printing wax hydrophobic channels on AChE strips.

[1] A.S Tsagkaris, D. Filippini, J.P. Salvador, M.P. Marco, J. Pulkrabova, J. Hajslova (2019), *The enzymatic paper-on-a-chip concept: Towards the on-site organophosphate and carbamate pesticides detection in fruits and vegetables*, 56th North American Chemical Residue Workshop, Book of abstracts pp. 36, Naples, FL,USA

Keywords: *lab-on-a-chip, acetylcholinesterase, smartphone, pesticides, screening method*

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LECTURES

L78

NANOMATERIALS, SCREEN PRINTED CARBON ELECTRODES AND THEIR USE FOR IMMUNOASSAY DEVELOPMENT IN THE AREA OF FOOD SAFETY

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Nowadays there is a high demand for cost-effective and user-friendly on-site screening tools in the food safety industry. Electrochemical sensors are the perfect candidates for this purpose since they combine the qualities of being miniaturized and relatively cheap at the same time. The use of carbon-based screen-printed electrodes (SPEs) is particularly ideal for on-site analysis, because of their well known low production cost, which drastically decreases the costs of the analysis. Furthermore, these SPEs can easily be modified with nanomaterials, which can result in a significant increase of their performance.
In this study a characterization of in-house SPEs modified with CB and their application for the detection of different pesticides is described. Different materials have been investigated during the printing process and the working electrode has been modified with nanoparticles directly in the ink or by dropcasting either manually or using the Biodot dispenser.
After an electrochemical characterization, the best performing SPE has been selected and its performance has been evaluated by using it either directly as a platform for the indirect competitive immunoassay or just as a transducer when the assay was performed using magnetic beads (MBs) as a platform for an Enzyme-Linked-Immunomagnetic-Electrochemical (ELIME) format. Both of the approaches have shown similar LOD and comparable sensitivity despite the fact that different enzymes (horseradish peroxidase and alkaline phosphatase) and different electrochemical techniques (Chronamperometry and Differential Pulse Voltammetry respectively) were applied, from which the latter approach also allows for multiplexation.
Thus, the described system is a promising candidate for the development of a portable smartphone-connected device for on-site analysis.

Keywords: *electrochemistry, pesticides, biosensor, food safety*

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LECTURES

L79

RETRIEVING PEROXIDASE-LIKE ACTIVITY OF LIGAND-CAPPED GOLD NANOSTARS FOR THE DETECTION OF MYCOBACTERIUM BOVIS

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Tuning and controlling size, shape and surface characteristics of plasmonic gold nanoparticles can help define their photonic, catalytic, and spectroscopic properties, and therefore are crucial for the development of biosensors. This strategy is demonstrated in the present study. Gold nanostars (Au NST) chemically synthesized through a seeding approach exhibited not only Vis-NIR tunable localized surface plasmon resonance (LSPR), but also strong peroxidase-mimicking activity which can catalyse the oxidation of tetramethylbenzidine (TMB) into an oxidized chromogenic product (oxTMB). The catalytic activity of gold nanostars was found to correlate well with their size and LSPR mode, and to be strongly affected by capping ligands (i.e. poly and oligo ethylene glycol, 11-mercaptoundecanoic acid, 4-mercaptobenzoic acid). When capped with molecular binders (i.e. peptides and antibodies), the peroxidase-mimicking activity was suppressed by almost 100% under the examined conditions. However, a gold deposition reaction catalysed by the nanostars themselves (so-called autocatalytic enlargement) was not affected by the capping, resulting in increased particle size and increased numbers of newly formed nuclei acting as independent centres for the catalysis. The autocatalytic enlargement thus overcame the barrier of bioconjugation-induced suppression and paved the way for biosensor development. The particle characterizations and catalytic processes were thoroughly analysed by absorption spectroscopy, HR-TEM, ζ-potential measurements.
To prove its applicability, the sensing approach was employed to develop a colorimetric biosensor for the detection of *Mycobacterium bovis*. *M. bovis* has been described as one of the main hazards of raw drinking milk in the EU, leading to more than 1,000 cases of zoonotic tuberculosis each year. More importantly, due to their slow-growth nature, the detection of pathogenic *Mycobacterium* species using culture methods is very time-consuming and thus extremely challenging. To date, no effective sensors have been developed to detect the presence of this bacteria in food samples, as most regulations only apply to the presence of the bacteria in the animal.
Towards this end, a binding immunoassay was implemented to select the antibody with the highest avidity for *M. bovis*. The selected antibody was then immobilized onto the Au NST, using thioctic acid-N-hydroxysuccinimide ester as a heterobifunctional crosslinker. This bionanoconjugate was enlarged by adding Au^{3+} ions to a final concentration of 10^{-4} M, thus restoring bare gold surfaces available for TMB oxidation. This step has resulted in the potential to reach higher sensitivity through signal amplification compared with conventional approaches. Integration of this colorimetric biosensing approach with a smartphone reader to allow the development of a semi-quantitative and user-friendly biosensor for in-field applications will also be reported.

Keywords: *gold nanostars, catalysis, mycobacterium bovis*

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LECTURES

L80

THE OMNIPHONE: ONE SMARTPHONE APP FOR UNIVERSAL COLORIMETRIC ANALYSES USING A RANDOMISED COMBINED CHANNEL APPROACH AND MACHINE LEARNING

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Smartphone based colorimetric biochemical assay quantification is of increasing interest in food safety analysis. As a result several systems have been developed enabling accurate predictions for specific tests with a smartphone using red green blue (RGB), hue saturation value (HSV) or lightness and chromatic axes A/B (LAB) colour space. However, these systems are test (and often smartphone) specific and have not been used to quantify colour in existing commercial assays. Mainly only one of the three available channels in a colour space is used. For instance, L is commonly used for lateral flow assay (LFA) quantification and has been claimed to work in a superior manner to all other channels mentioned here. However, to the best of our knowledge, this claim has not been validated by robustly comparing (L) performance with other channels. Moreover, digital representation of true colour is strongly simplified by utilizing only one of the three channels of a colour space. Other issues encountered are background illumination variation caused by various light settings and inter-phone variation in the channel values. To tackle these issues a light shielding box as well as camera calibration algorithms have been suggested. Although these solutions are acknowledged to be capable of correcting for large variations, simpler background correction techniques might take care of most of the variations expected during normal use. In the present study, a more universal approach towards smartphone colorimetry was developed. Prediction accuracy of each individual channel of the RGB, HSV and LAB colour spaces was determined for the quantification of colour variation, using pH strip, and colour intensity variation, using filter paper with dropped gold, latex or carbon black nanoparticles. R and B channels were identified as best performing for colour change and colour intensity respectively. A simple background correction was shown to be capable of avoiding variation caused by illumination as long as no direct sunlight was used, thus eliminating the need for a box. Moreover, the same background correction enabled, to a large extent, the elimination of inter-phone (n=6) variation thus permitting the quantification of colour with phone A using a calibration curve constructed on phone B. To validate the universal nature of the optimised system it was successfully used to quantify gluten in buffer (B channel), bovine milk in goat milk (B channel) and pH values in soil extracts (R channel) using commercial assays and various phones (all obtained prediction curves with $R2 > 0.9$ and mean average errors < 30%). Next a machine learning algorithm and app was developed to allow automated background subtraction and random channel combination of all colour spaces in 2 or 3 channel multidimensional calibration plots. This allowed error reduction and the establishment of a universal, user friendly app able to successfully quantify colour in various commercial LFAs and pH strips.

Keywords: *smartphone; colorimetric; nanoparticles; allergens; machine learning*

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LECTURES

L81

FOODTESTCHAIN: INTEROPERABLE AND IMMUTABLE FOOD DIAGNOSTIC DATA VIA BLOCKCHAIN

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The generation and interpretation of food analysis data is crucial for ensuring food integrity, safety, and quality. However, current food testing data is mainly stored in conventional non-distributed databases where the stored data suffers from a lack of accessibility and is vulnerable to data tampering. In order to enhance interoperability and immutability of the stored food testing data, we proposed a distributed and permissioned blockchain network, named FoodTestChain, for storing and sharing food testing data among verified stakeholders whilst securing the shared data from being tampered.
In order to demonstrate the applicability of the developed blockchain network, a data generator and a data consumer application were developed. Food testing results of real food matrices together with location information generated from smartphone food analysers were sent to and stored in the blockchain network. An open access webpage with a map-view that can be visited from computers and smartphones was also developed to visualise the locations and results of the food testing being conducted by the smartphone food analysers.

Keywords: *blockchain, food diagnostic data, interoperable, immutable*

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LECTURES

L82

FIGHTING THE INCOMPATIBILITY FOR IMPROVED FOOD SAFETY TESTING: LATERAL FLOW IMMUNOASSAY HYPHENATION WITH MASS SPECTROMETRY

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The presence of contaminants in food may lead to loss of quality or potential risk for consumers. The current strategy, for monitoring the presence of contaminants, often consists of a two-step approach. The first step involves screening of samples, for example, using a lateral flow immunoassay (LFIA). The screening assay is performed in the laboratory on a relatively small number of preselected samples. The screening aims to test the presence or absence of contaminants and reduce the number of samples that undergo the more expensive second step. The second step is confirmatory analysis of the suspect samples. For confirmation, analytical techniques such as liquid or gas chromatography coupled with mass spectrometry (LC- or GC-MS), aim at the identification and quantitation of the contaminants present. However, the current trend in food analysis is to make the screening assays easier, so eventually, they can be used by citizens. The development of user-friendly screening assays will lead to a shift towards simplified on-site testing, without the need of a laboratory. This development will eventually lead to an increase in the number of samples screened and consequently, more suspect samples that need to undergo laboratory confirmatory analysis. In the current study, we are working towards the hyphenation of an MS technique with screening assays. In principle the solvents used in LFIAs are not favourable with MS detection, so such hyphenation is a challenge. As a proof of concept, an in-house LFIA model system for the binding of the mycotoxin deoxynivalenol (DON) was developed, by assessing surface plasmon resonance (SPR) data. The developed LFIA is a simplified system, consisting out of anti-DON monoclonal antibodies. Working towards the hyphenation, promising results were obtained by analyzing different types of DON standard solutions with different types of running buffers used in LFIAs and by taking different direct ionization MS approaches. The development of this technique can act as an intermediate between screening and confirmatory analysis. Moreover, it can provide additional information that will be critical in the emerging new era of consumer-friendly screening for food contaminants. This will allow a faster and more cost-effective approach by producers or decision-makers, as no additional sample pretreatment is required.

Keywords: *lateral flow immunoassay (LFIA), direct mass spectrometric analysis, deoxynivalenol (DON), hyphenation*

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LECTURES

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TRUE MOBILE MASS SPECTROMETRY FOR ON-SITE ANALYSIS OF FOOD CONTAMINANTS

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Nowadays we are used to measure all kinds of mostly health-related parameters using our smart-phone and -watches using optical sensors. These same principles will be used in the near future by both consumers as well as food inspectors to check food for unwanted contaminants. The first methods that will become available for on-site inspection will be based on tests targeted a single or few contaminants and will not be multi-targeted. Additionally, these tests still produce initial screening results. In practice suspected samples still will need to be transferred to a laboratory where skilled technicians will perform mass spectrometry (MS) based confirmatory analysis. The next-generation methods for on-site analysis will be more advanced and will bring the high-end laboratory equipment like mass spectrometers in a miniaturized version available for field analysis. Currently, there are very few MS instruments available that can be used on-site for food analysis. The majority of these MS instruments are transportable and not truly portable, making on-site application limited and not flexible. It is realistic to envisage that true portable MS, already successfully applied for warfare agents and in forensic applications, in the future can also be used in food and feed control.
In recent years, we have invested in research which should lead to effective on-site MS. This was done by the development of on-site measurements by elimination of tedious sample clean-up, simplified sample introduction, and data evaluation tools that wirelessly communicate via smartphones with servers of relevant stakeholders, including the consumers. After an evaluation of the available portable MS instruments recently one was purchased by WFSR. This MS is fully self-sustaining in the field, containing a battery, small gas cylinder, computer, GPS, WiFi, gas chromatograph and connected to the WFSR via the cloud. The MS is operated under high-vacuum and is operational in 10 min after switching the system on. The ionization is based on electron ionization and the mass analyzer on a quadrupole. Different sample inlet options are available, e.g. membrane inlet MS (MIMS), thermal desorption and split/splitless injection. This truly portable MS is currently evaluated for various food applications. Depending on the application an inlet method is chosen. For example, MIMS for chemical incidents (volatiles), thermal desorption for food authenticity-related questions and splitless injection for pesticide analysis. The results of the evaluation of this MS system for food safety related applications will be presented and discussed.

Acknowledgement: *This research was funded by the Dutch Ministry of Agriculture, Nature and Food Quality, project number 126.73337.01*

LECTURES

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HUMAN BIOMONITORING OF EMERGING CHEMICALS: CURRENT TRENDS AND IMPLICATIONS IN THE EXPOSOME

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Human biomonitoring methods are well established for the exposure assessment of many chemicals. Such methods have resulted in establishing measures for regulations, following time trends of concentrations in human populations and in establishing reference values and ranges for a selected group of 250–300 known persistent and non-persistent chemicals.
Yet, as more and more chemicals are added to the market, there is an increasing need to estimate the human exposure to these emerging contaminants. Recent efforts and advances in mass spectrometry have seen the unprecedent rise of screening techiques with the aim to identify emerging contaminants and/or their metabolites present in humans. Such analytical approaches based on high-resolution mass spectrometry (HRMS) are: 1) target screening; 2) suspect screening and 3) non-target (or untargeted) screening. Using these advanced tools, we can capitalize even more on the identification of lifestyle-specific exposure profiles, i.e. compounds that may differ in relation to specific behavioural and dietary patterns.
Furthermore, the use of HRMS screening techniques allows the coupling of human biomonitoring with the exposome approach. An exposomic approach (exposomics) theoretically includes all exposures of potential health significance, whether they are derived from exogenous sources (e.g., diet, air, water, indoor environment) or endogenous sources (e.g., hormones, human and microbial metabolites). Since levels of chemicals in biological samples reflect a wide range of exposures (biomarkers of exposure), but also consequences of exposures (biomarkers of effect), exposomic biomonitoring offers an efficient means for characterizing the overall individual exposure profiles. Incorporating the exposome paradigm into traditional biomonitoring approaches offers a means to improve exposure assessment in many ways.
With only a few hundred chemicals routinely measurable through targeted methods and with limitations for short-lived compounds, exposomic approaches are critical to understanding the daily exposure to thousands of chemicals and the consequences of exposure in exposome-wide association studies (EWAS). The processing of rich sets of data from untargeted analyses offers a path for discovering health-impairing exposures that have thus far escaped scrutiny, a largely unrecognized benefit of exposomics. This should give guidance towards more accurate prevention measures that protect against exposure to (emerging) environmental contaminants and their substitutes in new materials and products and consequently in food.

Keywords: *human biomonitoring, diet, exposome, emerging contaminants*

LECTURES

L85

PESTICIDES BIOMARKERS IN 24H URINE VERSUS PESTICIDES IN DUPLICATE DIETS USING SUSPECT SCREENING AND TARGET ANALYSIS

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Food is a major route of exposure of the general population to (mixtures of) pesticides. Exposure assessment is typically done based on residue data from food monitoring and food consumption databases. Human biomonitoring (HBM) is an alternative and can bring added value for chemical risk assessment because it can reduce the assumptions regarding consumption rates, residue occurrence and processing effects, and it integrates exposures from different sources (diet, house-hold use, environment). An important aspect in use of HBM data for pesticides is to assess links between HBM and food data. This was done in a study in which 24h urine and 24h duplicate diets were collected from the same 35 persons, and analysis of both types of samples.

Upon uptake, many pesticides are rapidly metabolised and excreted via urine. This poses several challenges: i) the most suited biomarkers in humans are often not known, ii) a lack of analytical reference standards, iii) the unavailability of dedicated analytical methods. To address this challenge, a generic non-target measurement strategy for urine, based on 96-well SPE and LC-full scan HRMS, was developed and applied to analysis of 24h urine samples. The raw data were preprocessed to generate molecular formulas of all compounds present in the urine [1]. These formulas were then matched against a custom-made database containing phase-I/II metabolites of a large number of pesticides which had been compiled from DARs (EFSA), JMPR monographs and literature. This resulted in a long list of tentative hits. Strategies to mine the output and to reduce the number of tentative hits to a short-list of candidates for follow up for identification will be presented. Using our workflow, over 40 pesticide biomarkers were identified (ID-level 1 and 2 according to Schymanski et al [2]). As a next step, for biomarkers of selected pesticides (pyrethroids, chlorpyrifos/-methyl, chlorpropham), a quantitative determination was performed using dedicated methods.

In parallel, the corresponding duplicate diets were analysed for pesticide residues, using established quantitative/screening methods [3]. The data obtained from urine and duplicate diet analysis were compared in a qualitative way, and quantitative relationships were investigated for selected pesticides. Matching results were obtained for many pesticides/biomarkers, but further research on toxicokinetics and quantitative relationships are needed.

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[3] Zomer P., Mol H., Simultaneous quantitative determination, identification and qualitative screening of pesticides in fruits and vegetables using LC-Q-Orbitrap-MS, *Food Additives & Contaminants*, 2015, 32:1628–1636.

Keywords: *human biomonitoring, pesticides, suspect screening, LC-HRMS, biomarkers*

LECTURES

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DIFFICULTIES IN THE URINE SAMPLE PREPARATION FOR LC-MS/MS ANALYSIS OF MYCOTOXIN BIOMARKERS – HOW TO DEAL WITH THEM?

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Biomonitoring is helpful tool in the exposure assessment of toxins for humans and animals. Pigs are exposed to several mycotoxins at the same time and additionally particularly vulnerable to the effects of mycotoxins - can be good indicator for human health risk. A sensitive method is required for simultaneous determination of a wide range of mycotoxins and their metabolites in urine (biomarkers) at a very low level (ng/mL) for the effective exposure assessment.
The aim of the study was developing and optimizing the LC-MS/MS method for the determination of 40 toxins including deoxynivalenol (DON), zearalenon (ZEN), ochratoxin A (OTA), aflatoxins (AFLs), T-2 toxin (T-2), HT-2 toxin (HT-2), fumonisin B1 and B2 (FBs), their metabolites and additionally: citrinin, dihydrocitrinone, nivalenol, fusarenon-X, diacetoxyscirpenol, sterigmatocystin, beauvericin, enniatins and Alternaria toxins (alternariol, alternariol monomethyl ether, altenuene and altertoxin I) in pigs urine.
Because of the chemical diversity of analysed mycotoxins optimization of sample preparation is crucial. For this purpose liquid - liquid extraction (LLE) with several organic solvents with salt and acid addition at different pH was examined. Next, different solid-phase extraction (SPE) and multi-toxin immunoaffinity columns (IAC) were investigated.
Some mycotoxins (e.g. ZEN) are mainly excreted as glucuronides in pigs urine, therefore enzymatic digestion was used for the pre-treatment of urine before biomarkers determination. For the first time different sources of β-glucuronidases (Helix Pomatia, E.coli, Abalone) were tested. Only β-glucuronidase from E. coli enabled T-2 determination and was chosen for the enzymatic hydrolysis.
The high diversity of pigs urine samples was also challenging. Depending on the density of the sample, which is measured by the level of creatinine, different sample preparation schemes have been tested for "clean" urine with low creatinine (<1 mg/mL) and "dirty" urine with high creatinine (>1 mg/mL).
For both urine types, the IAC columns show high recoveries (80-110%) and low LOD (0.004-0.4 ng/mL) for DON, ZEN, T-2, HT-2, AFLs, OTA and FBs. Sample clean-up with SPE columns for most compounds was not sufficient.
For "clean" urines, LLE with ethyl acetate and sodium chloride addition shows high performance (LOD: 0.02-2 ng/mL, recovery: 70-110%) excluded FBs (additional clean-up was needed). For "dirty" urines and more polar analytes such as DON co-eluting and interfering matrix components were observed what decrease sensitivity of the method. Therefore dilution to constant creatinine level was needed.
Despite the IAC columns enabled reliable clean-up for all urine samples and lowest LOD, they are very expensive and limit the number of analytes to the cross-reactivity range. After standardization to low creatinine level, LLE could provide inexpensive and sufficient clean-up for a wide range of mycotoxins as an alternative for IAC.

Keywords: *mycotoxin biomarkers, biomonitoring, creatinine adjustment, enzymatic hydrolysis, LC-MS/MS*

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LECTURES

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IDENTIFICATION AND ANALYSIS OF POTENTIAL BIOMARKERS FOR TOMATO INTAKE

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Maintenance and improvement of human health as well as prevention of diseases are substantially influenced by the diet. The ability to conclusively establish a correlation between intake of different food items and observed health effects is consequently of high relevance. A major challenge faced herein is the objective and quantitative determination of the dietary intake outside of a controlled study environment. The analysis of dietary biomarkers, i.e. food constituents or derived metabolites quantifiable in biofluids after consumption of the respective food, has great potential in this regard. However, the number of established dietary biomarkers is very limited so far and in many cases a comprehensive validation is still necessary.
The objective of this project was to identify potential biomarkers for the intake of tomato products. For that purpose, a dietary intervention study was conducted to compare the urine metabolome of a study cohort between a tomato-free diet and after intake of a single dose tomato juice based on an LC-HRMS metabolomics approach [1]. Herein, novel metabolites of the steroidal glycoalkaloids esculeoside B-1 and B-2 were putatively identified as potential biomarkers. Furthermore, the *β*-carboline alkaloids tangutorid E and tangutorid F as well as their glucuronidated derivatives were characterized as potential biomarkers. The occurrence of both *β*-carboline alkaloids was additionally investigated in several food samples within this project by applying an LC-MS/MS-based stable isotope dilution assay; tomato products were found to contain the highest amounts of tangutorid E and F [2]. Lastly, novel imidazole alkaloids were detected in the study participants' urine after tomato juice intake and showed a huge potential as biomarkers for tomato intake. These alkaloids were successfully synthesized, structurally characterized and quantitatively determined in several tomato products for the first time [3]. The occurrence of the novel imidazole alkaloids in different foods and their potential biological activities besides the already established cytotoxic properties should be investigated in future research. Currently, the applicability of all identified metabolites as valid biomarkers for tomato intake is evaluated in the course of a further dietary intervention study.

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[3] Hövelmann, Y., Hahn, M., Hübner, F., Humpf, H.-U. *J. Agric. Food Chem.* 2019, *67*, 3670-3678.

Keywords: *dietary biomarkers, tomato, alkaloids, mass spectrometry*

LECTURES

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LIFETIME DIETARY RISK ASSESSMENT: A NEW METHOD TO CONSIDER CHANGES IN EATING HABITS, FOOD CONTAMINATION AND ACCUMULATION OF CHEMICALS THROUGH LIFE

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Introduction: Classically, dietary exposure to chemicals is assessed from punctual measurements of food consumption and food contamination. The dietary exposure, estimated from these short-term data, is compared with a chronic health based guidance value (HBGV) which is a dose below which, a risk for health can be excluded. According to this approach, the evolution of eating habits with age, changes in food contamination and potential lifetime accumulation of chemicals in the body, are not considered. In this study, we propose a method to assess lifetime dietary exposure trajectories, as well as the health risk related to long-term exposure.
Method: Sociodemographic parameters correlated with punctual dietary exposures are studied and virtual individuals are simulated, for whom significant lifetime sociodemographic variables are known. Then, dietary exposures are predicted at each time of life, according to individual parameters. Lifetime exposure trajectories can be corrected according to the year of birth, in order to consider the evolution of food contamination. External exposure trajectories are transformed into body burden trajectories by using physiologically based toxicokinetic (PBTK) models. Lastly, exposure and body burden trajectories are compared with external and internal HBGVs respectively.
Results: The method simulating lifetime exposure trajectories based on sociodemographic parameters allows the consideration of individual profiles and of possible "at-risk subpopulations". According to the selected HBGV and the approach used to interpret fluctuating exposure trajectories, conclusions in terms of risk assessment vary. In the case of dietary exposures to cadmium and PCBs, the strategy considering external HBGVs, usually based on a no observed adverse effect to which safety factors are applied, appears more conservative compared with internal HBGVs, usually based on body burden values above which an effect can be expected. Additionally, the internal exposure approach, in the case of accumulating pollutants, takes into account accumulation in the body over the years.
Discussion and conclusions: Because HBGVs are in most cases derived considering a constant scenario of exposure through life, comparisons with fluctuating realistic lifetime exposure trajectories are not trivial. This study raises the question of the relevance of the use of chronic HBGVs and punctual measurements of exposure to assess the health risk related to food chemical exposure, particularly for persistent organic pollutants. This method proposes alternatives to assess lifetime trajectories taking evolutions of exposure into account and possible approaches for interpretation in terms of health risk.

LECTURES

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QUALITY ASSURANCE PROGRAM IN HBM4EU: FIRST RESULTS AND FUTURE CHALLENGES.

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Human biomonitoring (HBM), as a tool for exposure assessment of hazardous substances in the general population, has increasingly gained recognition in the public health sector and to support policy making. The HBM4EU initiative is aiming to coordinate and advance HBM in Europe. The initiative is a joint effort of 28 countries, of 109 partner organisations, co-funded under Horizon 2020 and will run from 2017 to 2021. HBM4EU will contribute to fill-in the data gaps for the exposure to prioritized chemicals, through the chemical analysis of human samples. Achieving comparable HBM data is a prerequisite for European-wide political activities in the field. Therefore extensive quality assurance/ quality control (QA/QC) programme within the analytical phase was necessary to guarantee the reliability and comparability of analytical data. In addition, when human biomonitoring studies are accompanied with epidemiological questionnaires, QA/QC also allow ensure that differences found are the result of variations in exposure factors such as diet or life style, instead of variability in the analytical method. QA/QC programs for the analysis of food contaminants, are based on the use of certified reference materials, the application of official interlaboratory validated methods and proficency testing. However, these useful approaches are very limited in human biomonitoring studies, mainly due to the lack of reference materials for many emerging human biomarkers as well as differences in legislative and regulatory framework that controls the presence of chemicals in food compared to human samples. In order to overcome these limitations in HBM studies, the participation in Interlaboratory Comparison Investigations and External Quality Assurance Schemes (ICI/EQUAS) becomes essential for the complete analytical method validation of human samples. An ambitious program of ICI/EQUAS has been designed in HBM4EU: a total of 76 individual human biomarkers for the first round of priority substance groups (phthalates, flame retardants, cadmium, chromium, perfluorinated substances, polycyclic aromatic hydrocarbons, bisphenols and anilines). Laboratories from 26 out of the 28 member countries and with different background and experience are participating in QA/QC program. Successful participation in at least 2 ICI/EQUAS rounds is the minimum requisite for European labs to analyze HBM4EU samples. The program has an important component in capacity building for laboratories since the sustainability of the HBM4EU initiative cannot be based on only a few highly specialized laboratories. This presentation will show the design and implementation of the QA/QC measures developed and the links between the different key activities. Problems/ challenges and preliminary results related to the first list priority substances will be presented.

Keywords: *HBM4EU, QA/QC, chemical analysis, human biomonitoring*

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LECTURES

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SUSPECT AND NON-TARGETED SCREENING OF CHEMICALS OF EMERGING CONCERN FOR HUMAN BIOMONITORING AND ENVIRONMENTAL HEALTH STUDIES: CURRENT CAPABILITIES, PROMISES, AND CHALLENGES

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In connection with the new conceptual and methodological paradigms associated to the exposomic area, large scale suspect and non-targeted screening (NTS) approaches based on current and future generations of instrumentation dedicated to chemical profiling today open the door to holistic characterisation of biological samples. This revolution permits the simultaneous detection of a large number of chemical descriptors, among which markers of human chemical exposure. These markers are of interest for exposure assessment, biomonitoring and environmental health studies. Even more promising are the capabilities offered by these screening approaches for detecting Chemicals of Emerging Concern (CECs), for both supporting new research hypotheses and early warning support to policy. However, while of already significant and growing importance in the environment and food safety areas, respectively, this new field of suspect and non-targeted approaches remains poorly addressed in the field of human biomonitoring. In this context, the present work is proposing a review of the basic principles, current capabilities, promises and challenges of suspect and non-targeted screening approaches in the specific field of human biomonitoring studies. All pre-analytical to post-analytical steps of these workflows will be addressed. From this general picture, the development and harmonization activities conducted on this topic in the framework of the Human Biomonitoring for the European Union (HBM4EU) initiative will be emphasised. In particular, a focus on halogenated markers of exposure will be used to illustrate the capabilities of NTS to identify relevant markers of exposure and toxicological concern from human matrices including human milk, through the different steps of the analytical workflow from sample preparation, data acquisition and data processing.

Keywords: *human biomonitoring, suspect screening, non-targeted screening, HBM4EU*

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LECTURES

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INFLUENCE OF DIGESTION ON CONSUMER EXPOSURE TO PCBS IN MEAT

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In a risk assessment perspective, this work aims to assess the bioaccessibility of polychlorinated biphenyls (PCBs) in meat according to fat level, cooking intensity and physiological differences related to the age of the consumers. A standardised *in vitro* static digestion protocol was set up and coupled with extraction, clean-up and GC×GC-TOF/MS multiresidue analysis to monitor the fate of the 18 most relevant PCBs in meat during digestion. Starting with intentionally contaminated meat, average PCB bioaccessibility was 26 ± 2% in 11% fat meat. PCB bioaccessibility varied inversely with the fat level of meat, increasing to 48 ± 2% in 5% fat meat. The age of the consumer was a further key factor since PCB bioaccessibility decreased to 8 ± 1% and to 17 ± 2% in *in vitro* digestion physiological conditions mimicking infants and elderly, respectively. By contrast, meat cooking was shown to have less influence on PCB bioaccessibility, although intense cooking significantly decreased it to 23 ± 3%. The validity of the bioaccessibility data obtained with spiked meat is discussed in the light of bioaccessibility measurements carried out in naturally contaminated meat samples. Finally, a modular Bayesian approach (Tressou et al., 2017) using the PCB bioaccessibility data was implemented for determination of the mean uptake distribution according to fat level, cooking mode and consumer's age.
Tressou, J., Abdallah, N. B., Planche, C., Dervilly-Pinel, G., Sans, P., Engel, E., & Albert, I. (2017). Exposure assessment for dioxin-like PCBs intake from organic and conventional meat integrating cooking and digestion effects. *Food and Chemical Toxicology*, 110, 251-261.

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LECTURES

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IDENTIFICATION OF NOVEL RED MEAT-ASSOCIATED COMPOUNDS INVOLVED IN WESTERN CHRONIC DISEASES USING UNTARGETED POLAR METABOLOMICS AND LIPIDOMICS IN COLON CELL LINES AND RAT AND PIG INTESTINAL TISSUE

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The consumption of red as opposed to white meat has been associated with diabetes mellitus type II, cardiovascular disorders and particularly colorectal cancer (CRC). Thus, it is of paramount importance to investigate the compositional differences between red and white meat as well as the metabolic products that result from their digestion, allowing to gain more insights in pathogenesis of the abovementioned diseases. Whereas the nutritional composition of red and white meat has already been analyzed in detail, gastro-intestinal end product formation and absorption remain unexplored. Yet, exactly the latter is crucial for understanding the potential contribution of red meat to disease development. Therefore, we set out to elucidate red meat-associated mechanisms, initiated in colon cells upon gastro-intestinal digestion and absorption of meat, by applying a validated mass spectrometry-based polar metabolomics and lipidomics fingerprinting method. Owing to its untargeted and unbiased nature, this workflow allows pinpointing novel molecules associated with red meat intake and linking gastrointestinal metabolites with metabolic profiles of colon cells.
To do so, both *in vitro* and *in vivo* experiments were performed, whereby colon cell lines (the cancer cell line HT29 and immortalized, non-cancer cell line CCD 841 CON) were incubated for 24h with human in vitro colon digests (obtained from three human volunteers) of beef or chicken meat, whereas rats (n = 20) and pigs (n = 32) were fed a red or white meat-based diet. More specifically, pig's feed consisted of red/processed or white meat in addition to a western or recommended background diet that were representative for average human dietary intake.
Red meat intake was specifically associated with increased levels of L-carnitine, acylcarnitines and 3-dehydroxycarnitine in gastro-intestinal colon digests, intracellular extracts and colon tissue. Also, trimethylamine-N-oxide (TMAO) was elevated upon red meat intake in rat colon tissue samples. In vitro colon digestions with L-carnitine have demonstrated that 3-dehydroxycarnitine, which is much more abundant in red meat compared to white meat, can be transformed into 3-dehydroxycarnitine and trimethylamine. In the liver, the latter compound is metabolized into TMAO that has been associated with atherosclerotic plaque formation, inflammation and oxidative stress.
To conclude, by linking gastro-intestinal with colon cell metabolic profiles using a comprehensive metabolic fingerprinting approach, we have identified new red-meat associated metabolites that may have relevance to Western diseases and could serve as novel biomarkers and/or nutri-/therapeutic targets.

Keywords: *red meat consumption, western diseases, comprehensive metabolomics/lipidomics profiling, colon tissue, colon cell lines*

Acknowledgement: *This research was supported by the Institute for the Promotion and Innovation through Science and Technology in Flanders (IWT, project n° 151535) Vlaanderen (IWT merged with the Flemish Institute for Scientific Research (FWO) in 2016).*

LECTURES

L93

DIGITALIZATION OF FOOD SIDE STREAM USING LC-SWATH-MS

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A substantial part of the raw material processed by food industry remains as unwanted by-products. On the one hand, these side-streams can cause disposal issues, on the other hand, they are an unexploited source of valuable compounds such as secondary plant metabolites, cell wall components or fibres. To enable companies to utilize the ingredient potential of such waste streams, efficient tools for metabolite profiling with high selectivity and robustness are required, as a broad chemical space of target analytes from a plethora of matrices needs to be captured. With the aim to combine the selectivity and the quantitative character of targeted tandem mass spectrometry and the compound coverage achieved by untargeted high-resolution MS, data-independent SWATH-MS (sequential window acquisition of all theoretical mass spectra) was implemented as principal analytical tool. Starting with the generation of a spectral database originating from a natural product metabolite library and the development of a bead-beater based high-throughput workup for solid materials, optimization of liquid chromatography and MS parameters such as SWATH window widths finally led to a highly versatile profiling platform. Validation was performed by means of spiking selected individual metabolites from various compound classes into extracts generated from materials such as seeds and husks. Furthermore a data analysis workflow facilitating the generation of *pseudo*-MRM chromatograms from fragmentation spectra and high-resolution SWATH-MS data was adapted to enable a targeted data extraction similar to traditional LC-MS/MS. Application of this methodology on a large set of industrial food side-streams will deepen the compositional knowledge catalyzing the identification of relevant side-streams for valorization. Finally the reuse of side-streams will help to reduce food waste and improve resource efficiency, which is imperative to achieve a sustainable circular economy.

Keywords: *SWATH-MS, food side-stream valorization, data-independent LC-MS, metabolomics*

Acknowledgement: *Work on this project was supported by EIT Food as part of the project "Digital Markeplace for Side Streams"*

LECTURES

L94

IDENTIFICATION OF HIGH ADDED VALUE MOLECULES FROM THE WASTES OF TUNA FISHERY INDUSTRY THROUGH MS BASED ANALYTICAL METHODS

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Tuna fish processing industry produces more than 50% of waste and by-products (meat, scales, head, viscera and roes) that are usually discarded or used to produce low added value products such as fish meals. However, these waste materials still contains molecules essentials for the human diet, such as essential metals, vitamins, omega-3 fatty acids and aminoacids and proteins of high nutritional value. The re-use of such wastes for the production of dietary supplements for human consumption would perfectly fit with the concept of *Blue Economy*, defined as the sustainable use of ocean resources for economic growth, improved livelihoods, and jobs while preserving the health of ocean ecosystem. Moreover, waste products revenue will be a key factor in maintaining long-term profitability of industrial food processing.
In this work, Yellowfin tuna (*Thunnus albacares*) was used as a model for fish processing by-products, as it is a large epipelagic species widely distributed in the tropical and subtropical waters of the major oceans. We used LC-MS/MS to investigate the presence of Vitamin D and the total lipid composition of different fish part usually considered as waste from the fishery industry (e.g., muscle, skin, heart). Moreover, we performed a semi-quantitative analysis of the fatty acid content of such wastes, with particular interest on docosahexaenoic acid (C22:6n3, DHA) and eicosapentaenoic acid (C20:5n3, EPA), using a GC-FID-MS/MS instrument. Both these instruments were coupled to fully automated preparative stations able to perform the extraction of the lipid fraction from the samples and the trans-esterification of the fatty acids (for the GC analysis) prior to the injection in the instrument, increasing the reproducibility of the analysis and minimizing the errors due to the handling of the samples by the operators.
Finally, we also quantified the amount of essential and heavy metals using ICP-MS, after mineralization of the wastes. The ICP-MS method was validated using certified
Referencematerials.

Keywords: *mass spectrometry, omega-3, vitamin D, blue economy, food supplement*

Acknowledgement: *The authors thank Shimadzu and Merck Life Science KGaA for the continuous support.*

LECTURES

L95

DIRECT-MS SCREENING & IDENTIFICATION OF E-WASTE IN FOOD CONTACT ARTICLES

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Polymer recycling is an important mechanism to reduce pollution, energy use, and waste. Mixed recyclate streams, 3rd-party manufacturing, and difficulties with spectroscopic polymer quality checks in black recyclate all contribute to hazardous substances contaminating new polymeric products, products which may be used in more intimate human contact (toys, food articles). Waste electronics and electrical equipment (WEEE) have been documented to sporadically contaminate food contact articles with brominated flame retardants (BFRs). Because WEEE contains multiple hazardous substances not approved for food contact use (heavy metals, rare-earth elements, Sb/BFRs) contaminated food contact articles may represent a sporadic, but potentially significant and poorly characterized food safety risk. The frequency and extent of WEEE contamination of food contact articles is necessary to place this relative risk in context. Because the frequency of WEEE contamination is not expected to be high, and because the exact identity of the hazardous and non-allowed substances is key to understanding the relative risk, a sensitive and specific screening method is needed to detect food contact articles containing WEEE, and identify the BFR. Identifying WEEE contamination quickly is not only important to forming a risk characterization, but also to identify the likely mechanism and source of polymer contamination. Thus, identification of more than one WEEE component simultaneously would be more useful for a screening technique.
X-Ray Fluorescence (XRF) for bromine has been the primary screening approach. However, XRF sensitivity/accuracy is widely variable depending upon the instrumentation (benchtop vs handheld, incident vs direct, energy vs wavelength), its operation, and sample-to-sample morphology. XRF also does not specify the molecular form of the bromine. Extraction GC-MS and LC-MS techniques may identify some BFRs, but not others, and can suffer variable extraction/sensitivity between different polymer samples. Extraction steps also lower sample throughput. ICP-MS/OES techniques can quantify antimony or bromine, or heavy metals, or rare-earth elements, but not BFRs and are not quick methods of analysis.
Direct Analysis in Real Time (DART), a thermal desorption and chemical ionization source for mass spectrometry, has recently been shown to correctly identify several BFRs in WEEE impacted consumer goods. There was also evidence Sb could be detected in some articles. In this work we developed DART-MS analysis for a broader sampling of retail food contact articles, identified DART-MS ions specificity for 20 different BFRs and potential interferants, and benchmarked DART-MS screening performance by comparing DART-MS to GC-MS, LC-MS, XRF, ICP-MS, and laser ablation ICP-MS (LA-ICP-MS) of samples and reference materials. The likely origin/mechanism of DART antimony ions and their limits for antimony screening in food contact articles will be presented.

LECTURES

L96

INVESTIGATION OF MIGRATING SUBSTANCES FROM TEXTILE USED AS FOOD CONTACT MATERIAL

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With the polarized debate about paper and board packaging materials, food contact materials (FCM) made from fabrics/textiles has gain importance on the market. This trend is expected to rise with the prohibition of the Walloon and Brussels-Capital Region for single use plastic bags. In this context, the aim of the research was to evaluate the safety of these "new" food fabrics.
A simple ultra-sound assisted extraction of substances from the fabrics was done with acetonitrile to avoid any compound losses. Both a GC-MS full scan screening method and targeted quantitative assays were used to determine the levels of substances migrating from food fabrics. These protocols were carried out for 43 food fabrics carefully selected on the Belgian market. Well known food contaminants such as butylated hydroxy toluene, phthalates and photo-initiators were detected, but at very low levels. On the contrary, one bread bag sample contained higher amounts of bisphenol A.
It is well characterized that the migration of contaminants from food contact materials in foodstuffs is challenging due to the complexity and large variety of foodstuffs. To overcome this issue, migration experiments were carried out using food simulants. Consequently, the bread bag was brought into contact with the simulants for bread using accelerated time and temperature conditions to simulate migration. Analysis of the simulants afterwards showed no significant migration for Bisphenol A. Although this particular bread bag contains Bisphenol A, the migration thereof is limited and the article is considered to be food safe.
In conclusion, 43 food fabrics surveyed on the Belgian market were investigated for the migration of possible contaminants towards foodstuffs. Different compounds were detected but measured levels are very low and do not trigger safety concerns.

Keywords: *food contact materials, textile/fabric, screening, migration, market survey*

LECTURES

L97

SOLID STATE FERMENTATION OF BLACK SOLDIER FLY PUPARIUM, PREPUPAE AND ADULTS: TAILORING THE MOLECULAR COMPOSITION OF A RESIDUAL BIOMASS TOWARDS ANTIMICROBIAL PROPERTIES

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In a continuous search for new strategies for valorizing waste, *Hermetia illucens* (Black Soldier Fly, BSF) is a non-infesting and non-pathogenic insect that is often used in the bio-conversion processes of residual biomasses. Black soldier fly larvae grow well on organic wastes producing molecules with high biological value (proteins, lipids, chitin). During these bioconversion processes, large amount of insect-derived wastes (pupal membranes and adult insects at the end of life cycle) are also produced and accumulated. In this work, for the first time, these insect-derived waste materials were investigated in order to explore their antimicrobial potential, using solid state fermentation with Lactic Acid Bacteria (LAB). The difference in the molecular composition between fermented and unfermented insect-derived waste materials was studied, focusing on fatty acid profiles, protein fraction and chitin. Solid state fermentation was conducted with 3 LAB strains isolated from food, belonging to *Lactobacillus* species. The antimicrobial properties of fermented and unfermented biomasses were tested against foodborne pathogens. Their grow was monitored by plate count agar in selective medium in presence and absence of insect-derived biomasses. The bulk evaluation of fat, protein, moisture and ashes composition of insect-derived biomasses was carried out using standard procedures (AOAC, 2002). Then, fatty acid profiles were determined by GC-MS, whereas total amino acid analysis and the formation of chitin oligomers and glucosamine monomer was carried out by LC/ESI-MS methodologies, Results showed that the lipid fraction is particularly influenced by fermentation: the fermented biomass had a higher percentage of lipids and a more complex fatty acid profile as compared to unfermented mass. Also the protein fraction seemed to change upon fermentation: it was possible to observe a decrease in sulfur amino acids and a significant increase in tryptophan, probably due to the activity of the LABs involved in fermentation. Moreover, fermented insects show a high antimicrobial activity against foodborne pathogens with a reduction of 5 Log UFC/g. This study shows how fermentation can valorize insect-derived waste, exploiting the typical metabolic behavior of fermenting bacteria. Moreover, the fermentation process seems to favor the development of bioactive molecules, such as natural antimicrobial metabolites. Further studied are undergoing in order to identify the exact compounds exerting the antimicrobial behavior.

Keywords: *black soldier fly, LAB fermentation, molecular composition, antimicrobial behavior, chitin oligomers*

LECTURES

L98

AN UPDATE ON THE CURRENT ACTIVITIES AND MAIN CHALLENGES OF THE EU REFERENCELABORATORY FOR MARINE BIOTOXINS

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The responsibilities, tasks, duties and requirements of the EU Reference Laboratories are clearly specified in the EU Regulation (EC) No 882/2004 on official controls. The EU Reference Laboratories (EURLs) aim to ensure high-quality, uniform testing in the EU and support Commission activities on risk management and risk assessment in the area of laboratory analysis with the finally objective of ensuring the human health protection. The area of the EU Reference Laboratory for marine biotoxins covers not only the control of these contaminants naturally present in bivalve molluscs as well as the harmonization of practices for the phytoplankton control as the source of the contamination, but also a recent new activity focused on the harmonization of the guides for the microbiological control of production areas which has been inherit from the former UK EURL on microbiological contamination of the bivalve molluscs as a consequence of BREXIT. The intense activity of the EURLMB has been increased not only due to the increased competencies, but also due to the challenging times for the natural contaminants affected by environmental conditions, in particular climate change as one of the responsible for the worldwide expansion of this kind of contaminations. This presentation will be focused on a revision of the current activities of the EURLMB and the main challenges as a consequence of the presence of new or emerging marine biotoxins in the EU. Efforts in terms of ensuring the EU harmonized response for the reliable and efficient characterization of these risks will be presented and discussed.

Keywords: *EU reference laboratory*

LECTURES

L99

THE EU REFERENCE LABORATORY FOR MYCOTOXINS AND PLANT TOXINS: NEW TASKS COME WITH NEW CHALLENGES

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March 1st, 2018, Wageningen Food Safety Research (formerly RIKILT) was designated as the new EU Reference laboratory for mycotoxins and plant toxins. As EURL for mycotoxins it continues the tasks conducted by the Joint Research Centre in Geel, but the plant toxins are an important extension to the mandate, as laid down in Regulation (EU) 2017/625. Tasks and activities of the EURL MP are to provide technical and scientific assistance on analysis to the National Reference Laboratories in the EU member states and to the European Commission.
Legal limits on mycotoxins and plant toxins in food and feed in the EU are laid down in Regulation (EC) No 1881/2006, Directive 2002/32/EC and Recommendation 2006/576/EC and their amendments. The EU regulation on mycotoxins focusses on aflatoxins, deoxynivalenol, zearalenon, ochratoxin A, T2/HT2, fumonisins and ergot sclerotia in food and feed and on citrinin and patulin in food. EU harmonised food regulation focusses on the inherent plant toxins erucic acid and hydrocyanic acid, and on tropane alkaloids from co-harvested weeds. For the near future new or extended EU legislation is foreseen for the mycotoxins ergot alkaloids; and for the plant toxins pyrrolizidine alkaloids and tropane alkaloids. Regulation of alternaria toxins, DON metabolites and opium alkaloids is being considered as well.
The extension of the mandate comes with new challenges, one in particular with respect to the pyrrolizidine alkaloids (PAs). These genotoxic plant metabolites form a complex group of compounds that are found in nature in a very wide array of structures, including many isomeric analogues and two different chemical forms (tertiary amine and N-oxide). They can be present as contaminants in a broad variety of plant-based products such as herbal teas, (kitchen) herbs, cereals and food supplements and too often at relatively high concentrations. Certain animal derived products (honey, pollen, milk) can be contaminated as well, but often at lower levels. EU regulation on PAs in herbal teas, herbs and food supplements is in its final stage of development. The introduction of legislation requires setting up official control programs by the member states to enforce maximum limits and this in turn requires the availability of robust analytical methods. In this respect the number of PAs to be regulated is a relevant issue. The list will contain at least 21 substances, but this may increase to 35 as for many of the 21 PAs, isomeric analogues have been identified that may or may not co-elute, depending on the chromatographic conditions of the analytical method.
The results of a proficiency test, on deoxynivalenol and its metabolites conducted in 2018, and a research study on pyrrolizidine alkaloids, organised by the EURL-MP in 2019, to evaluate the capabilities of NRL laboratories for these groups of toxins, will be discussed in more detail. New work items for 2020 will be presented as well.

Keywords: *EURL-MP, mycotoxins, plant toxins, pyrrolizidine alkaloids, EURL-NRL network*

Acknowledgement: *The authors acknowledge the funding support from the European Commission, DG SANTE*

LECTURES

L100

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

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The EURL for pesticide residues in Cereals and Feedingstuff in Copenhagen (EURL-CF) has since 2007 organized 12 proficiency tests (EUPTs) on cereals and feeding stuff. The PTs are offered to EU and EFTA National Reference Laboratories and Official Laboratories both in EU and from Non-EU Countries. This has resulted in a high number of participants in each EUPT (up to 178). Barley, maize, oat, rice, rye, wheat, hay and compound feed for hens have been used as test material. The cereals have been grown and field treated with pesticides primarily in Denmark, only rice was produced outside, namely in Brazil. The test materials have trough out the years contained residues of 76 different pesticides, either incurred or spike post-harvest.

Robust statistics were used to calculate the assigned values and the standard deviation (Algorithm A mean and standard deviation). The participants were primarily evaluated on the correctness of the results and if they submitted false negative or false positive results. The participants received z scores for the individual results and a summed average z scores, AZ^2. However, the AZ^2 score were only calculated for Category A laboratories that analysed for more than 90% of the pesticides on the Target Pesticides List and detected more than 90% of the pesticides present in the PT test material.

The overall PT results show that the robust standard deviation of the results has decreased from 31% to 17 % which indicates that the monitoring data produced by the European laboratories are more comparable now than earlier. The number of acceptable z scores has increased to >90%. The number of good AZ^2 scores for laboratories in Category A has also increased significantly from 70 to >90 %. The PTs elucidated that for many pesticides residues, the flour samples should be added water before the extraction with solvent. Otherwise, the extraction efficiency was low. This observation has resulted in recommendation in the SANTE 11945/2015: Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed, and now almost all the laboratory has incorporated this in their method.

Besides the overall outcome of the analytical performance achieved from the EUPTs, the presentation will focus on the difference between pesticides scopes and performance obtained by National reference laboratories (NRLs) and Official Laboratories (OfLs). In general, the NRLs analyse for a broader scope of pesticides. However, no significant difference can be seen in the number of acceptable z scores. Finally, factors affecting the development, positively and negatively, will be discussed.

Keywords: *EURL, proficiency tests, pesticide residues, cereals and feeds*

LECTURES

L101

EUROPEAN UNION REFERENCE LABORATORY FOR PROCESSING CONTAMINANTS

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The purpose of the European Union Reference Laboratory for Processing Contaminants (EURL-PC) is to provide the National Reference Laboratories (NRLs) with analytical methods for processing contaminants in foods, to ensure their analytical quality, disseminate relevant knowledge to the NRLs and advise to the Commission. Finally, the EURL-PC is part of the European contingency for food safety with its scope.
A key part of the task is proficiency test to develop and document the analytical performance by the NRLs and official control laboratories as described in EU 2017/625 [1] and EU 2004/882 [2].
Most of the activities of the EURL-PC aim to implement:

- Efficient methods for determination of processing contaminants
- Boosting the communication in the EURL, NRL and official laboratories network
- Strengthen education and training
- Address knowledge gaps
- Ensuring harmonization in analysis performance

The National Food Institute at Technical University of Denmark (DTU Food) hosts the EURL-PC.
Focus for the Processing Contaminants in foods are compounds like acrylamide, furan and alkylated furans, 3-MCPD, 3-MCPD esters and glycidyl esters and PAH as these compounds are of health concern. Analytical method used for determination of these compounds includes mainly LC- and GC-mass spectrometry.

[1] Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products.
[2] Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

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

LECTURES

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RECENT ADVANCES IN CERTIFIED REFERENCE MATERIALS FOR FOOD ANALYSIS AT THE JOINT RESEARCH CENTRE OF THE EUROPEAN COMMISSION

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Certified Reference Materials (CRMs) are highly demanded from all fields of analysis. They are internationally recognised as the most suitable tools to control the quality of analytical data. It is however not always possible to find appropriate CRMs in the market for particular applications, especially when searching for matrix reference materials.
Over the past years the Joint Research Centre of the European Commission (EC-JRC) has produced CRMs mainly to support the proper implementation of European legislation, such as the genetically modified organisms. CRMs are furthermore produced in response to other needs, for example, in response of the emerging food crisis situations. The contamination of dioxins in food occurring in Belgium in the late nineties triggered an intensive activity in the topic and the production of dedicated CRMs as well as the fipronil crisis from 2017 which lead to initiation of the production of the egg powder material.
Currently more than 660 reference materials cover diverse fields of application where clinical, food, environmental and engineering materials are the core areas. Among the CRMs available at the JRC catalogue https://crm.jrc.ec.europa.eu/, approximately 26 % are related to food analysis. A large variety of food matrices, including drinking water, are certified for the content of elements, GMOs, contaminants, natural toxins, natural composition, drugs, etc. Some examples comprise the vitamins in Brussels sprouts, pesticides in milk powder, trace elements in brown bread or ethanol in wine and beer.
Recent developments cover heavy metals in dark chocolate, pesticides in different foodstuffs like cucumber, soya beans and pork fat, or GMO Bt11 in maize. Furthermore CRMs of mycotoxins in maize powder and in pistachios, veterinary drugs in pork muscle and PFASs in Pike-Perch were developed. These CRMs are all produced under ISO 17034 requirements. As an outlook are among others CRMs in the food authenticity field.
Practical aspects on the selection and use of CRMs will be illustrated by using recently produced CRMs for food analysis.

LECTURES

L103

PORTABLE AND CONSUMER SPECTROSCOPIC DEVICES FOR ADVANCING FOOD SAFETY AND AUTHENTICITY ENDEAVORS

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The last two decades have seen many technological innovations which have been advantageous not only to the general population but also to scientific instrumentation. Portable analytical devices in particular capitalize on the wireless communication, touchscreen displays, virtual keyboards, GPS capability, rapid processors, fingerprint scanners, and advanced cameras commonly found in standard smartphones. In addition, advances in spectrometer miniaturization have transitioned from the scientific realm into consumer-focused devices. Both analytical-grade and consumer-marketed technologies could be potentially harnessed by food safety professionals for improved screening of foods and dietary supplements; however, device efficacy must first be understood. As such, we have been exploring the feasibility of vibrational spectroscopy for evaluation of FDA regulated products using both laboratory-grade and lower-cost devices. Our primary research focus has been on those products where field testing would prove most beneficial, such as those that may be prone to mislabeling or those that are at high risk for economic adulteration. This presentation will overview near-infrared (NIR) and Raman portable spectrometers as well as two "pocket-sized" micro-spectrometers currently under evaluation in our laboratory. Device advantages and limitations will be highlighted through brief case studies of FDA regulated products (e.g., milk powder authentication, marine oil dietary supplement label verification, and seafood decomposition evaluation) to elucidate where the technology shows promise for supporting advances in food safety.

Keywords: *portable device, authenticity, food fraud, screening, chemometrics*

LECTURES

L104

MACRO-SCALE RAMAN IMAGING FOR FOOD SAFETY EVALUATIONS

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Integrated commercial Raman systems generally conduct imaging and spectroscopy measurements at subcentimeter scales. Such small spatial scale is not suitable for inspecting food samples with large surface areas. To remedy the lack of macro-scale Raman chemical imaging (RCI) tools for food safety and quality research, researchers at the USDA Agricultural Research Service (ARS) have developed two point-scan RCI systems, one using a 785 nm point laser and the other a 1064 nm point laser. Each system uses a whiskbroom method for hyperspectral Raman image acquisition from food samples carried by a two-axis positioning table. In addition, a more efficient line-scan RCI system was also developed to implement high-throughput Raman imaging using a 785 nm line-laser. The line-scan system accumulates hyperspectral data using the pushbroom method for samples on a one-axis translation table. Dispersive Raman spectrographs are used in both the point-scan and line-scan systems, which can all be configured to backscattering RCI mode for surface inspection and to spatially offset Raman spectroscopy (SORS) mode for subsurface inspection. LabVIEW software and MATLAB programs developed in-house are used to automate system control and data acquisition, and image data processing and analysis, respectively. The ARS Raman systems are flexible and versatile, and have been used to evaluate safety and quality attributes of a variety of various food and agricultural products. Examples are presented of RCI and SORS applications that have been investigated using these laboratory systems, including detection of chemical adulterants mixed in food powders and through-package inspection of foods and ingredients.

Keywords: *chemical imaging, hyperspectral imaging, food safety, food adulterants, raman imaging*

LECTURES

L105

RAPID DETECTION OF FOREIGN OBJECTS IN FRESH-CUT VEGETABLES USING REAL-TIME SPECTRAL IMAGING

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Fresh-cut vegetables may contain foreign objects such as small plastic pieces or biological contaminants (bugs and worms). In order to comply with requirements for product safety and maintain consumer confidence, there is a need for high-throughput, non-invasive technique for foreign objects detection in and real-time sorting of fresh-cuts. In this work, a liquid crystal tunable filter (LCTF) based multispectral fluorescence imaging system in the spectral region of 420–730 nm was developed for the detection of foreign objects in the fresh cuts. A 365 nm fluorescent light was used to illuminate the samples over a wide area. A custom-built software was developed to acquire spectral images which also facilitate the real-time visualization and integration of classification algorithms. Spectral images throughout the whole spectral region were first collected for both fresh-cuts and foreign materials. The optimal wavebands for discriminating between foreign materials contaminated and sound fresh-cuts were investigated using analysis of variance (ANOVA) method. The results suggest that only five wavebands can be used to detect foreign objects on the fresh-cut surface. Therefore, only five waveband images were collected for each sample and consequently processed to visualize the presence of any aforementioned foreign material on the fresh-cut surface. A conveyor belt and sorting unit were than synchronize with sensing module to transport the input fresh-cuts and to sort the foreign object included fresh-cuts, respectively. The overall results demonstrated that the developed system has a potential for rapid and real-time detection of different kinds of foreign materials in fresh-cuts and further incorporating a sorting unit, sound fresh-cuts can be separated.

eywords: *food safety, foreign objects, vegetables, spectral imaging, rapid measurement*

LECTURES

L106

EVALUATION OF AUTOMATED SAMPLE PREPARATION FOR MYCOTOXIN ANALYSIS IN FOODS

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Interest in replacing manual operations using robotic tools justifies the need of automation systems that can handle repetitive and high-volume tasks. In the field of food analysis, automated sample preparation is still in its early stage, though such practices have been commonly used in areas of drug screening and routine clinic sample analysis. For food analysis, samples need to be processed prior to instrumental measurements through multiple steps such as homogenization, weighing, solid-phase extraction (SPE), solvent exchange, shaking, heating/cooling, evaporation, filtration, centrifugation, and derivatization. This makes automated sample preparation a challenge as each step requires different tools, labware, and sample vessels. A highly automated sample preparation workflow requires integration of required devices and capability of transportation and storage so that samples can be processed and moved through each sub-step. In this proof of concept study, an automation system, Chemspeed Swing XL®, was evaluated focusing on sample preparation for the determination of mycotoxins using liquid chromatography–mass spectrometry (LC-MS). The system is equipped with two robotic arms, various modular tools and storage racks, and can conduct transport, gravimetric and volumetric dispensing, shaking, capping/decapping, filtration, and centrifugation without human assistance. Fortified corn, peanut butter, milk, and certified reference materials were used to evaluate the performance of the system. Our results suggest that automation is a promising alternative to manual operation and has potential for application to food analysis. Furthermore, automation may assist laboratories achieve a high degree of standardization for routine mycotoxin analysis.

LECTURES

L107

MYCOTOXINS THAT CHELATE: A TOOL FOR PROBING TOXIN/METAL INTERACTIONS

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Certain toxic secondary metabolites of fungi (mycotoxins) chelate metals. In particular, ochratoxin A (OTA), citrinin (CIT), cyclopiazonic acid (CPA), kojic acid, and tenuazonic acid (TeA) are known chelators. These mycotoxins, which have a wide range of structures, also have widely different targets, including the liver, kidney, immune system, and the nervous system. OTA, CIT, CPA, and TeA are found in a variety of foods including cereal grains, meats and cheeses. Kojic acid is used in cosmetics as a depigmentation agent. Certain of the lanthanide metals, such as terbium and europium, can fluoresce. This fluorescence is generally low but can be facilitated by interaction with molecules that transfer energy to the metal. This property has made europium and terbium useful as fluorescent probes of molecular interactions. Three of the chelating mycotoxins (OTA, CPA and TeA) are able to form complexes with terbium and europium. When such complexes form light can be absorbed by the toxin and transferred to the lanthanide, enhancing the fluorescence of the metal. The effect of OTA was minor. The relatively poor enhancement with OTA was likely caused by the loss of energy from "self-fluorescence". However, both CPA and TeA were able to greatly enhance the fluorescence of lanthanides. Terbium (Tb^{3+}) was the more effective of the two lanthanides. The influence of environmental parameters on the formation, and fluorescence from, the mycotoxin-lanthanide complexes was examined in detail. The type of solvent used, the water content, and pH were all important contributors to signal development. Optimal conditions consisted of 90% methanol with 10% aqueous buffer at pH 3-4. The development of model systems of TeA-Tb^{3+} and CPA-Tb^{3+} permitted an exploration of how other metals bind to these toxins. An assay was established wherein other metal cations (Cu^{2+}, Al^{3+}, Au^{3+}, Fe^{3+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}) competed with Tb^{3+} for binding to the mycotoxins. Results indicated that both CPA and TeA interacted best with Cu^{2+}. After Cu^{2+} the metals in oxidation state 3 generally disrupted the interaction better than metals in oxidation state two. The two metals in oxidation state one (Na^{+}, K^{+}) were essentially inactive. Because the inhibition of certain enzymes, such as sarco(endo)plasmic reticulum Ca^{2+}-ATPase is suspected to involve chelation, an understanding of the relative affinity of TeA and CPA for metals may provide insights into the mechanism of action of these toxins.

Keywords: *mycotoxins, fluorescence, lanthanide metals, sensor*

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 agreement between the U.S. Department of Energy and the USDA.*

LECTURES

L108

THE SECONDARY METABOLISM OF ASPERGILLUS FLAVUS: SMALL MOLECULES WITH DIVERSE BIOLOGICAL FUNCTION

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Aspergillus flavus can colonize important food staples and produce aflatoxins, a group of toxic and carcinogenic secondary metabolites. A. flavus also produces many other secondary metabolites and harbors more than 50 putative secondary metabolite biosynthetic gene clusters that have yet to be characterized. Bioactive secondary metabolites that enhance the ability of the fungus to infect crops are of particular interest. We have recently shown that biosynthetic gene cluster 11 in A. flavus encodes for the biosynthesis of aspergillic acid, a toxic hydroxamic acid-containing pyrazinone compound that can bind iron, resulting in a red-orange pigment known as ferriaspergillin. A decrease in A. flavus pathogenicity and aflatoxin contamination was observed when aspergillic acid biosynthesis was blocked during maize seed infection. We have probed the available genomes of Aspergillus species for biosynthetic gene cluster 11 homologs. We find that all species possessing gene cluster 11 produce aspergillic acid or a closely related isomer. We demonstrate that the Aspergillus section Flavi species harboring biosynthetic gene cluster 11 produce a mixture of aspergillic acid, hydroxyaspergillic acid, and aspergillic acid analogs differing only in the amino acid precursors. Interestingly, many Aspergillus section Circumdati species, known mainly for their production of the problematic mycotoxin ochratoxin A, also harbor gene cluster 11 homologs but do not produce aspergillic acid. Instead, these species produce neoaspergillic acid and its hydroxylated analog neohydroxyaspergillic acid, indicating that cluster 11 is responsible for neoaspergillic acid biosynthesis in Aspergillus section Circumdati.

Keywords: *aspergillus flavus, secodary metabolites, aspergillic acid*

LECTURES

L109

PRACTICAL APPROACHES TO THE SINGLE LABORATORY VALIDATION OF ANALYTICAL METHODS IN THE ANALYSIS OF FOOD AND DIETARY SUPPLEMENTS

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Method validation is necessary for demonstrating that an analytical method is suitable for its intended use but it can be a daunting and complicated task, which can fail without proper planning and execution. Considering the importance of method validation, various international organizations and analytical communities have published a number of guidance documents on single laboratory validation. The most widely evaluated performance characteristics include accuracy (trueness), precision (repeatability and intermediate precision), specificity, limit of detection (LOD), limit of quantitation (LOQ), linearity, range, and robustness. The validation process should also cover system suitability and stability of reference standard and sample solutions. This seminar will provide an overview of practical approaches to the validation of targeted quantitative methods with emphasis on the assays employing chromatographic techniques coupled to mass spectrometry. The topics covered will spread from preparation of the validation plan to processing and evaluating the results against the applicable acceptance criteria with real life examples. The target audience are young researchers and PhD students but anyone is welcome to attend, share their experience and bring their questions!

Keywords: *method validation, chromatographic methods*

LECTURES

L110

USING THE POWER IN UNTARGETED ANALYTICAL TECHNOLOGIES FOR UNTARGETED MONITORING AND DIAGNOSIS OF NATURAL SAMPLES

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Contemporary analytical chemistry has unprecedented power in analyzing intact systems, bringing the lab to the sample and the diversity of chemical species that may be simultaneously detected and quantified in the same measurement. This greatly increases the potential value of such analytical technologies, but it also requires step changes in the quantitative analysis of the resulting data; especially when this new analytical potential is used to measure increasingly complex food systems. This is a clear-cut mission for Chemometrics, which uses the novel available knowledge about measurement and the food system, to provide data-driven predictions together with novel insight in the key drivers of chemical variability in such systems. Using this knowledge-supported approach to data-driven modeling has great value in every chemical analysis along the Farm to Fork pathway, encompassing on-line Multivariate Statistical Process Control (MSPC), *in situ* food authentication with handheld technologies, microplastics analysis and diagnosis of metabolic diseases. Many of these highly diverse data analysis solutions can be constructed from adapting and fine-tuning a limited toolbox of data analysis, pre-processing and model validation methods but may provide considerable, sometimes disruptive value to the measurement technology.

LECTURES

L111

TO TARGET OR NOT TO TARGET? DEFINITIONS AND NOMENCLATURE FOR TARGETED VERSUS NON-TARGETED ANALYTICAL FOOD AUTHENTICATION

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Analytical methods that can offer fast, cost-effective and reliable food authenticity testing at several points in the food production and retail chain are urgently requested. Targeted methods such as stable isotope ratio analysis [1] still have much to offer but it is increasingly acknowledged that food is a complex matrix and should thus be treated and analyzed by techniques that can embrace this complexity. The use of non-targeted analytical methods in food authentication has therefore rapidly increased during the past decade. Examples are authentication of fish, olive oil and spices by spectrometry and spectroscopy based techniques [2-3].
The increasing use of non-targeted analyses across several scientific disciplines has brought together a mixture of analytical traditions and terminologies and terms such as profiling, signature, fingerprinting, analytical marker etc. are inconsistently used. Consequently, the scientific literature on food authentication often includes different approaches and a variety of definitions and nomenclature for both targeted and non-targeted analysis.
While non-targeted fingerprinting methods are still taking the initial steps into the food authenticity community much more work is required to validate and harmonize these methods and the associated data. An essential prerequisite is a common understanding of the analytical principles of targeted versus non-targeted food authentications. At the conference, novel definitions and nomenclature of targeted and non-targeted authentication methods will be presented as a first step towards harmonization. Biological, chemical, and microscopy-based examples of targeted and non-targeted approaches will be presented while discussing the associated possibilities and limitations for analytical food authentication.

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[2] T. Wulff, M.E. Nielsen, A.M. Deelder, F. Jessen, M. Palmblad, Authentication of Fish Products by Large-Scale Comparison of Tandem Mass Spectra, Journal of Proteome Research, 12 (2013) 5253-5259.
[3] M.-A. Poiana, E. Alexa, M.-F. Munteanu, R. Gligor, D. Moigradean, C. Mateescu, Use of ATR-FTIR spectroscopy to detect the changes in extra virgin olive oil by adulteration with soybean oil and high temperature heat treatment, Open Chemistry, 2015.

Keywords: *analytical chemistry, food authentication, non-targeted, targeted, definitions*

LECTURES

L112

STATISTICS BEHIND QUALITATIVE CHROMATOGRAPHY ANALYSIS IN FOOD ANALYSIS: METHOD VALIDATION AND METHOD PERFORMANCE MONITORING.

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Nowadays, statistics tests are used across analytical quantitative method lifetime, from design of experiment in method development, uncertainty evaluation during validation but also control chart and z-score to monitor performance throughout lifetime of the method. However, statistics evaluation in qualitative analysis for method validation and method performance monitoring remains sporadic. Nevertheless, to reduce cost, extend scope and efficiency, more and more qualitative analysis is validated. Therefore, uncertainty associated with targeted and untargeted qualitative results must be guaranteed. This presentation investigates the different statistical evaluation of targeted and untargeted qualitative methods to guarantee statistical control of qualitative results throughout analytical method lifetime.

To guarantee statistically qualitative results, statistics evaluation is essential during method validation. In targeted qualitative analysis, decision limit, CCα, and detection capability, CCβ, as described by Currie in 1968, evaluate respectively statistics risk alpha, false positive, and risk beta, false negative. Though, statistics evaluation for targeted qualitative analysis are more than 60 years old and requires simple design and calculation, generally limit of detection only is evaluated during validation. Indeed, the limit of detection is often associated to the detection capability and allow evaluation of the false negative. But, validating the limit of detection only does not evaluate the risk of false positive and may have a financial impact for any laboratories. In addition, the detection limit is not always equal to the limit of detection and false negative evaluation may be biased. At least, for untargeted analysis, false positive and false negative cannot be evaluated. Therefore, conclusion in untargeted analysis must include the detection technique used as part of the statistics risk.

To guarantee statistics risk observed during validation, method performance must be monitored throughout lifetime of the qualitative analytical method. Generally, control quality used blank analysis, limit of detection standard or extracted sample to monitor the method performance but statistics risks are not often evaluated. Indeed, plotted quality control in control chart would provide statistical control of the results. In addition, proficiency test can also be used to recalculate false positive and negative risks.

LECTURES

L113

AN INTERNATIONAL COLLABORATION FOR BUILDING A CONSOLIDATED DATABASE OF CCS VALUES FOR THE CHARACTERIZATION OF STEROIDS BY ION MOBILITY MASS SPECTROMETRY. APPLICATION TO CHEMICAL FOOD SAFETY

Maykel Hernández-Mesa*(1), **Valentina D'Atri**(2), **Gitte Barknowitz**(3), **Mathieu Fanuel**(4), **Julian Pezzatti**(2), **Nicola Dreolin**(3), **David Ropartz**(4), **Fabrice Monteau**(1), **Serge Rudaz**(2), **Sara Stead**(3), **Hélène Rogniaux**(4), **Davy Guillarme**(2), **Gaud Dervilly**(1), **Bruno Le Bizec**(1)

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The recent commercialization of ion mobility spectrometry (IMS) coupled with mass spectrometry (MS) has opened new possibilities for the analysis of molecules, with interesting perspectives for food quality applications.[1] IMS can be easily integrated in traditional liquid chromatography (LC)-MS workflows, providing an additional separation dimension.[2] Consequently, both selectivity and peak capacity are generally improved. Isobars and isomers, which are not resolved in the LC chromatographic dimension and nor can be distinguished by MS, can be potentially separated by IMS. Sensitivity is enhanced because the analytes of interest are separated from background noise, increasing signal-to-noise ratio (S/N). Moreover, IMS provides additional information to retention time indices and mass spectra, the so-called Collisional Cross Section area (CCS). Despite the proposal to include CCS as an identification point (IP) in the current context of Regulation 2002/657/EC revision,[3] the use of CCS values as an identification criterion is still not more widely implemented due to the lack of both CCS libraries and robustness criteria for the measurement of this molecular descriptor.
This study explores the potential of IMS to extend the current boundaries of LC-MS workflows intended for the analysis of steroids in meat producing animals. For phase II steroid metabolites in bovine urine, the observed S/N was increased between 2 and 7-fold when IMS was integrated in our LC-MS method.[4] In addition, we have recently reported a CCS database for 300 steroids characterized by Travelling Wave IMS (TWIMS).[5] To validate the proposed values and establish an acceptable error threshold for CCS measurements, an inter-laboratory study involving four different TWIMS-MS platforms (i.e., Synapt G2-S, LABERCA, Nantes, France; Synapt G2-Si, BIBS-BIA, Nantes, France; Vion, EPGL, Geneva, Switzerland; and Vion, Waters Corporation, Wilmslow, UK) and 97 steroids (i.e., androgens, estrogens, corticosteroids, and progestagens) has been carry out. In general, CCS measurements fell within the currently accepted error threshold of 2% in comparison to CCS values in the original database. The results also show that measurement accuracy is improved if CCSs based on multi-platform measurements are established as references. In this context, the present work discusses the need to establish normalized CCS databases and develop a reliable score system inclusive of CCS measurements for unequivocal compound identification purposes.

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2. V. D'Atri, *et al.*, J. Sep. Sci., 2018, 41, 20-67.
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Keywords: *ion mobility spectrometry, collisional cross section, steroids, interlaboratory study, CCS database*

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LECTURES

L114

METROLOGY IN FOOD AND THE ROLE OF RESEARCH INFRASTRUCTURES

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Metrology represents the basic support for all the research needs related to food production and consumption and allows the promotion of competitiveness and sustainability, supporting the institutions involved in ensuring food security and acting against frauds. It enables the adoption of an integrated approach and promotes transparency and objectivity. Furthermore, it is a valuable support for the Agrifood sector in facing the so-called "digital revolution", which in fact requires the availability of reliable data with known uncertainty, new sensors and ICT resources for data collection and integration. In particular, high-quality data in the food chain are of fundamental importance to populate the expanding data technologies with useful contents and enable advanced research according to the FAIR *(Findable, Accessible, Interoperable, Reusable)* principles.
Research Infrastructures (RIs) are facilities, resources and services to conduct and support top-level research. They play a central role in the progress and application of knowledge in Europe and represent key investments in research in all areas, as they meet both the demand of the scientific community for state-of-the-art resources to support excellent science, and the demand of knowledge transfer for innovation at social and economic level. The establishment of a landscape of first-class sustainable RIs and services open to researchers, industry, and other interested groups is of strategic importance in the context of the European Research Area (ERA). RIs must be recognized as long-term strategic investments at all levels, indispensable both for enabling excellent research in their scientific domains, and for contributing to overall competitiveness. A robust long-term vision is essential to successfully and sustainably develop, construct and operate RIs.
METROFOOD-RI (ESFRI Roadmap 2018 - Domain Health and Food) provides high-quality metrology services, comprising an important cross-section of highly interdisciplinary and interconnected fields throughout the food value chain, including agrifood, sustainable development, food safety, quality, traceability and authenticity, environmental safety, and human health. It aims for a comprehensive approach to metrology in food, beyond the well-established and high quality methodologies by fundamental physical measurements, and by acknowledging the complexity of food systems in terms of their physical, chemical, nutritional and biological compositions and properties. The approaches adopted allow to comply with the FAIR and the *Responsible Research Innovation* (RRI) principles, taking into account impacts on environment and society and keeping high ethical standards in science. Its practical realisation as ready-to-operation infrastructure can represent a strategic and concrete solution to achieve the harmonization, quality and reliability required in metrology in food.

Keywords: *metrology in food and nutrition, harmonisation, services, interoperability, FAIR data*

LECTURES

L115

ANALYTICAL REQUIREMENTS TO SUPPORT HEALTH CLAIMS ON FOODS. THE CASE OF "OLIVE OIL POLYPHENOLS"

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Health claim approval of bioactive ingredients by the EU legislation after a positive scientific opinion release by EFSA is a time consuming process that should be supported by strong evidence for bioavailability and health benefits upon consumption. However, its effective implementation relies heavily on the availability of the most appropriate and unambiguous analytical procedures to avoid disputes that may cause concerns in the community about possible misuse of the claim that may violate consumer rights and fair trade rules. The almost universal interest in the phenolic compounds that are transferred from olive drupe to olive oil due to the presence of certain bioactive secoiridoids, derivatives of oleuropein and ligstroside, led to numerous publications on their analysis since the '60s though not a fully validated official method exists so far. Approval of the health claim on 'olive oil polyphenols' regarding 'protection of blood lipids from oxidative stress' [1] raised analytical concerns for the determination of the 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil using existing protocols among which the International Olive Council (IOC) recommended protocol for HPLC analysis of olive oil phenols [2]. This lack of an appropriate method is reflected to many efforts to fill the gap since 2012. Because the many efforts of different research groups continue to different directions in terms of analytical strategies, there is a need for consensus among all interested parties that will lead to a harmonized and standardized protocol suitable for commercial needs.
The presentation highlights the analytical gaps for the fulfillment of this particular health claim and the efforts made in the frame of the OLEUM project [3] for filling them by seeking both consensus among scientists and by providing an in house validated cost effective UHPLC protocol that it is also presented and discussed [4]. The pros of the UHPLC-DAD procedure that involves hydrolysis of the polar fraction of the oil are supported by validation data and comparison with results for the same olive oils using other analytical approaches [5].

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Keywords: *olive oil, phenols, EFSA health claim, UHPLC-DAD, in house validation*

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LECTURES

L116

SCIENTIFIC CHALLENGES OF METROFOOD-RI: METABOLOMICS FOR AUTHENTICITY, NOVEL BIOACTIVES IN FOODS AND NEW FOOD SYSTEMS

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METROFOOD-RI - *Infrastructure for Promoting Metrology in Food and Nutrition* aims to promote scientific excellence in the field of food quality and safety. In this regard, novel analytical platforms such as targeted and non-targeted metabolomics play a key role to assess integrity and the "foodome" in general [1].
A particular analytical focus of METROFOOD-RI will be Food Integrity as a general term for sound, nutritive, healthy, tasty, safe, authentic, traceable, as well as ethically, safely, environment-friendly and sustainably produced foods. In order to verify these properties, analytical methods with a higher degree of accuracy, sensitivity, standardization and harmonization and a harmonized system for their application in analytical laboratories are required [2]. Examples of preliminary work at METROFOOD-RI will be presented.

[1] Rychlik M., et al (2018) Ensuring Food Integrity by Metrology and FAIR Data Principles. Front. Chem. 6:49
[2] 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

Keywords: *food integrity, foodomics, method standardization*

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LECTURES

L117

METABOLITES OF PESTICIDES AS THE FOOD QUALITY / AUTHENTICITY MARKERS

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An intensive discussion has been running on health risks associated with pesticide residues occurring foods and human environment. A number of cases exists when pesticides are used either illegally or not in line with principles of Good Agriculture Practice (GAP). Analysis of pesticide residues using conventional analytical methods targeting active in gradient of respective pesticide preparation is commonly used to control present residues. However, most of modern pesticides rapidly degrades after their application due to both physicochemical factors and through biotransformation. Consequently, at the time of sample residues can be either undetectable or detected at very low concentrations (≤ 10 μg/kg that do not allow critical assessment of contamination source. Under such conditions, monitoring of pesticide metabolites in samples might be a conceivable solution enabling the documentation of earlier pesticide use. Analysis of metabolites might pose a real analytical challenge, as degradation of a single parent compound leads to the production of a number of metabolites, differing in some extent in their structure and polarity. This pilot study was focused on the introduction of a novel method allowing fast screening of both pesticide residues and their metabolites (not involved in Maximum Residue Limit, MRL, definition) in samples of fruit and products thereof, using ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPL-HRMS). The objective is supporting (i) the verification of the method of farming (pesticides should not be used for production of organic crops) or (ii) pesticides misuse (the presence of metabolites documents the use of pesticides even in case that re parent compound is not detected). Worth to notice, that in our experiments, a number of pesticide metabolites and degradation product was found in fruit from conventional farming. However, as far as these transformation products are not, involved in residue definition (what is the case of compounds which are of toxicological concern), they are not targeted in routine monitoring programs. Under these conditions, some overestimation of pesticide exposure might occur in biomonitoring studies focused on metabolites in consumers´ urine as part of them does not correspond to parent compound intake which is considered in a health risk assessment process.

Keywords: *pesticide residue, pesticide metabolites, high resolution mass spectrometry (HRMS), authentication of organic crops, documentation of pesticide treatment history*

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LECTURES

L118

OPEN LAB APPLICATION FOR THE CHARACTERIZATION OF NANOMATERIALS BY TRANSMISSION ELECTRON MICROSCOPY

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Applications of nanotechnologies in the food sector are rapidly growing in several areas such as food processing, packaging, nutraceutical delivery, quality control, and functional foods to even the use of nanosensors to assure food quality and safety.
Since it is widely expected that more and more nanotechnology based products will become available in the European Union over the coming years, the European Commission (EC) has developed a Recommendation that provides a basis to determine whether a material should be considered as a nanomaterial (NM) for legislative and policy purposes in the EU (2011/696/EU). The aim is to further apply and adapt this current Recommendation to sector specific needs such as food and food contact materials, biocidal products, cosmetics and medical devices. As a consequence there is a growing need for validated characterization methods and for certified materials facilitating the implementation of the EC Recommendation and sector-specific regulations.
The physical and chemical characterization of NM in complex matrices like food is an extremely challenging task requiring expert knowledge and modern instrumentation. The application of electron microscopy (EM) for the characterization of NM is advised in several international guidelines, including guidelines of the European Food Safety Authority (EFSA) and the Scientific Committee on emerging and Newly Identified Health Risks (SCENIHR). However, EM based methodologies are cost- and labor-intensive and such dedicated infrastructures remain limited to specialized research institutions.
Over the last years the National Reference Laboratory for Nanomaterials in Food of Sciensano (Belgium) has acquired high level expertise and instrumentation to measure the size, morphology, crystallographic structure and chemical composition of a wide range of NM by EM. Identification and measurement of particles can be performed in complex matrices such as food, cosmetics, medicines and environmental samples. A high degree of automation of the EM imaging and image analysis was recently developed to facilitate the measurement of particle size and shape distributions.
Within the context of the METROFOOD-RI "Infrastructure for promoting Metrology in Food and Nutrition" this facility will be further developed as a test case for an open laboratory application to be shared with interested universities, research institutes or companies, ultimately allowing remote operation of the EM and monitoring via VPN-connection.

Read more:
Verleysen, E. et al. Evaluation of a TEM based approach for size measurement of particulate (nano)materials. Materials 2019, 12,2274; doi: 10.3390/ma12142274.
https://www.sciensano.be/en/health-topics/nanomaterials/role
https://www.metrofood.eu/

Keywords: *nanomaterials, physico-chemical characterization, food analysis, Metrofood-RI, open laboratory*

LECTURES

L119

IMPROVEMENT OF QUALITY AND SAFETY DURING FOOD PROCESSING: PILOT PLANT EXPERIMENTS

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It is known that, along the food chain, food contaminants such as: physical, chemical and microbiological contaminants, can occur. That is why prevention management methods and methodologies were developed in order to minimize the level of these contaminants. Food contaminants can be contained by the raw materials that are used for producing food, can be formed during food processing but, can be also introduced into the food chain by food producing staff. So, food quality and safety is influenced by the quality of food ingredients and quality of food technologies on the basis of the Good Manufacturing Practices.
The pilot plant experiments that we present demonstrate how to chose the appropriate bakery technology in order to have a positive influence in the total quality of the bakery products from both of the nutritional and safety point of view. Concerning nutritional aspects the keeping, already existing vitamins and minerals, available into the final product, bread, is envisaged. From the safety issues point of view our target is to minimizing the acrylamide formation into the whole bread using different technological methodologies. Additionally to all of these issues, the consumer preferences about sensorial properties of bread will be tested and positive consumer acceptance will be taken into account.

Keywords: *bakery technology, acrylamide, mineral's availability, sensorial, whole bread*

Acknowledgement: *PN12480101 project, Contract 48N/2012, Researches concerning the influence of type of wheat flours on the acrylamide formation on bread.*

LECTURES

L120

DATA STANDARDS, DATA EXCHANGE AND THE ELECTRONIC PART OF METROFOOD-RI

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METROFOOD-RI is a research infrastructure (RI) for promoting metrology in food and nutrition and consists of a physical part (P-RI) and an electronic part (e-RI). In general, an e-RI should allow researchers, policy makers, the industry and the public to access and use data that was generated in the P-RI. This data are analytical results but also analytical information and knowledge about methods for certain food matrices and compounds, standards, best practices and publications. METROFOOD-RI created a basic e-RI as a pilot system. The first step was to create a data management plan addressing data and value documentation, data archiving and preservation (long-term storage), data format for exchange, policies for access and sharing. It also addresses details on what data are generated and collected, whether and how they will be exploited or made accessible for verification and re-use, and how they will be curated and preserved. In a second step, available data and information sources for analytical food chemistry were investigated and available data formats were identified. These data format standards are crucial to an e-RI because they harmonise data and enables exchange. Data standards also allow computer programs to interpret data and to offer data analysis functionalities to different user groups. Data standards are also needed to comply with the FAIR principle defining data to be findable, accessible, interoperable and re-usable. Data standards and data exchange are therefore core objectives of an e-RI. The next step was to write a specification describing the different parts of the METROFOOD-RI. According to the European Open Science Cloud which is a European Commission project to provide open data, an e-RI must consist of a central platform, data and information sources, services, catalogues, search functionalities, and an authentication and authorisation mechanism. A central METROFOOD-RI platform will be the entry point for users and provide catalogues and search functionality to find data and information sources for analytical chemistry. The platform also provides catalogues of tools and services that can be used in combination with data. It also provides information on access policies for data sources, tools and services. Many of the METROFOOD-RI data sources, tools and services will be self-hosted by data owners giving the e-RI a distributed topology. The distributed RI also needs an authentication and authorisation infrastructure so that users have to login only once and can use as many parts as desired without re-login on each data source. Finally, a first prototype of a METROFOOD e-RI was implemented and tested using FoodCASE, a food data management system. One important outcome of this work is that data producers need to get more aware of data life cycles and that produced data, information and knowledge is the main outcome of every analytical work which is needed for further research purposes and is lasting for a long time.

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LECTURES

L121

THE MICROPLASTICS ISSUE IN THE FOOD CHAIN, LESSONS LEARNT FROM WATER

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Synthetic polymers have become an increasingly important material in the past decades due to their exceptional properties. The versatility applications of synthetic polymers lead to an increased production, which results in a high waste volume due to largely single use usage. The exceptional durability and stability of synthetic polymers pose a problem as soon as they are introduced into the environment and/or entering the food chain. Large plastic objects undergo weathering, embrittlement and finally fragmentation generating plastic debris, the so called secondary microplastic (MP) (1 µm-5 mm). In contrast to secondary MP, primary MPs are manufactured as small particles such as beads, pellets or fibers. MP-particles were reported in different aquatic ecosystems as well as the food web. Published findings correlate well with the production volumes of the detected polymers as well as potential sources, such as e.g. PET bottles or PE lids. This tendency indicates that a significant fraction of MP is introduced into the environment and/or food web by improper consumer waste management or impurities during production. So far many analytical data mirroring the presence of MP in e.g. water, biota or food are debatable to lack of standardized protocols and thus the reliability and comparability of data is a critical issue. Many studies did not verify the material of every detected particle via analytical methods as the determination is cost-intensive and time-consuming. The MP burden is commonly extrapolated for the entirety of detected particles. When analyzing only a small excerpt of detected particles, the most promising particles are chosen, which prevents a representative sample and leads to false-high quantifications. Additionally blank values of MP are quite often disregarded or improperly studied. Due to the described issues, there is a quest for simple and reliable methodologies to determine MP in any kind of samples. Analytical methodologies, which are applied for MP analysis are presented along with their advantages and drawbacks during the presentation. For routine analysis spectroscopic (Raman and FT-IR) and thermo-analytical (Py-GC-MS and TED-GC-MS) are the most promising methodologies. MALDI-TOF-MSI and TGA-DSC need intense research to catch up and other methodologies as SEM or fluorescence microscopy are only applicable to special experimental setups. Recent publications describe adverse health effects especially for particles in nm-ranges, shifting attention from micro- to nanoplastics. MS based methods are not limited to particle sizes and may thus be more versatile applicable for plastic particle determinations. Spectroscopic methods are still suitable for large particles and will find applications for particle analysis of micro and macro sized particles. Further methods as SEM and fluorescence microscopy can be applied as it is now for special experimental setups.

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LECTURES

L122

MS2 NON-TARGET ACQUISITION FOR PESTICIDE RESIDUE ANALYSIS IN FRUIT AND VEGETABLES. BENEFITS AND PITFALLS IN VARIOUS HIGH-RESOLUTION INSTRUMENTS

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For a very long time, the triple quadrupole mass spectrometry has been a synonym of the sensitivity. Nevertheless, its sensitivity is inseparably bonded with the target character of the data acquisition. An indisputable advantage of high-resolution mass spectrometry is a very sensitive non-target acquisition. This approach helps to obtain complete information about the sample. In MS1, the full scan is the simplest and the most obvious mode. However, in MS2 the situation is more complex since some instruments permit for the segmentation of the mass range. It means that the mass range of interest can be divided into smaller subranges and each of them is fragmented in a separated fragmentation event and registered in an individual trace. By that, the number of possible interferences is diminished. Nowadays, only two types of high-resolution mass spectrometers are widely used in the field of pesticide residue analysis in fruit and vegetables. Both, Orbitrap and time of flight analyzer can provide high-resolution spectra. However, those two instruments use different laws of physics to separate the masses. And by that they provide different mass resolution, they have different acquisition speed, etc. All those differences can affect the performance of the analysis. To evaluate the non-target acquisition a representative group of 163 pesticides was selected. Matrices with a different degree of complexity were used. Tomato was chosen as an example of an "easy matrix", whereas orange and leek were more challenging because provided more interferences and higher suppression. In the study, spiked extracts were analyzed with acquisition methods that had a different number of mass segments (1; 5; 8; 20). The parameters that were controlled were: number of precursor ions detected in full scan MS, number of ions detected in MS2, mass accuracy, number of data points per chromatographic peak. Also, the difference between fixed and variable mass segments was investigated.

Keywords: *pesticides, QToF, Orbitrap, high-resolution mass spectrometry*

LECTURES

L123

THE IMPACT OF FLAME-RETARDANTS ON SEAFOOD SAFETY: FROM PLATE TO GUT

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Polybrominated diphenyl ethers (PBDEs) comprise one of the most relevant classes of brominated flame retardants and are well-known endocrine disruptors.[1] The dietary route, through contaminated seafood consumption, is the main contributor to human exposure.[1,2] Hence, the aim of this work was to provide thorough information on the dietary pathway of PBDEs and their biologically active metabolites – methoxylated PBDEs (MeO-PBDEs) – after consumption of contaminated cooked seafood.
Selected fish species with diverse lipid content (European hake and Atlantic salmon) were cooked using optimized household practices (steamed, grilled, and microwaved). Afterwards, a static *in vitro* method[3,4,5] that simulates four human adult digestion steps with distinct digestion times, pH, and composition (simulating saliva, stomach digestion, small and large intestine digestion) was applied to raw and cooked fish. Finally, the colonic volatile profile was studied to determine the possible impact of PBDEs and MeO-PBDEs on microorganisms living in the human gastrointestinal tract.
Analyses were performed by gas chromatography (tandem) mass spectrometry using environmental-friendly extractive methods validated for fish and samples from several digestion segments. Average calculated method detection limits were lower than 40 $pg \cdot g^{-1}$ wet weight (WW) for fish muscle, 250 $pg \cdot mL^{-1}$ for bioaccessible fractions and 500 $pg \cdot g^{-1}$ WW for non-bioaccessible fractions.
Cooking led to an overall reduction of all tested contaminants, ranging from 0.1 % to 17.6 % for PBDEs, whereas, for MeO-PBDEs, it varied from 4.3% to 32.3 %. Both grilling and microwaving provided good outcomes, especially for 2'-MeO-BDE-68 that accounted with a 32 % reduction.
Bioaccessibility of PBDEs and MeO-PBDEs in the small intestinal was low (below 24 %), which point to a likely heavy impact on gut microbiota as later confirmed by volatolomics. Nevertheless, gut microbiota was able to reduce the amounts of targeted contaminants (up to 82 %) in the colon.
This study contributes for the first time to the understanding of the influence of human digestive processes on PBDEs and MeO-PBDEs present in raw and cooked fish through several cooking practices, in order to provide new insights about their bioaccessibility and to simulate more accurately human exposure.

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Keywords: *PBDEs and MeO-PBDEs, Fish, Cooking, Bioaccessibility, Gut microbiota*

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LECTURES

L124

MINERAL OIL HYDROCARBONS IN THE FOOD CHAIN

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Mineral oils are complex mixtures of saturated (MOSH, mineral oil saturated hydrocarbons) and aromatic (MOAH, mineral oil aromatic hydrocarbons) hydrocarbons with a strongly varying composition. They can be present in foods due to their use in the agro-food chain, their presence in the environment or via migration from food contact materials. Because of their complex composition and the additional presence in foods of other hydrophobic substances which may interfere during analytics, the quantitative determination of mineral oil components is challenging. In a 2018/2019 study the presence of MOSH and MOAH in four categories of food products on the Dutch market has been investigated. MOSH was present in about 30% of the samples, especially in pasta and baby food, in concentrations over 100 mg/kg for MOSH. MOAH was present in about 20% of the samples, generally in lower concentrations than MOSH. All samples were analysed twice, the first food sample at the beginning of the study and a second sample from an unopened food package that was stored for a period of six months. The results clearly show that some MOSH/MOAH contaminations originate from the production of the foods while others originate from the migration of these contaminants from the food packaging. Analysis of the food packaging confirms the latter. Risk characterization allowed to conclude that especially the chronic exposure to MOAH is a public health concern, while for MOSH, only at the highest levels health effects are likely to occur. Some EU member states currently take individual measures to manage the risk.

LECTURES

L125

PERFLUOROALKYL SUBSTANCES (PFASS) – AN UNDERESTIMATED CHALLENGE FOR INTRERNATIONAL ENVIRONMENTAL AND FOOD REGULATIONS?

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Perfluoroalkyl substances (PFASs) are a diverse substance class consisting of a hydrophobic, alkylated, fluorine-saturated carbon-chain and a hydrophilic head attached at a terminal end. These compounds have been produced since the 1950s and are used in consumer products as protective coatings for textiles and paper, in the production of semi-conductors, as ingrediencies in aqueous film-forming foams (AFFF), as polymer additives, in herbicide and insecticide formulations and in cosmetics[1-3]. PFASs have unique chemical properties like hydrophobicity, oleophobicity, resistance to degradation processes. Recently, the Organisation for Economic Co-operation and Development (OECD) updated the first list of priority PFAS and reported on 4730 relevant PFAS for future environmental and food associated monitoring [4]. During the past 2 decades the list of relevant hazardous PFAS expanded, thus, from 2 (perfluorooctane sulfonate = PFOS & perfluoro octanoic acid = PFOA) to a comprehensive list of more than 4700 potentially harmful PFAS on the priority list for international screening programs. Already shortly after PFOS and PFOA were identified as environmental pollutants[5], product composition where adjusted in industrial productions leading to a continuous level reduction of PFOS / PFOA in the environment. Mainly short-chain PFAS (C3-C5) were reported as substitutes. Due to new information on exposure risk and toxicology, the European Food Safety Agency (EFSA) is currently revising the official tolerably daily intake (TDI) thresholds for PFOS and PFOA. Recently, the industry explores new production pathways for surface coating agents (i.e. Teflon®) . Thus, a new PFAS was recently identified in the Netherlands released from the current modified Teflon® production process. Gen-X (,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid = HFPO-DA)[6] is currently evaluated with respect to toxicity, transformation pathways and environmental fate[7-9]. After an initial early screening of PFAS in the Nordic environment[10] a new survey on novel PFAS revealed that the number of relevant compounds as well as the concentration levels of PFAS rose throughout the past 15 years in all Nordic countries [11]. Furthermore, the total extractable organic fluorine (EOF) measured in the Nordic samples revealed, that the updated target PFAS list represents in most samples only 30-50% EOF. PFAS are still used in surface coating for food packaging and in cosmetics and, as such, still validated as pollutants in consumer products. In addition to a historical overview, a comprehensive survey on PFAS in environmental monitoring and consumer products will be provided in the here planned presentation. Based on recent screening data and scientific studies, a general risk for human and environmental exposure will be discussed.

https://www.dropbox.com/s/5m2q6eyv51bo0bo/References-RAFA.docx?dl=0

Acknowledgement: *the Nordic Council of Ministers (NCM) funded the NORDIC PFAS screening projects; The Arctic Monitoring and Assessment Programme (AMAP) provided information on the PFAS levels in Arctic environments; KingsBay AS funded the local PFAS study in Svalbard (Norwegian Arctic).*

LECTURES

L126

ANALYSIS OF MINERAL OIL IN FOOD: AN ANALYTICAL CHALLENGE

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Mineral oil can enter the food chain in many different ways: (i) as a contaminant via e.g. environment or lubricants used in equipment or machinery, (ii) as an additive or technical auxiliary or (iii) as a residue via the migration from materials and objects that come into contact with foodstuffs. The analysis is very challenging since mineral oil is a very complex mixture. Among the many different substances present in mineral oil, two main types can be distinguished: the saturated hydrocarbons (MOSH) comprising a complex mixture of linear, branched and cyclic compounds and variable amounts of aromatic hydrocarbons (MOAH). Both MOSH and MOAH form "humps" of unresolved peaks in the chromatograms with the same range of volatility. Since these two fractions have a different toxicological relevance, it is important to quantify them separately.

Commonly, an online technique existing of a combination of Liquid Chromatography with Gas Chromatography (LC-GC) with Flame Ionization Detection (FID) is used for quantification of MOSH and MOAH. However, due to the limited availability and applications of this instrumentation, another technique (offline) can be implemented. The offline technique exists of separation of both fractions by Solid Phase Extraction (SPE) using silver nitrate/silica followed by evaporation and quantitative determination of both fractions by GC-FID with large-volume injection. An overview of both techniques with their advantages and disadvantages will be presented.

Besides the different techniques, the tested matrix has also an important impact, not only on data integration and interpretation but also on the sample preparation. Due to the presence of olefins and natural alkanes, some matrices require auxiliary methods such as epoxidation and clean-up with aluminum oxide.

Next, the optimized method was used to analyze a wide variety of 198 food such as dry food, vegetables, fish and meat products, sweets,…. An overview of the results of the market survey will be presented. Afterwards, the results are compared to the action thresholds as proposed by the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FAVV-AFSCA, Advice 19-2017).

Keywords: *MOSH/MOAH, online LC-GC, offline SPE, market survey, mineral oil*

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 RF 15/6296 MinOil.*

LECTURES

L127

REAL-TIME SCREENING OF SINGLE CORKS FOR TCA AND TBA CONTAMINATION BY VOCUS CI-TOF

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2,4,6-trichloroanisole (TCA) contamination of cork stoppers used in the wine industry remains an unsolved issue causing large economic losses. A contamination of the cork stopper with a concentration of TCA as low as 0.5-2 ng/L can lead to a wine affected by a cork taint. No current analytical technique is capable of reliably screen single cork stoppers for TCA contamination in a few seconds. Non-destructive methods based on fast gas chromatography or ion mobility are either too slow or show very high false positive rates and further require direct sample introduction techniques with time consuming extraction and concentration steps (Cacho, Journal of Chromatography A, 1475, 74–79, 2016). Chemical ionization - mass spectrometry (CI-MS) is a real time direct injection method for the detection of volatile compounds requiring no sample preparation. Recent innovation in the field of CI-MS as led to Vocus CI-TOF, which overcomes previous boundaries in LOD and sensitivity. The new technique can screen cork stoppers for TCA contamination in real-time. Comparison with the determination of releasable TCA by ISO 20752, which is the reference method employing soaking and SPME, provides good agreement with the results of Vocus CI-MS. The unprecedented performances of Vocus CI-TOF can help the cork and wine industry to solve the TCA contamination problem by virtually screening every single cork stopper. The same technique is also able to simultaneously screen single cork stopper for 2,4,6-tribromoanisole (TBA) contamination, which is an increasing problem for the cork industry since it originates from pesticides that are still employed (Tarasov et al., Talanta 175, 82–92, 2017).

LECTURES

L128

USE OF HIGH THROUGHPUT SEQUENCING FOR DETECTION OF GMOS AND PLANT SPECIES IN FOOD AND FEED

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High Throughput Sequencing (HTS) is a new way to detect and characterize genetically modified organisms (GMOs). Whole genome sequencing approaches are not always able to detect a genetic modification due to high variability of genome sizes from one plant species to another and because some regions of the plant genome can be deeply sequenced while others are not covered. This issue is exacerbated if the sample to be analysed contains a mix of several plant species. Within the framework of GMO analysis, it is in any case also interesting to determine which plant species are present in a sample.
A first strategy that was adopted triggered the sequencing to a set of defined DNA segments. Results focused essentially on data going beyond them. To that purpose, DNA was sheared in fragments of ~400 bp that were caught with capture probes of 100-120 bp using the SureSelect technology (Agilent Technologies, Santa Clara, CA). These probes were designed on the basis of a collection of sequences gathering structural elements (promoters, genes, terminators) that can be found in transgenic constructs or consisting of DNA segments that are specific to plant species.
To analyse the large amount of collected data, a bioinformatic workflow was created. The workflow was divided in two parts. A first part aimed at the detection of the sequences, filtering and assigning the reads according to their alignment scores, and a second part focussed on the creation of contigs in an attempt to reconstruct the whole transgene or the sequence of the gene specific to the plant species.
Compared to the 328 GM events listed in the GMOSeek matrix (Block et al., 2013), the adopted strategy would detect all of them except 2 GM events because they do not contain any of the 40 targeted structural elements used for enrichment. Furthermore, the methodology theoretically enables the detection of 20 plant species. Practically, the approach showed its ability to reconstruct partially or completely all of the 13 GM events tested that related to 7 plant species. This was possible even at GMO levels as low as 0.1%.
Moreover, this is the first step towards a more informative analysis as the collection of probes is constantly updated in order to always propose a more powerful tool. Enrichment can be extended to sequences corresponding to additional structural elements, plant species, allergens and contaminants.
In order to design a more general methodology, which would not be limited by the availability of specific plant species sequences, a second strategy based on metagenomics was proposed. It is built on three universal plant targets: one is of nuclear origin while the other two are of mitochondrial origin. This approach was able to detect a large panel of plant species in mastered samples (self-made mix of plant species) as well as in real-world processed samples, even at low rates.

Keywords: *GMO, plant species, detection, sequencing, bioinformatic*

LECTURES

L129

GREEN ANALYTICAL TECHNIQUE AND CHEMOMETRIC TOOLS APPLIED ON PLANT-BASED BEVERAGE AUTHENTICITY

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Plant-based beverages, as rice beverages, have been increasing consumption in the last years. These products present an appeal for a health food, lactose and gluten free, with sustainable characteristics. In this context, the variety of combinations of rice beverages with other ingredients, as soy beverage or soy extract is available. However, the addition of soy in "pure" rice beverages is not always desired, because it changes the original composition of these beverage, taste and technological properties. In addition, some soy components are considered allergic compounds, representing a risk for allergic people. Then, the addition of soy extract to the rice beverage could represent an adulteration of this product, and until now it was not related any method to detect the soy presence in rice beverages. This adulteration could be detected by traditional methods, however that are expensive, time-consuming and not environmentally friendly. Therefore, the near infrared spectroscopy (NIR) combined with chemometric tools could be a green, fast and accurate alternative methodology. The aim of this study was to develop an efficient green method capable of assure the authenticity of powdered rice beverage, applying NIR and chemometric tools. It was produced samples of authentic powered rice beverage (by freeze dry method) and adulterated samples with different percentages of soluble soy extract (1%, 5%, 10%, 15%, 20%w/w). In total, it was obtained 100 samples of authentic powered rice beverage and 25 of adulterated samples. It was performed an unsupervised method, the principal component analysis (PCA) and a supervised method of classification, Soft Independent Modeling of Class Analogy (SIMCA) to detect adulterated samples. For SIMCA about 75 authentic samples and 15 adulterated were used as calibration set. For the prediction 25 authentic samples and 10 adulterated were used as test set. The spectra were obtained using approximately 2g of each sample in the NIR (10000-4000cm^{-1}, resolution of 4cm^{-1}). The chemometrics analyses were performed with MatLab R2019a and 102 PLS-toolbox version 8.6 (*Eingevector Research Inc.*, 2010). The data were pre-processed (1st derivative by Savitzky-Golay method, Multiplicative Scatter Correction, mean center). For PCA analysis, the PC1 (85.75%) and PC2 (4.93%) together explain 90.68% of the information and they separated the samples in 2 groups: the authentic and adulterated samples. For SIMCA, the calibration and prediction results presented 100% of specificity, sensitivity and precision for classification samples as authentic or adulterated, indicating the high performance to adulteration detection. Then we can conclude that NIR associated with chemometric techniques was an excellent alternative to replace the traditional wet chemical analysis applied in authenticity studies related to plant-based beverages. Then it is possible to obtain a fast, accurate, safe and green method to detect adulteration in these beverages.

Keywords: *rice beverage, green technology, lactose free, NIR*

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LECTURES

L130

ION MOBILITY SPECTROMETRY ANALYSES TO SUPPORT, DIAGNOSE OR PRE-EMPT TASTE PANEL STUDIES

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Ion mobility spectrometry is an extremely sensitive analytical technique, ideally suited to the screening of volatile chemical fingerprints in the vapour phase at extremely low concentrations. Taste panels studies have the disadvantage of being regarded as subjective and often difficult to interpret as well as being expensive to conduct. Significant variability in taste panel results can be observed, often depending on the level of training of the panellists and their familiarity with the samples being studied. This variability can cause problems in interpreting the root cause behind taste panel observations not to mention the often low number of unique human observations making statistical analysis of the results and the identification of outliers difficult.
Identifying an instrumental analytical technique capable of accompanying taste panel studies has always been challenging. The sensitivity of the human taste panellists to very tiny changes in flavour molecule composition means the instrumental techniques need to be capable of reproducibly detecting volatile fingerprint changes down to sub µg/L levels. We have investigated the applicability of several different instrumental analytical techniques to support or pre-empt taste panel studies as well as for investigating off-smells in other product classes. We have concluded that the simplicity and robustness of ambient pressure and temperature ion-mobility spectrometry makes it an excellent tool to add to our arsenal of supporting techniques in product development. This talk will highlight the approach taken, some of the strengths and weaknesses of the application of ion mobility spectrometry in this field as well as alternative scenarios for validating the results obtained.

Keywords: *ion mobility spectrometry, taste panels, volatiles analysis, quality control, screening*

LECTURES

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OCCURRENCE OF MULTIPLE MYCOTOXINS IN VARIOUS TYPES OF RICE AND BARLEY SAMPLES IN THAILAND

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The prevalence of mycotoxins is often increased by climatic conditions prevailing in tropical regions. Therefore, consumers in tropical countries such as Thailand have a higher risk of mycotoxin exposure. Existing reports have revealed mycotoxin contamination in rice. This study was conducted to determine the occurrence of multiple mycotoxins in barley and nine types of rice sold in Thailand and to assess consumer health risk. A total of 300 samples collected from various markets in Thailand were analyzed for the presence of 16 mycotoxins using a QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure and a triple quadrupole mass spectrometer equipped with an electrospray ionization source. Of the 300 samples, 124 (41.33%) were contaminated with at least one mycotoxin, and 38.71% of the mycotoxin-positive samples were simultaneously contaminated with more than one toxin. The incidence of mycotoxin contamination differed among the rice and barley samples. Beauvericin, diacetoxyscirpenol, zearalenone, and aflatoxins were the most frequently found mycotoxins. However, the concentrations of regulated mycotoxins were below the regulatory limits. The assessed mycotoxin exposure does not represent a health risk for Thai consumers because the estimated exposure concentrations were lower than the tolerable daily intake values established by the Joint FAO/WHO Expert Committee on Food Additives. However, our findings suggest that continued monitoring of mycotoxin contamination in rice and barley and concomitant risk assessments are warranted.

Keywords: *Barley, Liquid chromatography-tandem mass spectrometry, mycotoxins, QuEChERS, rice*

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

LECTURES

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COMPREHENSIVE GAS CHROMATOGRAPHY COUPLED TO SIMULTANEOUS DUAL DETECTION (TOFMS/FID) AS THE CONFIRMATORY METHOD FOR MINERAL OIL DETERMINATION IN FOOD.

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MOHs are a very complex mixture of isomers mainly associated with two classes of compounds, namely MOSH (composed by linear, branched, and alkyl-substituted cyclo-alkanes) and MOAH (which includes mainly alkyl-substituted (poly)aromatic hydrocarbons at a different number of fused rings). The presence of mineral oil hydrocarbons (MOH) in food was first highlighted by Biedermann and co-workers in 1989 (Biedermann et al, 1989); however, the main discussion started 20 years later when the same authors related the presence of high amount of MOH to the use of recycled fibers for food contact material (Biedermann et al., 2009). The analysis of such a contaminant in food is a challenging task, mainly due to the high complexity of the matrices and the high affinity with the lipid fraction and many of its components. Moreover, FID detection needs to be employed since it gives a virtually equal response per unit of mass for all hydrocarbons, avoiding the calibration problems encountered with mass spectrometry (MS) (reference standards not available). On the other end, GC-MS alone fails to act as a confirmatory method since the MS spectra of the fractions considered and interferences are difficult if not impossible to be distinguished. Due to these challenges, the use of powerful confirmatory techniques is required. The European Food Safety Authority (EFSA, 2012) confirmed the use of on-line LC-GC/FID as the most effective method for the analysis of MOH, but the presence of false-positive and the doubts on the nature of the chromatographic hump originated with this analysis remain. Therefore, this analysis needs to be supported by a more powerful technique for confirmatory purposes. Although some authors look toward mass spectrometry, GC×GC-MS/FID seems to be the most promising solution, as also stressed by the EFSA. However, additional proof of concepts needs to be provided to convince the most skeptics. This contribution works on this direction to provide additional and conclusive support to confirm the valid use of GC×GC as an EU regulated confirmatory method.

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Keywords: *mineral oil saturated hydrocarbon (MOSH), mineral oil aromatic hydrocarbons (MOAH), food safety, comprehensive gas chromatography time-of-flight mass spectrometer (GCxGC-ToF MS), confirmatory method*

LECTURES

L133

CHANGES OF SILVER NANOPARTICLES INDUCED IN A FOOD MATRIX BY SIMULATED DIGESTION ANALYSED USING SINGLE PARTICLE ICPMS

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As the silver nanoparticles (Ag NPs) became quite common component of various consumer products recently, they enter the environment and consequently could appear in some food products. The amount of industrially produced Ag NPs is estimated to hundreds of tons a year. Although Ag in an ionic form is non-toxic, the safety of nanoparticulate Ag contained in food is still questionable. Expressed in terms of mass, the total content of Ag in the majority of food materials is very low and rarely exceeds $ng.g^{-1}$ levels; but even such low mass fraction represents a high number of particles, if a good deal of total Ag is present as NPs. A suitable technique capable of analysing metallic NPs is single particle inductively coupled plasma mass spectrometry (sp-ICPMS). It enables the quantification of NPs as well as the determination of their size distribution. This technique utilises a high frequency data acquisition that can resolve the signals of individual particles that are recorded as individual peaks. The number of peaks recorded during the analysis time is proportional to the particle number concentration N [ml^{-1}], while the area of each individual peak is proportional to the particle mass m [fg]. To analyse solid food material, the sample has to be brought into an aqueous dispersion of NPs in the dissolved food matrix. A high sample dilution ratio must be used to avoid an overestimation of particle size and an underestimation of particle number.
We applied sp-ICPMS for the determination of Ag NPs in the food samples enriched with the NPs that were treated by simulated digestion. The digestion consisted of two steps: firstly a simulated gastric digestion using pepsin in diluted HCl and secondly an intestinal digestion using pancreatin at pH of 7.5, both performed at 37 °C. Rather minor changes of Ag NPs were observed as a result of food matrix digestion; e.g. the experiments with wheat flour samples enriched with the 60 nm Ag NPs standard (Ag mass of 0.2 µg or 2 µg per sample portion) indicate that the majority of nanoparticles remains undissolved (i.e. the particle number concentration is decreased by 10–12 % only). The size distribution of Ag NPs is also a bit changed: the most frequent particle diameter is decreased from 60 nm to 54 nm. From the total mass concentration of Ag of 100 $pg.ml^{-1}$ in the analysed dispersion, the dissolved portion of Ag (Ag^+ ions) represents only ca 20 $pg.ml^{-1}$. If we accept this simulated digestion as a simplified model of real food digestion, these results mean that at least a portion of Ag NPs contained in a consumed food product persists over digestion and could be absorbed into inner organs of a consumer. With respect to possible undesirable effects of inorganic NPs inside a human body, the use of NPs in various technologies should be carefully considered. Further studies of the fate of inorganic NPs in the environment and in the food chain are therefore strongly needed.

Keywords: *silver, nanoparticles, food digestion, sp-ICPMS*

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LECTURES

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KICKING HRMS INTO GEAR FOR RISK-BASED FOOD MONITORING

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National and international food and feed safety authorities are shifting from routine- to risk-based monitoring. Risk-based monitoring requires flexibility in the scope of analytes, matrices, and sampling. Also, risk-based monitoring implies a desire for retrospective analysis in order to follow trends, perform statistics with later insights and identify new threats on food safety. Currently, the most common way of routine food measuring is by triple-quad LC-MS/MS analysis. This type of measuring uses a predefined set of analytes and a designated sample clean-up, which makes this technique less compatible with risk-based monitoring.
The current availability of sensitive and accurate high resolution mass spectrometry (HRMS) techniques provides the possibility for risk based analysis. HRMS analysis can be used in combination with simple and universal clean-up procedure and opens the window for untargeted data acquisition, retrospective data analysis and accurate detection of food contaminations. Besides current risk based monitoring the HRMS data can also be mined retrospectively using a different scope(s), in order to provide suggestions for future analyte analysis.
In our research, we investigated the applicability of HRMS methods for routine food analysis on veterinary products and hormones. By using Orbitrap and TOF techniques, we explored the use of HRMS for several applications. Namely for application as a screening method for analytes for the Residue Monitoring Plan, as a (semi)untargeted fragment-ion screening, and as a non-targeted follow-up for other screening methods. Besides, we inquired in which way retrospective analysis could contribute to risk-based monitoring.
By implementing HRMS for routine food and feed analysis the safety authorities could extend following facts by a predefined scope, and lead the way for risk-based monitoring.

LECTURES

L135

RECENT ADVANCES IN ION MOBILITY HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS – LC-IMS-QTOF - AS A POWERFUL TOOL FOR ORGANIC FOOD AUTHENTICITY

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High resolution mass spectrometry has become a powerful tool in non-targeted and targeted food metabolomics and food authenticity testing. Sensitivity and ability to include chromatographic separation increases the number of target compounds to several ten thousands. Chemometric approaches like PCS of PLS-DA helps to develop models for the individual objectives. In recent years food fraud have become a major issue, like dilution, sugar adulteration or false declaration. Organic products have become into focus since increasing organic marked. Pesticide contamination is sometimes an indication for food fraud, however, several other sources may also responsible, like overspray from surrounding fields.
Organic agriculture is not only related to pesticides. Artificial fertilizers are also not allowed to apply. Therefore, soil conditions and pesticide applications influences the metabolome of the species. Our recent results indicate variations in the metabolome between organic grown cultivars and conventional one. We applied ion mobility technique to further improve separation of isobaric compounds.
Metabolites like polyphenols are often used as marker compounds. However, lots of isobaric compounds make identification complicated even if the retention time is identical. Ion mobility overcome this problem. Collision cross section (CCS) values are a new dimension in mass spectrometry to characterize isobaric compounds. Recent advances of ion mobility high-resolution mass spectrometry techniques with applications to organic food authentication will be presented.

Keywords: *ion mobility, high resolution mass spectrometry, LC-IMS-QTOF-MS, FOODOMICS, organic food*

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LECTURES

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UNRAVELING THE MYSTERY OF ISOMERIC COMPOUNDS CRUCIAL IN FOOD AUTHENTICITY STUDIES UTILIZING TRAPPED ION MOBILITY COMBINED WITH LC-HRMS – SECOIRIDOIDS IN EXTRA VIRGIN OLIVE OIL AS A CASE STUDY

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Extra virgin olive oil (EVOO) has been declared as an integral part of a Mediterranean diet for its high nutritional, sensory and health protecting properties. Olive oil mainly consists of glycerides and fatty acids that represent more than 98% of the total oil weight. However, many of its distinctive properties are derived from the minor fraction of ~2% w/w that includes a number of phenolic compounds (PCs). Phenols and phenolic derivatives are classified among the most important constituents in olive oil, as their presence indicates an increasing potential for health protection. A new European legislative framework (EU 432/2012), highlights the contribution of polyphenol-rich EVOOs to "the protection of blood lipids from oxidative stress". Special attention has been paid to secoiridoids, including "hydroxytyrosol and its derivatives".

One of the most challenging parts of secoiridoids research, remains the separation and identification of isomeric compounds, detected in high abundance in olive oil. HRMS-based workflows, appear to be a powerful tool for the detection and identification of phenolic compounds found in olive oil. However, despite HRMS high applicability and accuracy, separation of isomeric compounds is not always possible, as they may pose identical chromatographic and spectral profile (retention time, mass accuracy, isotopic profile and MS^2 qualifier ions). Consequently, an additional dimension of separation is needed for reliable identification of isomers. Trapped Ion Mobility Spectrometry (TIMS) has proved to be a very promising technology for the separation of isomers, based on their three-dimensional size and charge in the gas phase.

In the present study, a novel methodology utilizing TIMS combined with LC-HRMS (timsTOF, Bruker Daltonics) was applied in order to separate and identify secoiridoid isomers, which are crucial for the health claim of EVOOs. Exploiting the capability of mobility dimension, and in particular the ability to provide collision cross section (CCS), TIMS separation of secoiridoid isomers was achieved. More specifically, isomers that could not be discriminated in terms of retention time, were successfully separated and identified for the first time, to the best of our knowledge. Different mobility peaks were detected, and further studied through data dependent acquisition (DDA). Interestingly, MS^2 DDA data were acquired for each mobility peak, thus enabling structure elucidation. Moreover, samples of different variety and geographical origin were analysed, leading to noteworthy differentiations, regarding the profile of isomeric secoiridoids. It should be noted that different mobility peaks were detected in samples of different variety, providing a strong indication that isomers could be used as potential authenticity markers. In conclusion, LC-TIMS-HRMS is a powerful technique for the chemical characterization of isomers, which is crucial in many food authenticity studies.

Keywords: *extra virgin olive oil, phenolic compounds, isomers, LC-TIMS-HRMS, authenticity*

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DATA FUSION OF GC-IMS DATA AND FT-MIR SPECTRA FOR THE AUTHENTICATION OF OLIVE OILS AND HONEYS – IS IT WORTH TO GO THE EXTRA MILE?

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The potential benefit of data fusion based on different complementary analytical techniques was investigated for two different classification tasks in the field of foodstuff authentication. 64 honey samples from three different botanical origins and 53 extra virgin olive oil samples from three different geographical areas were analyzed by attenuated total reflection IR spectroscopy (ATR/FT-IR) and headspace gas chromatography–ion mobility spectrometry (HS-GC-IMS). The obtained datasets were combined in a low-level data fusion approach with a subsequent multivariate classification by principal component analysis–linear discriminant analysis (PCA-LDA) or partial least squares–discriminant analysis (PLS-DA). Performing a back-projection of PCA loadings, the influence of variables in the FT-IR spectra (one-dimensional) and the GC-IMS profiles (two-dimensional) on the discrimination was visualized within the original axis of the two data sources. Validation results of the classification models were compared to the results that could be obtained by using the individual data blocks separately. For both the honey and olive oil samples, a decreased cross validation error rate and more robust model was obtained due to the low-level data fusion. The results show that data fusion is an effective strategy for improving the classification performance, particularly for challenging classification tasks such as the discrimination of olive oils with different geographical origin.

Keywords: *data fusion, ion mobility spectrometry, MIR spectroscopy, olive oil, honey*

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LECTURES

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EXTENDING OVER LONG-TERM STUDIES THE UNTARGETED AND TARGETED FINGERPRINTING OF EXTRA-VIRGIN OLIVE OIL VOLATILES BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY

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Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) is a technique that provides highly informative fingerprinting for food characterization. However, 2D fingerprints consistency might be affected by variables correlated to both the analytical platform and the experimental parameters adopted for the analysis making challenging cross-comparative studies extended over long-time range.
This study focuses on the combined untargeted and targeted (UT) fingerprinting of volatiles from extra-virgin olive (EVO) oils and proposes an effective work-flow to correct fluctuations that may occur during long-term studies and that affect patterns alignment and response consistency.
Volatiles from high-quality EVO oils (*Picual* cultivar, from Granada Altipiano, Spain) are sampled by headspace solid phase micro extraction (HS-SPME) with a DVB/CAR/PDMS d_f 50/30 µm 2 cm length fiber and analyzed by GC×GC-TOF MS featuring tandem ionization on a polar × medium polarity column set-up. 2D-pattern misalignments are simulated by changing chromatographic settings (modulation period M_P and ^{2}D column dimensions) while MS response fluctuations are induced by adopting different tuning and acquisition modes.
Misalignments, caused by chromatographic setup changes, impact on ^{1}D and ^{2}D retention times (^1t_R, ^2t_R) and peak-width while MS tuning and acquisition mode impact on analytes absolute and relative response. A strategy is then proposed to define a) a minimal signal-to-noise ratio (SNR) threshold, for consistent extraction of MS features to be adopted for UT fingerprinting, b) a minimal direct match factor (DMF) similarity value to improve the specificity of the matching, and c) a minimal distance threshold to guide the matching transform toward a 100% of true-positive matches. Once designed, the work-flow is applied to a target template of known analytes with a full supervision of the analyst. On the other hand, a fully automated and unsupervised procedure is then applied to automated for the UT feature template by adopting previously optimized parameters. For both targeted and UT templates, the percentage of matching between misaligned patterns reaches 95%.
Finally, to compensate for detector response fluctuations, different normalization approaches are examined: normalization on total or partial response and normalization on multiple Internal Standards ISs. Although the first two approaches result attractive, being simpler and less time-consuming, results are not satisfactory as those obtained by multiple ISs normalization. The latter performs better for those analytes showing higher response fluctuations due to the pressure-drop applied.
In conclusion, this study shows that, thanks to a careful optimization of "smart templates" parameters, a full metadata transfer for targeted and untargeted features can be achieved even when dramatic misalignment occurs on complex 2D-patterns.

Keywords: *GCxGC-TOFMS, extra-virgin olive oil volatiles, template matching, combined untargeted and targeted (UT) fingerprinting*

LECTURES

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DEVELOPMENT OF A NON-TARGETED LC-MS METABOLOMIC APPROACH FOR THE SEARCH OF BIOMARKERS OF COCOA POWDER ADULTERATION

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Cocoa is a high valued product largely consumed all over the world due to its fascinating characteristics and pleasant aroma and flavour. With the rapid growth of cocoa industry, the demand of cocoa has increased in the last years; however, its production is being tightened due to climatic changes, deforestation, etc. In this sense, the addition of undeclared low-cost raw materials such as carob and soy flour or chicory for an economic profit has also been increased [1]. Although metabolomics has been employed to the search of markers of geographical origin, growing region, and fermentation process [2-4], as far as we know, it has never been employed in the study of cocoa adulteration.
In this work, a non-targeted metabolomics approach based on the use of reversed-phase liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (RPLC-(QTOF)MS) was developed in order to find metabolites which could be pointed out as potential adulteration markers of cocoa powder samples. Metabolic analysis was performed using six groups of samples: high and low levels of chicory, and carob and soy flour. To obtain the maximum number of molecular features, different extraction protocols and chromatographic conditions were evaluated. Data, obtained both in positive and negative MS modes, were analyzed using non-supervised and supervised multivariate statistical analyses. Variable importance on projection (VIP) values obtained in supervised partial least square discriminant analysis (PLS-DA) models were employed to select the most relevant metabolites allowing group discrimination which were subsequently selected for further identification through their retention time and MS/MS spectra and taking into account metabolites database.

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Keywords: *adulteration markers, cocoa powder, liquid chromatography-mass spectrometry, non-targeted metabolomics*

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LECTURES

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COMPREHENSIVE ANALYSIS OF VARIOUS CANNABIS PRODUCTS BY GC-HRMS

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Cannabis plant has been utilized by populations throughout the world over thousands of years for various purposes such as: (i) food (seeds), (ii) source of fuel, (iii) source of fibers and (iv) herbal remedy. Nowadays, multiple health benefits offered by Cannabis plant to patients suffering of various disorders have been extensively investigated. Cannabinoids, terpenes and terpenoids are the major bioactive secondary metabolites in this plant, therefore, the information on their occurrence in cannabis based products is of high concern. In this study, we developed and validated a new efficient method for the quantification of 37 terpenes and terpenoids in hemp, hemp resins and essential oils. As solid phase micro extraction (SPME), that was used in the first phase of our experiments did not enable to isolate sufficient amounts of less volatile terpenoids, simple extraction of matrix using ethyl acetate followed by analysis employing gas chromatography coupled to high resolution mass spectrometry (GC–HRMS) was chosen. The analytes profiles fairly differed among the tested samples depending both on a hemp cultivar and the way of its processing. In most cases, myrcene, ß-caryophyllene and α-bisabolol were the dominant components in hemp. On the other hand, in hemp essential oils and resins were detected predominantly sesquiterpenens such as trans-caryophyllene, caryophyllene oxide and α-humulene. With regards to a high complexity of the hemp samples, comprehensive two–dimensional gas chromatography coupled to mass spectrometry with tim- of-the-flight mass analyzer (GC×GC–TOFMS) was also investigated with the aim to separate as many terpenes, terpenoids and matrix components as possible. The major problem encountered in terpenes/terpenoids analysis were large differences in target analytes concentration what needed careful optimization of injected amount of sample to enable detection of both minor components and major ones thus avoiding exceeding linearity range in the latter case.

Keywords: *cannabis, hemp, terpenes, terpenoids, GC-HRMS*

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TRANSCRIPTOMICS, SENSORIAL ANALYSIS AND VOLATILOME FINGERPRINTING OF FRESH PRODUCE: A MULTI-TRAIT APPROACH TO IDENTIFY PREDICTORS OF FOOD QUALITY.

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Fruit quality is determined by numerous traits including sweetness, colour, aroma, acidity and firmness. Quality has become an important characteristic for the consumer. Therefore, the fruit industry needs to ensure and optimize product quality throughout the supply chain. Volatilome fingerprinting combined with gene expression profiling can provide evidence for fruit quality differences and changes which can be related to genotype selection, geographical origins, post-harvest storage and supply chain processing (such as washing, drying, and trimming). In this context, next generation sequencing and metabolomics technologies can be very useful by allowing comprehensive, simultaneous characterization of metabolite and gene expression data from diverse genotypes of the same species as well as assessing effects of post-harvest storage conditions.
Here, comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (TOF-MS) was used to analyse the volatilome of peach (*Prunus persica* (L. Batsch) fruits. An RNA-sequence transcriptomic approach was employed to identify differentially expressed genes (DEGs) amongst post-harvest treatments focusing on those associated with volatile organic compound (VOC) metabolism in order to better understand mechanisms underlying their modulation post-harvest.
Peach fruits are characterized by a rapid deterioration at room temperature meaning that cold storage is widely used to delay post-harvest ripening of the fruit and extend its commercial life. It is, therefore of considerable scientific and economic interest to improve our knowledge of the mechanisms by which fruit respond to cold stress. Our study, focussed on one peach (*cv* Sagittaria) and one nectarine (*cv* Big Top) cultivar: fruits were analysed immediately after harvest and after 1, 5, 7 and 14 days of cold storage at 1°C. A total of 159 VOCs were identified for Sagittaria, while 89 VOCs were detected for Big Top. Canonical Analysis of Principal coordinates (or CAP) on VOC profiles showed a discrimination between cultivars and post-harvest storage periods. A combination of sensory evaluation and VOC profiles showed the same trend reported by CAP analysis. Furthermore, correlation between the expression profile of flavour-related genes and VOCs was shown. For example genotype specific activation of some VOC biosynthetic pathways, such as that related to sesquiterpenoid and triterpenoids biosynthesis, was observed in Sagittaria. Differences were also detected in sensory characteristics.
Overall the combination of sensory evaluation, VOC profiles and gene expression could help breeders to understand which traits/aroma are more relevant to consumer perception. Furthermore, understanding of metabolic and genetic changes occurring in fruit VOC patterns post-harvest could contribute to providing a suite of simple diagnostic checks to monitor fruit quality throughout the supply chain.

Keywords: *volatilome, transcriptomics, sensory, post-harvest, peach*

Acknowledgement: *This work is supported by Fondazione con il Sud, call Brain2South, as part of the FRUITY collaborative project (2015-0245).*

LECTURES

L142

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

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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.
The Regulation (EU) 2017/625 (the new "Official Control Regulation") entering into force in 14 December 2019, contains detailed provisions on procedures to be applied for official control, including on methods of analysis to be used.
Following requests of the European Commission, the Panel on Contaminants in the Food Chain (CONTAM) from the European Food Safety Authority (EFSA) has completed in recent years several scientific opinions on contaminants in feed and food, reviewing the possible risks for animal and human health due to the presence of these substances in feed and food.
In the presentation, recent developments on EU legislation on contaminants in feed and food shall be presented in particular on the presence of ergot alkaloids, pyrrolizidine alkaloids, tropane alkaloids, dioxins and PCBs, glycidyl fatty acid esters and 3-MCPD fatty acid esters, chlorinated paraffins, …
Particular attention shall be paid to the analytical requirements and the analytical challenges that are faced for an effective EU policy on contaminants in feed and food.
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: *food and feed, contaminants, analytical challenges, EU policy, human health protection*

LECTURES

L143

LASER SPECTROMETER FOR FOOD SAFETY

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The food safety testing market is expected to grow at a compound annual growth rate of 7.2% (2016-2021) and is projected to reach 18.54 billion $ by 2022. The utmost importance of the global problem of food safety explains why The Consumer Goods Forum - organization that gathers consumer goods retailers and manufacturers - launched the Global Food Safety Initiative that brings together key actors of the food industry to collaboratively drive continuous improvement in food safety management systems around the world.
The Diagnostics and Metrology Laboratory (FSN-TECFIS-DIM) of the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) applied for years laser photoacoustic spectroscopy (LPAS) to food safety. Up to now, CO_2 lasers were used, as in the prototype developed in the framework of the SAL@CQO project. These sources are good candidates for this research because they are discretely tunable from 9 to 11 μm, inside the fingerprint region.
In recent years, quantum cascade lasers (QCLs) tunable in the fingerprint region have become commercially available. Although their power is still lower than CO_2 lasers, they can be deployed in the field, because significantly smaller, and their tunability is continuous and broader, thus allowing to record spectra that provide more information on the sample.
Having in mind that control authorities that routinely verify food quality will greatly benefit from a portable and user-friendly system, we decided to develop a QCL based photoacoustic spectrometer fully managed by a dedicated software.
The continuous wave emitted by the QCL is chopped at an audio frequency and irradiates a food sample inside a photoacoustic cell. The radiation is absorbed by the sample, with the consequent temperature increase, adiabatic expansion and pressure wave generation. Acoustic resonance amplifies the signal that is detected by a microphone coupled with a lock-in amplifier. A small part of the laser beam is sent to a power meter by a beam splitter. A personal computer (PC) controls the experiment.
A dedicated software fully manages the spectra acquisition. The user has to input on the interface:
- minimum wavelength, maximum wavelength and wavelength step,
- number of measurements at a given wavelength (each measurement lasts 1 s),
and simply start the instrument from the PC that records microphone signal and laser power per each measurement. Usually, each point of the LPAS spectrum (photoacoustic signal) is given by the ratio of the average of microphone signals and the average of laser powers.
In this paper, examples of detection of food contaminants/frauds will be given. Usually, it is impossible to achieve this goal simply looking at the LPAS spectra but, fortunately, principal component analysis can easily perform this task.

Keywords: *QCL applications, laser spectroscopy, photoacoustic technique, agrifood chain, food safety*

Acknowledgement: *The authors are grateful to Aldo Pizzuto, Roberta Fantoni and Antonio Palucci for constant encouragement. This research has been carried out in the frame of the work package 1 - SafeFood of the TecHea project funded by ENEA.*

LECTURES

L144

SIMULTANEOUS, MULTICLASS, AND QUALITATIVE/QUANTITATIVE GC×GC METHOD FOR CANNABIS PRODUCTS

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The recent trend towards the commercialization of legal cannabis in several countries has generated novel opportunities to understand the potential benefits for medical purposes. Together with the growing need for safer cannabis products, the quality control inspections and methods for their characterization increased exponentially. Generally, quality control analyses include
multi-chemical class testing for potency, terpenes, pesticides, residual solvents, heavy metals, mycotoxins and microorganisms.
In this presentation, the importance of the profiling and the determination of terpenes, cannabinoids, and pesticides will be discussed. In addition, a unified method for their qualitative and quantitative analysis in a single analytical run will be provided. The method involves a fast sorption-based extraction followed by comprehensive two-dimensional gas chromatography coupled to (high resolution) mass spectrometry, *i.e.* GC×GC-(HR)ToF MS. The extraction method was optimized to have optimal recovery for the chemical classes of interest. A factorial design of experiments was used to determine the most advantageous combination of the extraction conditions (solvent type, salt addition, extraction time and temperature).
The overall method was validated on a variety of recreational cannabis flowers and cannabis oil samples. It will be demonstrated that the method allowed to efficiently highlight the difference between the various cannabis strains based on the multi-chemical class information provided.

Keywords: *cannabis analysis, comprehensive two-dimensional gas chromatography, qualitative/quantitative analysis*

LECTURES

L145

TOWARDS DIETARY INTAKE ASSESSMENT USING IMAGE ANALYSIS

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Personal(ized) sensors or "wearables" can be applied in the near future to ultimately arrive at personalized nutritional advice. A challenge here is to assess dietary intake. To date, this has mainly been done with questionnaires or interviews, for example, food frequency questionnaires or 24-hour recalls. However, these methods are prone to bias due to conscious or unconscious misreports. More objective measurement methods are therefore desirable. Therefore, we aim to enable consumers to measure their food and energy intake themselves, using state-of-the-art miniaturized vibrational spectroscopy and image analysis techniques. In the current study, a first demonstrator is developed, which can be further integrated into smaller devices such as wearables or smartphones. In a subsequent study, the demonstrator will be tested in a cohort study.
The analytical challenge for this work is comprised of: (i) vibrational spectroscopy to determine macro and micro components (molecular level), to quantify food intake and to differentiate between types of food and (ii) image analysis to recognize types of food (object level) and to estimate food and energy intake. Cameras and sensors were tested, trained and validated for effectiveness and applicability at both levels. For this purpose, multivariable statistics were used to get workable outcomes from the multidimensional data and to be able to create a basis for a user interface. At both analysis levels, the application of hardware can also work symbiotic (for example for food recognition and estimating the energy intake) and thus increase the robustness of the measurements.
For testing the ability of the demonstrator for dietary intake assessment, two food cases were chosen as a test: (i) margarine, low-fat margarine and butter applied to a sandwich and (ii) raw and prepared vegetables. For the butter case, a Specim IQ hyperspectral imaging system was applied to 30 butter samples with varying total fat and saturated fat content. The system was able to differentiate between (standardized) bread and butter and was able to predict the total and saturated fat content when the butter was applied to the bread by multivariate modelling. Furthermore, the sample intake could be estimated by pixel counting. Estimating the layer thickness of the butter remains challenging. For the vegetable case, a RealSense camera with Inertial Measurement Unit was used. By applying structure from motion techniques, depth maps of the scenes with vegetables could be generated for identification by shape and to quantify the intake. The aim is to eventually reach technology readiness level 4, which means that the demonstrator can be operated in a controlled environment by trained persons.

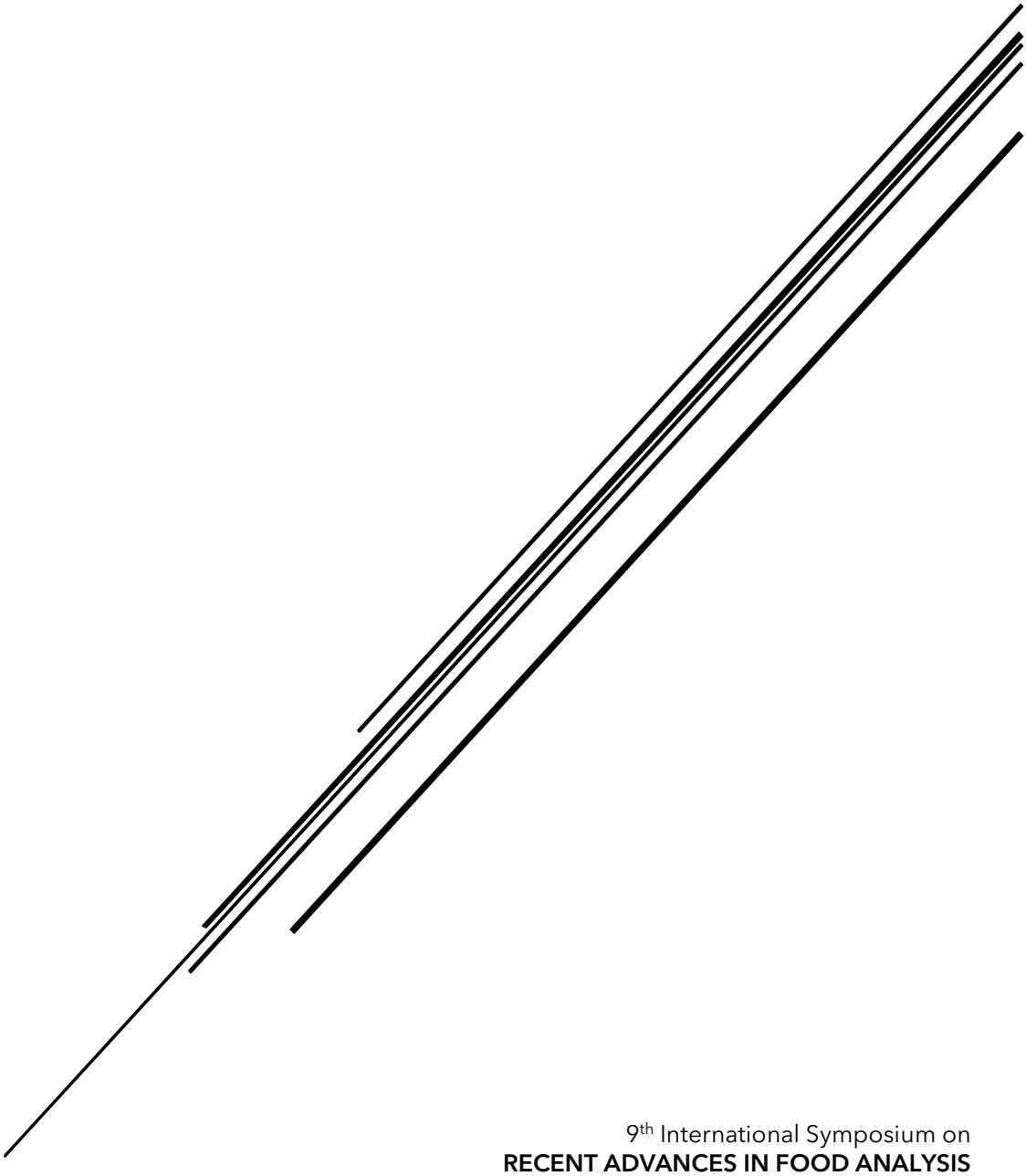
Keywords: *dietary intake, hyperspectral sensing, structure by motion, citizen science, wearables*

Acknowledgement: *This research is part of the Dutch Research Council (NWO) program " Dutch National Research Agenda: Start Impulse Measuring and Detecting of Healthy Behaviour" under grant number 400.17.604. www.Metenendetecteren.nl*

POSTER SESSIONS

ALLERGENS

POSTER SESSIONS



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A1

EFFECT OF STORAGE CONDITIONS ON THE MAIN PROTEINS PRESENT IN BOVINE MILK WHEY CONCENTRATE

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The availability of large amount of whey protein (WP) products on the market and their growing use in food has led to increased research activities focused on whey components. The aim of this study is to investigate the effect of storage conditions on whey protein products, in particular the induced chemical modifications that could affect protein structure. Twenty samples of freeze-dried WPs, stored at different conditions, were provided by FrieslandCampina. Sweet whey was freeze-dried and stored for different days (3, 8, 10 and 14 days) at different temperatures (20, 30, 40, 50 and 60°C). Initial bulk analyses were performed to determine protein content. Protein analysis and amino acid content were performed with UPLC-MS and SDS-PAGE. Finally, *in vitro* simulated gastro-intestinal digestion studies were performed to investigate the effect of the induced chemical modifications on digestibility. Bulk analyses confirmed that protein content is not affected by storage conditions. The effect of the Maillard reaction on α-lactalbumin and β-lactoglobulin, in particular the lactose condensation on lysine residues, were investigated with UPLC-MS. It was discovered that increasing the temperature of storage increases the amount of proteins in the lactosylated form. Interestingly, the same trend was observed while increasing the number of days of storage. To investigate the composition of the WPs, the amino acid content was determined. Only few residues (methionine, cysteine and tryptophan) seem to be affected, with a low extension, by storage conditions. After the digestion studies, following Minekus et al. (2014) protocol, in the digestive phases were identified different β-lactoglobulin peptides in the lactosylated form. The results indicate that storage temperature and duration affect the degree of protein lactosylation, which alters protein structure and decreases the number of available free lysines. The identification of lactosylated peptides reveals that this modification is stable through the digestion.

Keywords: *whey, proteins, storage, lactosylation, allergens*

A2

MEASUREMENT OF SPECIFIC MILK ALLERGENS IN BAKED FOOD CHALLENGE MATERIALS

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Rationale: Oral food challenges (OFC) are considered the 'gold standard' to diagnose a true food allergy. Allergists use baked milk food preparations for OFC under the assumption that they contain decreased allergen levels due to baking. However, the effects of baking on specific allergens has not been thoroughly investigated. The aim was to compare levels of major milk allergens and IgE reactivity in uncooked and baked milk challenge materials currently used in clinical practice.
Methods: Uncooked and baked muffins were prepared using recipes from Mount Sinai (Jaffe Food Allergy Institute) and the UK National Health Service (NHS). Allergen levels were compared using a two-site monoclonal antibody ELISA for beta-lactoglobulin (Bosd5) and for beta-casein (Bosd11). IgE reactivity was assessed using sera from milk-allergic patients in direct binding and inhibition ELISA.
Results: Bosd5 (β-lactoglobulin) concentration decreased from 680µg/g in uncooked muffin mix to 0.17µg/g in baked muffin, representing >99% reduction in Bosd5 allergen. The level of Bosd11 (β-casein) decreased by 30% from 4,249µg/g in uncooked muffin mix to 2,961µg/g in baked muffin. Bosd11 levels in the Mount Sinai muffins (n=30) were higher compared to the NHS muffins (n=15) and varied depending on whether the baked muffin was sampled from the top, middle or bottom. Baked muffins retained ~70% of the IgE reactivity in uncooked muffin mix while baked muffin extracts inhibited IgE antibody binding to uncooked muffin by up to 80%.
Conclusions: The level of major milk allergen Bosd11 remained high in baked muffins used in oral food challenges. These findings emphasize the potential risk for adverse reactions to baked milk challenges, especially in patients who have high anti-casein IgE antibodies. Measurements of specific milk allergens, together with IgE molecular diagnostics, should improve the safety of food products used for OFC and reduce the risks associated with milk challenges in clinical practice.

Keywords: *oral food challenge, milk allergen, casein, beta lactoglobulin, ELISA*

A3

LUPINE ALLERGENS IN FOOD PRODUCTS: EFFECT OF PROCESSING AND FOOD MATRIX ON THEIR DETECTION AND IMMUNOREACTIVITY

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Lupine (*Lupinus albus*) is widely used as a functional food and as an ingredient in all kind of food products (bakery, confectionary, snacks) due to its nutritional value and technological properties. However, it is also a source of allergens capable of inducing allergic reactions in sensitised individuals, representing a potential risk even with the ingestion of trace amounts. Food processing commonly used in this type of foods, such as heat treatments can alter the structure/stability of proteins, subsequently affecting their allergenicity.
In this work, two approaches were presented: a normalised real-time PCR system with a TaqMan™ probe to detect and quantify a lupine allergen in food products and the evaluation of lupine flour immunoreactivity in processed foods by immunochemical assays.
Referencemixtures of known quantities (10% to 0.0001%) of *L. albus* in rice and wheat flours (with and without baking) were prepared. A normalised calibration approach using real-time PCR was developed targeting an encoding sequence of the PR-10 protein of *L. albus* and an eukaryotic Referencegene. Protein profiles were determined by NATIVE- and SDS-PAGE (in denaturing conditions), while lupine immunoreactivity was tested by immunoblotting using polyclonal anti-gamma-conglutin antibody. A total of 27 commercial samples were purchased at local markets and evaluated by both approaches. Model mixtures of lupine in rice presented the best sensitivity results, suggesting that the performance of the method is affected by food matrix and baking. The real-time PCR assay showed absolute and relative sensitivities of 1 pg of lupine DNA (1.7 DNA copies) and 0.0005% (w/w) of lupine in rice, respectively. The normalised calibration model based on the ΔCt method, in the range of 10-0.0005%, exhibited optimal performance parameters, being successfully validated. From 27 tested commercial foods, it was possible to quantify lupine in 5 samples that were in compliance with labelling. Native- and SDS-PAGE showed a clear effect on lupine protein profiles caused by thermal treatment and food matrix. Immunoblottings for lupine γ-conglutin demonstrated a decreased in its immunoreactivity in rice flour mixtures, but particularly in baked wheat mixtures (breads). In native conditions, the antibody identified a protein of approximately 200 kDa corresponding to γ-conglutin. Different lupine species (*L. albus, L. luteus, L. mutabilis* and *L. angustifolius*) seems to present distinct amount of gamma-conglutin. In denaturing conditions, several bands at different molecular weight are present with distinct patterns among species, which corresponds to the subunits of the protein. Gamma-conglutin was also identified in 3 commercial samples by immunoblotting.
This work showed the high specificity and sensitivity of DNA-based methods for the detection of trace amounts of lupine allergens in foods and the importance of the evaluation of commonly used food processing on lupine allergenicity.

Keywords: *allergens, lupine, real-time PCR, Immunoassays*

Acknowledgement: *This work was supported by UID/QUI/50006/2019 with funding from FCT/MCTES through national funds and by the projects AlleRiskAssess – PTDC/BAA-AGR/31720/2017 and NORTE-01-0145-FEDER-00001. Caterina Villa is grateful to FCT grant (PD/BD/114576/2016) financed by POPH-QREN (subsidised by FSE and MCTES).*

A4

MODEL BREAD SAMPLES FROM HYDROLYZED DURUM WHEAT FLOURS: DISCLOSING IMPLICATIONS FOR CELIAC DISEASE PATIENTS BY UNTARGETED HR-MS/MS ANALYSIS

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In this communication, we present the preparation of model bread samples from hydrolyzed flours of three specific *Triticum turgidum* wheat genotypes that were selected in our previous investigation for their potential low toxic/immunogenic activity for celiac disease (CD) patients [1]. Sourdough was produced as follow: 15 g specific durum wheat flours and 35 g of tap water (30% w/w) containing 9 log CFU/g (final density in the dough) of lactic acid bacteria strains mixture (*Lactobacillus brevis* SS25, *Lactobacillus paracasei* SS-A4, *Lactobacillus casei* SS1), with the addition of E1 (*Aspergillus oryzae)*, E2 (*Aspergillus niger)* (200 ppm), Veron HPP (10 g/100 kg of protein) and Veron PS (25 g/100 kg of protein) enzymes. Doughs were incubated for 48 h at 37°C with stirring conditions. Gluten free bread (DY, dough weight x 100/flour weight, of 200) was prepared using rice and corn flour (ratio 1:1 on dry matter). Sourdough was added in the final recipe of bread (30% of total amount of dough). Baker's yeast was added at the percentage of 2% w/w, corresponding to a final cell density of ca. 9 log cfu/g in all the bread. Doughs were mixed at 60 x g for 5 min with an high-speed mixer and fermentation was at 30 °C for 1.5h. All breads were baked at 220 °C for 30 min.
The model hydrolyzed bread samples were characterized by R5 competitive ELISA in order to quantify the residual gluten in the final baked product. Noteworthy, all the hydrolyzed samples displayed a lower absolute amount of gluten than control samples even if with a different efficacy in gluten degradation; indeed, two of them were classified as gluten-free and low-gluten bread, respectively.
In order to investigate the proteolytic degradation occurred to gluten proteins, we performed an advanced proteomic characterization of hydrolyzed and control bread samples, by high-resolution tandem mass spectrometry. The gluten proteins were extracted with a strongly denaturing and reducing buffer solution previously optimized [2] and the identified peptides were screened for intact CD epitopes. Noteworthy, the MS analysis confirmed the same trend observed by R5-ELISA. Indeed, two out of three hydrolyzed breads presented an exhaustive degradation of the epitopic sequences relevant for CD immune stimulatory activity. Finally, we performed *in-vitro* simulated human gastroduodenal (GD) digestion experiments in order to identify GD resistant peptides that would be released under physiological digestion conditions and could present immune-stimulatory potential for CD patients. The persistence of CD full-length epitopes was assessed, providing a qualitative evaluation of the residual toxicity/immunogenicity potential of the model hydrolyzed bread samples.

1. R. Pilolli et al. Sci Rep, 9 (2019) 1646.
2. Colgrave et al. J. Proteome Res. 2015, 14, 2659.

Keywords: *durum wheat, gluten, sourdough, high resolution mass spectrometry*

Acknowledgement: *The research was funded by the Ministry of Education, Universities and Research (MIUR-Italy), program SIR 2014 "S. Wheat Pro. - Proteomic characterization of Selected durum Wheat cultivars for PROduction of low toxicity-food products towards celiac disease patients (RBSI14QQ1W)".*

A5

ALL IN ONE SWEEP - A LEGUME ALLERGEN DETECTION ASSAY (LADA)

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Food allergies represent an impairment for a growing number of people and affects nowadays up to 5-7 % of the population worldwide. The increasing number of individuals with multiple food allergies and the rising availability of yet unusual food components raises the demand for test systems that can detect different allergens simultaneously. The three legume crops soy, lupin and peanut, belong to the 14 food allergens, that have to be declared in the ingredients list of any food in order to protect consumers. However, detection of traces of allergens presents still a challenge despite numerous test systems which are on the market.
The allergens soy, lupin and peanut are regularly detected by commercial test kits, based on enzyme-linked immunosorbent assays (ELISAs), lateral flow immunoassays (LFIA) or on polymerase chain reaction (PCR). ELISA and LFIA are either employed with extensive labor or lacking of sensitivity to detect all three crops at the same time.
Using multiplex technologies to detect multiple allergens in one sample are time and sample saving. However, most techniques for multiplex are based on mass spectrometry requiring well trained staff, expensive equipment and have the problem of unsatisfying sensitivity. The often-used multiplex PCR technologies will not solve the problem, because of the trend towards the use of processed protein ingredients that contain very little DNA. Furthermore, some of the allergens like egg white and milk proteins are not accessible by PCR. Therefore, the advancement of immunological techniques in multiplex format represents a good option.
Such a strategy has been pursued by the multianalyte profiling (xMAP®) technology developed by the Luminex® Corporation. The xMAP® is a fluorescence-based microbead assay offering the opportunity to examine up to 500 analytes in one well. Monoclonal antibodies are linked covalently on polystyrene microspheres and used as analyte specific capture antibodies. Another analyte-specific detection antibody gives the quantification by fluorescence-based reporter system. As each microsphere carries a unique signature, the detection system can identify to which set of capture antibodies it belongs. The xMAP® technology is used intensively in biomarker detection in the medical sector, but has been rarely applied in food analysis.
Our goal is to combine the xMAP® technology with well-characterized monoclonal antibodies of stable quality, raised against soy, lupin and peanut protein. This one-shot approach provides the possibility to develop a cost and time efficient high-throughput legumes test kit. The open platform format gives the possibility to include almost all known food allergens, dependent on the possibility to discriminate them with antibodies.
Here, we present the development of a novel multiplex assay for detection of three legume allergens soy, lupin and peanut as a fast, labor saving and highly sensitive alternative to current available assays.

Keywords: *food allergens, soybean, lupin, peanut, multiplex*

Acknowledgement: *This study is funded by the Fraunhofer Foundation Project FoodAllergen.*

A6

MASS-SPECTROMETRY-BASED ANALYSIS OF MULTIPLE ALLERGENIC INGREDIENTS IN INCURRED MATRICES : OPTIMIZATION OF SAMPLE PREPARATION

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Nowadays, 4 to 8 % of children and 1 to 2 % adults suffer from food allergies. It is therefore essential to develop sensitive and reliable analytical methods to detect the presence of allergens in complex and processed foodstuffs. An objective of the ThRAll project, funded by the European Food Safety Authority, is the development of a harmonised targeted mass spectrometry-based prototype Referencemethod for the quantification of multiple food allergens in standardised incurred food matrices. Several tasks are needed to reach this objective, as production of test materials, selection of marker peptides, optimization of sample preparation and finally validation of the analytical method. First, six priority allergenic foods (cow's milk, hen's egg, hazelnut, peanut, almond and soybean) were then incurred in two model foods (chocolate bar and broth powder) selected as hard to analyze. Signature peptides for the detection of the six allergenic foods were afterward selected with a dual approach combining the critical evaluation of proteotypic peptides already reported in previous independent studies and their experimental validation by discovery analysis on the specific incurred matrices under investigation.
In this communication, we will describe the next step of the method development, namely the optimization of sample preparation.
Several steps improving method performances were discussed and analytical strategies from previous studies were listed, keeping as final objective the development of a single sample prep strategy for multi allergens in processed/difficult matrices. Extraction of targeted allergens was tested regarding composition of the extraction conditions (type of buffer, use of detergents/chaotropic agents, ..) as well as the use of sonication. Afterward purification was tested either at protein level with use of size exclusion chromatography at peptide level using C18 solid-phase extraction. Targeted UHPLC-MS/MS analysis was used to evaluate the effect of the sample preparation conditions on the detection of candidate peptides.

Acknowledgement: *This project has received financial support from the European Food Safety Authority (EFSA), Grant GP/EFSA/AFSCO/2017/03. The present communication, however, is under the sole responsibility of the authors. The positions and opinions presented in this article are those of the authors alone and do not necessarily represent the views/any official position or scientific works of EFSA. To find out more about EFSA guidance documents and other scientific outputs of EFSA, please consult its website at: http://www.efsa.europa.eu.*

A7

THE FIRST LATERAL FLOW TEST FOR THE QUANTIFICATION OF HISTAMINE IN SEAFOOD SAMPLES

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Introduction: High levels of Histamine (HA) may cause scombroid poisoning in humans and therefore the concentration of HA should not exceed 50 ppm in seafood, according to the EU regulations. So far, there is not an established lateral flow method for the quantification of HA, because of the limited immunochromatography technology. For the moment, ELISA is one of the most reliable methods for HA quantification and usually lasts 20-60 minutes.
Aim: The aim of this study was the validation of a quantitative 5-minute Lateral Flow assay by the determination of HA recovery in spiked fish samples and quality control materials as well as the comparison of these results with those derived by ELISA method.
Methods: The quantification of HA in fish samples was conducted with Lateral Flow Symmetric Histamine (S9048, Lot S9048004, Prognosis Biotech S.A.) and ELISA Bio-Shield Histamine (B6096, Lot B6096007, Prognosis Biotech S.A.). Both methods do not use the acylation step and the test time is 5 and 20 minutes, respectively. The sample preparation consists of extraction followed by dilution normalization. Fresh and thawed frozen raw Histamine-free fish samples (anchovy, sardine, tuna, cod, mackerel, fish meal) were spiked at two levels (50 and 100 ppm). Quality control materials provided by FAPAS (T27229QC and T27234QC) were also analyzed and the recovery levels were calculated.
Results: The lateral flow method's recovery and coefficient of variation of all the spikes and the quality control materials were within an acceptable range and almost identical to ELISA method levels. Additionally, there was no significant difference in the results between fresh and thawed frozen raw fish samples.
Conclusion: This innovative Symmetric lateral flow kit provides results comparable to those of a quantitative ELISA, reducing considerably the analysis time. Thus, Symmetric Histamine is the first specific Lateral Flow product to quantify HA in seafood.

Keywords: *lateral flow, histamine, seafood, ELISA, quantification method*

A8

IDENTIFICATION OF PROTEOTYPIC PEPTIDES TRACING FOR MULTIPLE ALLERGENIC INGREDIENTS IN INCURRED MATRICES

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The European legislation in place (EU 1169/2011) issues the mandatory labelling of fourteen allergenic ingredients whenever used in different food formulations. Among these, six main allergens, namely milk, egg, peanut, soybean, hazelnut and almond, can be prioritized in light of their higher occurrence in food recalls for undeclared presence with serious risk decision. An objective of the ThRAll project, funded by the European Food Safety Authority, is the development of a harmonised targeted mass spectrometry-based prototype
Referencemethod for the quantification of multiple food allergens in standardised incurred food matrices. The six priority allergenic foods were incorporated in two selected model foods (1) chocolate bar having high contents of fat and polyphenols and (2) broth powder as an extensively processed food.
In this communication, we will describe the first step of the method development, namely the identification of reliable peptide markers. The latter is a very crucial step since the robustness and sensitivity of the overall analytical method will strictly depend on the reliability of the proteotypic peptides tracing for each allergen. The selection was accomplished with a dual approach combining the critical evaluation of markers already reported in previous independent studies and their experimental validation by discovery analysis on the incurred matrices under investigation.
Several critical aspects affecting peptide markers reliability were discussed. Candidates from previous studies selected according to specific criteria as sequence length, occurrence of amino acids prone to natural and chemical modifications have been studied and evaluated. The peptides that were never validated in either chocolate or incurred highly processed matrices were rejected. This list of makers was then evaluated experimentally. The incurred matrices prepared at the highest inclusion levels were used for discovery experiments. Conditions for protein extraction and purification were optimised using technical aids such as sonication, size exclusion chromatography and C18 solid phase extraction. After tryptic digestion the peptide pools were analysed by untargeted HR-MS/MS on a hybrid quadrupole/Orbitrap™ MS platform and the fragmentation spectra processed with a commercial software. The discovery analysis resulted in the optimization of the extraction conditions to obtain the best detection of proteotypic peptides, the final aim being to maximize the coverage of target protein and expand the list of candidate markers.
The information gathered from literature review and discovery analysis was then collated to produce a list of highly reliable candidate peptides that will be included in the targeted method for final validation.

Acknowledgement: *This project has received financial support from the European Food Safety Authority (EFSA), Grant GP/EFSA/AFSCO/2017/03. The present communication, however, is under the sole responsibility of the authors. The positions and opinions presented in this article are those of the authors alone and do not necessarily represent the views/any official position or scientific works of EFSA. To find out more about EFSA guidance documents and other scientific outputs of EFSA, please consult its website at: http://www.efsa.europa.eu.*

A9

COMPARISON OF ELISA AND QPCR KITS FOR DETERMINATION OF GLUTEN IN REFERENCE MATERIALS

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Cereals containing gluten, namely: wheat, rye, barley, oats, spelt, Khorasan wheat or crosses between these species, and products thereof can lead to major hypersensitivity reactions (e.g. celiac disease). This problematic is a real challenge for healthcare systems, legislative forces but also for the related fields of food analysis.
To guarantee consumer protection, screening analytical methods for detection of gluten in the food chain are available; moreover, official threshold levels for labelling the absence/very low presence of gluten have been established (Commission Implementing Regulation 828/2014).
The aim of this work was to evaluate the performances and fitness for purpose of several ELISA kits and one qPCR kit for analysis of gluten detection. This evaluation has been performed on cookies deliberately contaminated with wheat, rye and barley. The Reference materials were analyzed both by the National Reference Laboratory (NRL) and by the kit providers themselves.
The results showed large variability between assays especially for cookies prepared with rye and barley. The under- or overestimations are due to several factors such as possible modification of the allergens (proteins) during food processing, matrix interferences, presence of inhibiting agents, weak extractability of target (DNA or proteins) but also choice of antibody, calibrant, extraction procedure,...

A10

DETECTING LUPIN MAJOR ALLERGEN CONGLUTIN B FROM VARIOUS LUPIN SPECIES USING NEWLY ESTABLISHED MONOCLONAL ANTIBODIES

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Modern nutrition relies on plant-based protein, especially from legumes, as meat replacements or fat substitute in cold meat. Therefore, the consumption of innovative legume products e.g. made of lupin has increased notably.
There are three main species that have agricultural significance for human nutrition: blue lupin *(lupinus angustifolius*, central Europe), white lupin (*lupinus albus*, Mediterranean countries) and yellow lupin (*lupinus luteus,* central Europe). With worldwide production of more than 1.3 million t, blue lupin is the most widely cultivated of them.
Lupin protein offers a wide range of health benefits along with technological advantages however possess a high allergic potential as well. Out of a total of 14 listed food allergens in the European Union [Regulation (EU) 1169/2011] lupin is one of three legumes that needs labelling when used in foodstuff. So far four lupin proteins have been listed as allergens, among them lupin conglutin β, which is also known as the major allergen Lup a 1.
Even small amounts of lupin can cause adverse reactions in sensitised patients: symptoms ranging from mild itching to severe breathing difficulties including life threatening anaphylaxis.
Since people who are suffering from food allergies need to be protected from unintentional traces of allergenic proteins in food, there is a strong demand for highly specific detection methods.
The aim of this work was to develop a reliable immunoassay to detect even traces of specific lupin antigens in a wide range of processed food. Due to the mentioned various cultivars there was particular attention to determine lupin proteins from blue, white and yellow crops simultaneously.
We purified native lupin proteins using different chromatographic separation steps and used a logical approach to differentiate/demarcate appropriate extraction and test buffer conditions by nanoDSF technology.
Mouse monoclonal antibodies against different lupin proteins were developed and screened for their binding to the purified protein fractions.
These antibodies are specific for single lupin antigens, and moreover show no crossreactions to other legume crops such as peanut, soy, pea, lentils, chick peas and mung beans. The best sets of lupin immunoglobulin G antibodies were chosen to build up specific sandwich ELISA systems in which the optimized buffer conditions result in maximal extractability and recovery.
With this immunoassay it was possible to detect conglutin β in extracts from various lupin subspecies from *l.angustifolius, l.albus* and *l.luteus* in the same range as well as in food samples that are treated by different food processing steps below the regulatory demand of the EU.

Keywords: *lupin, ELISA, allergen detection, food allergy, processed food*

Acknowledgement: *Fraunhofer Zukunftsstiftung*

A11

INTRODUCING AUTOMATION IN ALLERGENS TESTING- IMPROVEMENT ON MEASUREMENT UNCERTAINTY WHILE MINIMISING CONTAMINATION

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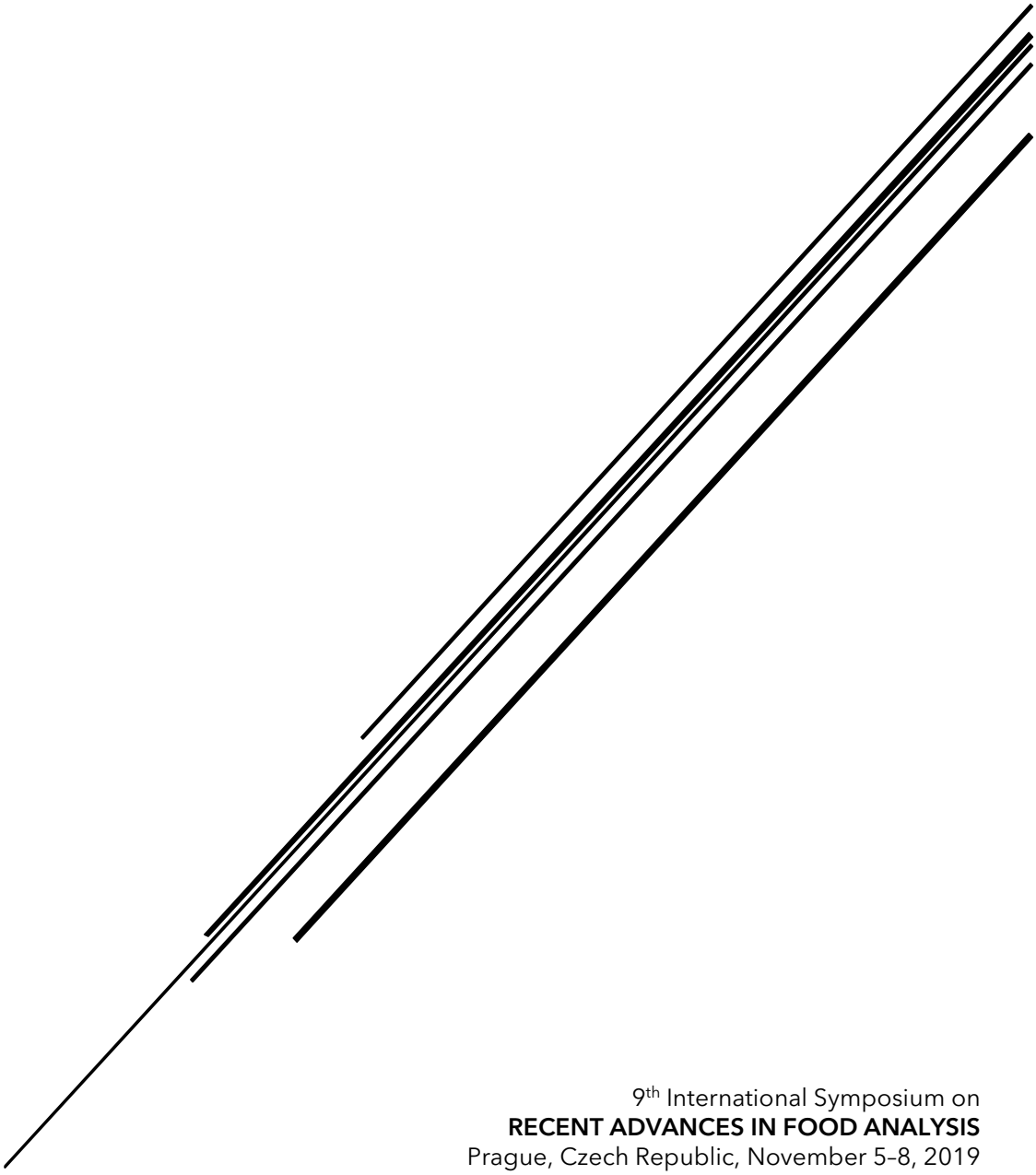
Using enzyme-linked immunosorbent assay (ELISA) is the widely recognised method for the detection of most of the allergenic proteins in food. As a technique, ELISA has been used in other industries in fully automated way for several years however allergens testing in food remains a laborious manual exercise.
Commercial ELISA kits provide the plate, standards and reagents required. The laboratory is responsible for the sample preparation as well as the extraction and measurement. A typical ELISA plate (96 well) will take approximately 4-5 hours to be completed which is critical when samples are tested for positive release. Sample intake for ELISA is usually small and in most cases can be scaled up to 1g without significant issues. Due to this small amount, sample homogeneity is key and it is the major contributor of uncertainty with the other one being the measurement itself. Generally ELISA methods have wide method uncertainties which are dependent on the kit, concentration and matrix. The project looked into optimising the analysis by automating all the post extraction steps, reducing plastic use and improving reproducibility.
A range of commercially available samples (mix of
Referencematerials that contained gluten as well as free form products) were used for the study. All the samples were extracted using the Sensi Spec Ingezim R5 Gluten kit (Ingenasa, Eurofins Technologies) following the kit validated protocol. The same extracts were measured using manual ELISA and automated by Thunderbolt® Analyzer (GSD, Eurofins Technologies). Sample matrices varied from infant formula to chocolate.
The results showed a significant improvement in reproducibility from 7% RSD to 3-4% RSD as well as improved calibration deviation. Repeated testing showed that there is possibility to maximise capacity by using a single curve for longer measurements (96 wells x 2 format). In addition challenge testing using high/low/high gluten samples of different viscosity run simultaneously show no cross contamination between wells. Drift time is measured by well which also allows for a more accurate quantification

Acknowledgement: *Cristina Romero (Eurofins Ingenasa), Max Davantes (Gold Standard Diagnostics)*

AUTHENTICITY, TRACEABILITY, FRAUD

POSTER SESSIONS



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B1

A NEW LC-MS/MS SCREENING METHOD TO DETECT FRAUDULENT ADDITION OF DYES IN HERBS AND SPICES

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The unauthorised addition of colours to herbs and spices for fraud purpose is a recurrent issue affecting food business operators. Such a practice aims at improving food visual attractiveness, masking poor product quality, and/or compensating for natural colour variation with the ultimate goal to increase profits. To detect such a fraud, a new LC–MS/MS method was developed for screening > 50 illegal dyes in these commodities.
This extended list of targets was established based on requirements from international spices organizations, past issues identified by web scouting and by notifications from the European Rapid Alert System for Food and Feed (RASFF).
The method is intended to quickly detect fraudulent addition of dyes with Screening Target Concentrations (STCs) ranging from 0.1 to 2.5 mg/kg. Validation was performed according to the European Community
ReferenceLaboratories Residues Guidelines 20/1/2010. False positive and false negative rates were below 5% for 58 out of 59 targeted compounds.
Applicability of the method was further demonstrated by analysing 117 samples collected worldwide. None of the surveyed dyes was found in herbs (n = 28, 16 varieties) whereas 6% of spice samples (n = 89, 21 varieties) were found contaminated with one or two dyes at levels ranging from 0.12 to 255 mg/kg (quantification by the 3-pt standard addition approach). Four out of the nine detected compounds have never been reported in the RASFF, thus demonstrating the usefulness of this analytical approach.
Small peaks were often seen in chromatograms but well below our STCs. Findings of unauthorized colorants at low µg/kg levels are justified by incidental contaminations from either colours used in pesticide formulations, inks used for sack labelling, contaminations from packagings, or colored lubricants in equipment. Authorities in the USA, UK, and Scotland have set some action limits at 0.5 or 1 mg/kg to discriminate between adulteration and incidental contaminations. A guidance document at the CODEX level is highly desirable to avoid this misalignment of control policies worldwide that ultimately may cause disruption of international trade.

Keywords: *dyes, food fraud, spices, LC-MS/MS, colorants*

B2

IDENTIFICATION OF FOOD FRAUD BY ADDITION OF AZO-DYES IN RED SPICES BY UHPLC-API-MS/MS

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Consumer products of high commercial value are frequently the objective of manipulation and adulteration practices. The replacement of main and valuable ingredients by cheaper and inferior quality ones, unfortunately, is becoming a common fraud practice, which represents not only an economical problem but also a human health risk because the intake of adulterant/substituent could be of food safety concern. Among the wide range of synthetic dyes used in industrial applications, azo-dyes are a group of additives banned within the food industry due to their related human health concern [1]. In 2003, France informed through the Rapid Alert System for Food and Feed (RASFF) about the detection of Sudan I azo-dye in hot chilli products, but in the same period, other Sudan dyes were also detected in spices such us curcuma and curry [2].
In this work, the chromatographic separation of eight azo-dyes related compounds is achieved in less than 8 minutes using an Accucore C18 column (100 mm × 2.1 mm id., 2.6 µm particle size), a binary mobile phase of acetonitrile:water (formic acid/ammonium formate, 20 mM, pH 3.75) and a flow-rate of 600 µL min^{-1}. This chromatographic method is used to analyze these compounds by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Positive and negative ion mode is used to identify and confirm the presence of these target compounds in red spices to detect adulterated/fraud samples. Moreover, the ionization efficiency is studied using electrospray (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) to evaluate these ionization sources and to select the most sensitive and selective one. A sample treatment based on simple and fast solid-liquid extraction procedures is proposed for the analysis of spice samples. Among the different extractant solvents tested, acetonitrile allowed to maximize the extraction efficiency and minimize the matrix effect.
Several chili, curry, saffron, and curcuma have been analyzed and quantified and the results show the applicability of the method for the identification of fraud spice samples due to the addition of banned azo-dyes. The performance of the proposed method shows satisfactory quality parameters providing low limits of detection (0.01 – 0.03 mg L^{-1}), good precision (RSD% <10%), high extraction efficiencies (>95%), low matrix effects (<15%) and accurate quantification (relative error % <10%).

[1] EFSA Journal. 263 (2005), 1-71
[2] P. Botek, J. Poustka, J. Hajslova, Czech J. Food Sci. 25 (2007), 17-24

Keywords: *red spices, food fraud, azo-dyes, UHPLC-API-MS/MS*

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B3

LC-MS/MS SCREENING METHOD FOR DETECTION OF UNAUTHORIZED COLORANTS IN SPICES

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Economically Motivated Adulteration (EMA) is the fraudulent addition of nonauthentic substances or removal or replacement of authentic substances without the purchaser's knowledge for the economic gain of the seller. Spices and spice mixes are traded in large volume and at premium prices, and commonly sold in a granulated form. The combination of these factors makes spices one of the most susceptible targets for EMA. Therefore, an LC-MS/MS screening method was developed and validated to detect 25 unauthorized or undeclared colorants in spices, including turmeric, chili powder, paprika, curry, and sumac. The performance metrics, including selectivity, recovery, the limit of detection, and linearity, were determined. The method was applied on spice samples on the market, including rejected samples, and detected one or more unauthorized or undeclared colorants in different samples. This validated method will be proposed as a Food Chemicals Codex standard.

Keywords: *spice, colorant, fraud, LC-MS, EMA*

B4

RELAXATION OF THE ANIMAL BY-PRODUCTS FEEDBAN? ANALYTICAL CHALLENGES AND FORESEEN SOLUTIONS TO ENSURE HIGH LEVEL OF FEED SAFETY

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In the 90's, a feed ban on the use of animal by-products was introduced in order to manage the risk of spread of Bovine Spongiform Encephalopathy (BSE). Indeed, the consumption of meat and bone meal containing carcasses of infected animals was incriminated.
Since 2001, a positive trend in the BSE epidemic is observed and a gradual lifting of the ban has been put in place. However, even today, uncertainties do remain in relation to the pathogenesis and epidemiology of the disease. Occasional cases of classical BSE still occur in animals born after the reinforcement of the feed ban (BARB cases) and it is not clear whether these cases are due to an incorrect implementation of the ban or to spontaneous incidents.
One of the key points to consider when revising the current feed ban is the availability of control tools to ensure proper enforcement of the regulation. The persisting challenge in this context is the development of complementary methods or the adaptation of official methods in order to refine the identification of feed materials.
The aim of this presentation is to give an overview of the complexity of the feed ban in its current state and to outline the analytical challenges to safely implement a further relaxation of the ban. It also underlines the analytical limitations that should be taken into account in regulatory provisions aiming at such a further lifting of the feed ban without increasing the risk of fraud that would have consequences for feed safety. Finally, the analytical solutions currently envisaged are presented with a highlight on the most promising one: the detection of specific peptides by ultra-high liquid chromatography combined to mass spectrometry. Indeed, this method is perfectly adapted to regulation requirements by providing information about the tissue and species of origin based on the detection of one or more specific peptide sequences. Results from these current developments will be presented to illustrate what future operational patterns might look like.

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

B5

A JOINTLY USED DATABASE – A LONG WAY IN NON-TARGETED ANALYSIS

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In the field of non-targeted analysis, spectroscopic methods, e. g. Fourier Transform Infrared (FT-IR) spectroscopy, combined with subsequent multivariate data evaluation are increasingly used to investigate different food authenticity issues. For this purpose, a so-called chemical fingerprint of the particular food is acquired and deviations from the expected product composition can be identified. However, the routine use of this approach is currently restricted due to a multitude of prerequisites which need to be fulfilled for a standardized/harmonized analysis and finally, for the use of a common database. In this presentation, two main requirements and their possible solutions will be discussed, investigated in the BMEL-funded project FoodAuthent: i) developing of a uniform strategy for quality assurance in non-targeted analysis and ii) ensuring the comparability of spectral data. To tackle these challenges, various multivariate approaches were tested using FT-IR spectra of edible oils. In case of quality assurance strategies, general aim is to monitor the analytical procedure and to enable the timely identification of temporal and/or instrumental trends or outliers by numerical values documented in a quality control chart. As a proof of concept, a well-established quality control sample (refined rapeseed oil) was measured by FT-IR spectroscopy under different laboratory conditions, for instance various measurement temperatures. In a first step, explorative data analysis was performed. For this, principal component analysis in combination with Hotelling's T-squared distribution was performed. Secondly, density-based (local outlier factor) and distance-based (nearest neighbors) models were tested to identify outliers. Here, concrete numerical values, especially mean and standard deviation, were generated in accordance to a classical *Shewhart* control chart [1] used in targeted analysis to build up a respective multivariate solution.

[1] Shewhart, W. A., et al. (1939), Statistical method from the viewpoint of quality control. Washington, United States of America: Lancaster Press

Keywords: *standardization, harmonization, comparability, quality assurance*

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B6

"A2 MILK" AUTHENTICATION USING ISOELECTRIC FOCUSING AND DIFFERENT PCR TECHNIQUES

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Milk protein polymorphism has received great interest within the dairy industry because of possible relationship to milk production traits, milk composition (e.g., protein content), and technological properties (e.g., heat stability, renneting and cheesemaking properties) of milk. Interestingly, the distribution of milk protein genotypes was shown to vary considerably between the dairy cattle breeds. Moreover, it has been reported that the frequencies of some milk protein loci have changed during the last years, probably unintentionally or due to breeding programs (e.g. κ-Cn B, β-Cn A^2; β-Lg B) that have already been performed in several countries (e.g., Austria, Italy, Switzerland, New Zealand). β-Casomorphins (BCMs) are opioid-like peptides (chain length of 4–11 amino acids) released from β-casein of bovine or human milk during technological processes and/or enzymatic digestion in the intestine. And according the "A1/A2 hypothesis", milk can be classified into two types, i.e., A1 "like" milk having a histidine at position 67 that determines the enzymatic cleavage of the peptide bond releasing b-casomorphin-7 (β-CM-7), and A2 "like" milk that does not cleave due to the presence of a proline at this position. A number of studies have suggested that bovine β-CM-7 can act as a causative agent of cardiovascular disease, type 1 diabetes, sudden infant death syndrome, autism and schizophrenia. However, it appears highly unlikely that evolutional (natural) selection retained a harmful product as the main nutritional component for mammalian infants, and most data indicate the positive action of milk-derived peptides. Finally, a scientific report of EFSA concluded that a cause-effect relationship between the oral intake of β-CM-7 or related peptides and aetiology or course of any suggested non-communicable diseases cannot be established.
As such dairy products (e.g., A2 milk and yoghurt) are on the market and are advertised offensively as "risk-free" A2 milk or "indigenous" milk, the authentication of these high-prized alternative foods is crucial to avoid misleading consumers by adulteration. Therefore, the aim of this study was to establish reliable analytical methods for the detection of genetic variants using isoelectric focusing (IEF) of milk proteins, and appropriate polymerase chain reaction (PCR) techniques.
The simultaneous separation of genetic variants of caseins and whey proteins using IEF was successfully applied for the phenotyping of dairy cows. Moreover, allele-specific PCR was performed to identify the β-casein alleles A^1, A^2, B, C and I. In addition, κ-Cn and β-Lg B genotypes were determined by PCR-RFLP and/or AS-PCR using specific primer-pairs and appropriate restriction enzymes. DNA extraction techniques from various sources (i.e., milk, yoghurt, blood, saliva, hair, and sperm) were optimized. Authenticity of "A2 milk" products in Austria was monitored by analysing both individual cows and retailed yoghurt and liquid milk products.

Keywords: *A2 milk, authentication, isoelectric focusing, AS-PCR, PCR-RFLP*

B7

MONITORING OF ILLEGALLY ADDED COMPOUNDS AND DRUGS IN FOODS-FOCUSED ON PROHIBITED INGREDIENTS

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Illegally added compounds and drugs have been detected in general or functional foods up to recently. Especially, unknown compounds mimicking anti-impotence drugs including icariin and anti-obesity drugs such as 7-keto-DHEA were frequently detected in various food products.
The purpose of the current study was to monitoring illegally added compounds and drugs in foods to ensure food safety.
We monitored 156 food items advertised as slimming products or sexual enhancement products in offine markets. Samples (1 to 10 g) were methanol and sonicated for 30 min, followed by filtration using 0.2 μm syringe filter. 48 illegal compounds were detected by liquid chromatography equipped with photo diode array(LC/PDA) and confirmed with LC coupled with tandem mass of spectrometry(LC/MS/MS).
7-keto-DHEA, Icariin, Synephrine or Yohimbine was detected in 21 food items with range of 0.15~0.85 mg/g, These results indicated that a sustainable monitoring of illegal compounds in various food products is required for the safety.
These results indicated that a sustainable monitoring of illegal compounds in various foodstuffs is required for the safety management. In conclusion, The results of this study will contribute to reinforcement the food safety.

Keywords: *illegal adulterants, icariin, 7-keto-DHEA, anti-impotence drug, anti-obesity drugs*

B8

DNA BARCODING REVEALING SEAFOOD MISLABELING IN FOOD SERVICES FROM SPAIN

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Consumers are increasingly concerned about the origin and sustainability of supply seafood chain. Commercialization of seafood is among the most complex food chain internationally and it is therefore one of the food commodities most likely to face challenges around fraud and authenticity. Since seafood is a very appreciated and valuable food product, economic motivation, to provide lower cost products from fisheries and aquaculture, is one of the main factors behind fraud. Consequently, some studies have revealed a high percentage of fraud for species substitution at retail and food service level worldwide including European Union. Spain is one of the European countries with the highest consumption of fisheries and aquaculture products, at home and outside the home, and therefore a very interesting region to evaluate the impact of seafood fraud in food services. This study is the first large-scale attempt to study the rate of seafood fraud in food services across Spain. A total of 313 samples were collected in 204 mass caterer outlets in 15 of the most important Spanish Autonomous Communities. DNA barcoding revealed that 50% of the food services establishments sold mislabeled seafood. The highest mislabeling rates were found in dusky grouper, butterfish, tope shark, sole and bluefin tuna. Those species, and others, were fraudulent substituted with a great variety of species revealing the carelessness in the manipulation of seafood products and the absence of transparency in the Spanish food services affected also by the exemption of including the commercial name as a mandatory information in the menus. These findings evidenced also the complexity of international seafood supply chains that is also supported by the fact that many of the species reported substituting local species, are coming from Asian, South American and South African regions.

Keywords: *food service, mass caterer, seafood, fraud, mislabeling*

Acknowledgement: *The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° (613688) and from the Department of Food, Fisheries and Agriculture from the Basque Government. Samples were collected thanks to the efforts of all the collectors across Spain.*

B9

HIGH-RESOLUTION MELTING OF MULTIPLE BARCODE AMPLICONS FOR PLANT SPECIES AUTHENTICATION

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Authentication of plant species is important in a variety of different areas such as the trade of illegal and endangered species, herbal medicine, and food authentication, where one species is replaced with a cheaper one.
Authentication of plant species can be performed through microscopy, detection of metabolites, species-specific peptides, and DNA based techniques. Several DNA markers exist, including satellites, insertions and deletions (InDels), single nucleotide polymorphisms (SNPs), and barcode regions. Barcode regions are particularly popular because of a high variability flanked by conserved regions suitable for primer design.
Information reflecting the size and sequence of individual amplicons can be extracted with high-resolution melting (HRM). Discrimination or identification of species with HRM of amplicons was achieved in several examples [1-3], but with an application limited to a small number of species. Interestingly, it was concluded in a thorough HRM investigation of barcode regions in plants that a barcode combination could provide an identification rate of 99% [4]. In this presentation, we show how to expand the technology and allow HRM to operate on several barcode amplicons from a multiplexed PCR reaction. Theoretically, this renders a concurrent universality and a high discrimination power. Multiplexed HRM profiles will inevitably be more complex than simplexed HRM curves. To embrace this complexity, multivariate data analysis applied to the high-resolution melting profiles would be an advantage. The ability of this profiling approach [5] to accomplish universality through the plant kingdom alongside the ability to distinguish closely related species will be presented.

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Keywords: *Authentication, Food fraud, High-resolution melting, Species identification, Plants*

Acknowledgement: *This project (WBS H6681) was supported by the European Commission's (JRC) Exploratory Research Program 2018.*

B10

IS VIBRATIONAL SPECTROSCOPY AN ADEQUATE TOOL FOR ASSESSING THE GEOGRAPHICAL ORIGIN OF HONEY?

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Ha price premium. Hence, the potential for economic fraud is clear. Nowadays, pollen analysis (melissopalynology) is typically used for the honeys' floral origin identification, but it can hardly be used for geographical discrimination. Furthermore, this technique is extremely time-consuming and strongly affected by the analyst competence.
While a range of techniques have been proposed for the classification of monofloral honey, mainly based on the typical volatile signature and/or the phenolic profile, the correct classification of multifloral honey is still challenging.
The aim of the present study was to investigate the capability of vibrational spectroscopy as valid tool for the geographical origin discrimination of multifloral honey.
Multifloral honey samples were collected among the three main honey-producer regions of Argentina: Buenos Aires, Catamarca and Misiones. The sampling was repeated over four harvesting seasons (2014, 2015, 2016, 2017). The total number of samples was n=502. Analysis were performed by three different spectroscopic techniques: Raman, Near Infrared (NIR) and Mid-Infrared (MIR).
Partial Least Squares Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM) were used as supervised discriminant techniques. Cross-validation leaving one-year-out was performed and the resulting model has been used for the prediction of an independent test set. In order to improve the model discrimination ability, different data preprocessing methods were tested and variable selection was carried out. Since data coming from three different instruments were available, data fusion approach was tempted. The purpose was to gain better classification and lower error rate.
Principal component analysis revealed clustering between the geographical regions over all the considered years. The same discrimination was obtained with all three spectroscopic techniques. Discriminant analysis shown very good performances in discerning Misiones and Buenos Aires samples, whereas higher error classification rate was found for Catamarca. This might be explained by the environmental effect, especially in term of climate conditions and geographical features. While Misiones has its own characteristic subtropical weather and vegetation, Catamarca exhibits several different microclimates.
The results from this study demonstrated the feasibility of vibrational spectroscopic techniques as fast and effective methods for honey authentication and provenance confirmation.

Keywords: *honey, vibrational spectroscopy, geographical origin, chemometrics*

B11

AUTHENTICITY OF HONEY: IS DIRECT-MS AN EFFECTIVE SCREENING TOOL?

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In terms of authenticity, honey is among the most challenging food products, being addition of non-permitted substances (i.e. syrups or sugars), bees overfeeding, mislabeling and abuse of veterinary drugs, among the main adulteration practices.
Nowadays, melissopalynology still remains the
Referencemethod for many of the authenticity issues in honey, such as floral and geographical origin, undeclared blending and/or filtration, etc. However, such an approach is highly time-demanding and strongly conditioned by the analyst skills. Therefore, a lot of effort has been focused on developing alternative analytical methods for authentication purposes. In particular, fast and reliable screening techniques are highly claimed for effective management of a fast-paced global food network.
Since ambient mass spectrometry meets the requirements in terms of speed and ease of use, substantial literature has been reported so far about the potential and limitations of Direct Analysis in Real Time-Mass Spectrometry (DART-MS) in food analysis [1].
In a previous work (unpublished data), the capability of several analytical techniques (namely, NIR, NMR, ICP-MS, and untargeted LC-HRMS) in discriminating honeys according to their geographical provenance, was evaluated. Under a data fusion approach, results coming from different platforms were merged, leading to a 100% correct classification rate. Although extremely powerful, the approach was, however, time- and labour intensive. Therefore, with the aim of exploring alternative methodologies, the ability of direct MS to discriminate the same set of honey samples in terms of geographical origin, was challenged.
The sample set, i.e. 10 Italian and 10 non-Italian Acacia honeys, was fingerprinted by means of a DART ion source (IonSense, Saugus, USA) coupled to a QDa single-quadrupole mass detector (Waters Corp., Manchester, UK). The sample preparation was minimal, with a total analysis time of 5 minutes. Data pretreatment was aimed to improve the overall data quality, overcoming pitfalls in data reproducibility. Thereafter, multivariate data analysis was carried out, using data obtained by DART-QDA instead of those obtained by HR-LCMS. An overall sample discrimination of 100% was again obtained.
The results of the present work, proved the capability of DART-MS as fast and cost-effective screening platform for honey authenticity analysis.

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Keywords: *Food Fraud, Honey, Direct-MS, Chemometrics*

B12

TRACKING SUGAR ADDITION IN FOOD AND BEVERAGE USING ISOTOPE FINGERPRINTS

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Complexities in the food and beverage supply chain from the production site through to the consumer have presented significant, and at times relatively easy, opportunity for economically motivated fraudulent activities to occur and be undetected. Consequently, there is an increase in retailer and consumer demand to proof that food and beverage products are what the label claims them to be, including origin, authenticity and ingredient verification.
One of the most known adulteration processes involves the addition of sugar to food and beverages. Detecting the added sugar can be achieved using stable isotope measurements because stable isotopes can differentiate between the sugar already present in the sample from the sugar which is added artificially. Carbohydrates carry an isotope fingerprint, a unique chemical signature which identifies their origin. To visualize this fingerprint, Isotope Ratio Mass Spectrometry (IRMS) can be used, identifying the isotope fingerprint of the product.
In this presentation the application of stable isotope fingerprints in detecting sugar addition to food and beverage samples is explored. Data 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 official methods using isotope fingerprints for food and beverage analysis are also provided.

Keywords: *isotope fingerprints, IRMS, stable isotopes, origin, authenticity*

B13

ELEMENT COMPOSITION AND STABLE ISOTOPE RATIO OF LIGHT ELEMENTS COMBINED WITH AMINO- AND FATTY ACID COMPOSITION FOR AUTHENTICITY AND GEOGRAPHICAL ORIGIN CHARACTERIZATION OF SPIRULINA DIETARY SUPPLEMENTS

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Spirulina products are a good source of nutrients and are among the leading food supplements on the market. High cost of a highly controlled closed system production environment, as well as variations in quality due to changing environmental conditions in an open production system and the market demand for a high quality products make these products more likely to be deliberately adulterated. In this study, a combination of elemental composition, stable isotope ratio of light elements (carbon, nitrogen, sulfur, hydrogen and oxygen) together with total amino acid and fatty acid composition was used to differentiate between samples of different geographical origin and to determine their authenticity.
Spirulina dietary supplement samples (47) were collected from the Slovenian market. The samples were in powder, capsule or tablet form, fresh or dried and labelled as produced in Hawaii, India, China, Japan, Italy, Taiwan and Portugal. For this study, elemental analysis - isotope ratio mass spectrometry (EA-IRMS) and high-temperature thermal conversion isotope ratio mass spectrometry (TC/EA-IRMS) were used to obtain stable isotope ratios of carbon, hydrogen, sulfur, oxygen and nitrogen. The levels of micro- and macroelements were determined using X-ray fluorescence (XRF) while the trace element content was obtained using inductively coupled plasma mass spectrometry (ICP-MS). Fatty acid derivatives were prepared using the esterification method and amino acid derivatives by using the EZ:faast Amino Acid Hydrolysate kit from Phenomenex. The total fatty acid and amino acid content was determined by gas chromatography-mass spectrometry (GC-MS).
Carbon stable isotope values ($\delta^{13}C$) ranged between -32.3‰ and -16.7‰, $\delta^{18}O$ ranged between 12.9‰ and 27.2‰, $\delta^{15}N$ between -5.4‰ and 13.8‰, $\delta^{34}S$ between -1.8‰ and 13.8‰ and $\delta^{2}H$ values ranged between -208‰ and -97‰. Macro- and microelement distribution according to their concentrations in the samples was as follows: potassium > phosphorus > sulphur > silicon > calcium > chloride > iron > manganese > titanium > zinc > bromide > vanadium > selenium. The amino acid composition of the Spirulina samples was varied between samples, but all the essential amino acids and the majority of the non-essential amino acids were present in the samples. Distribution of the fatty acids was as follows: palmitic acid > linoleic acid > γ-linolenic acid > stearic acid > oleic acid > palmitoleic acid. Omega-3 fatty acids were found only in small concentrations. The large deviations in fatty acid composition suggest some of the samples had been adulterated.
Combining stable isotope ratios and elemental composition with total fatty- and amino acid composition in the Discriminant Analysis (DA) resulted in improved discrimination of the samples' based on their geographical origin as well as revealing possible adulterations.

Keywords: *spirulina, isotope ratio, amino acid, fatty acid, authenticity*

B14

A NOVEL UHPLC-MS/MS METHOD TO DETECT UNDECLARED BLOOD PLASMA ADDITION IN SAUSAGES

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Large amounts of porcine blood are produced during the slaughter of pigs, being the most relevant slaughter animal species in the European Union. In contrast to whole blood, which can be added to meat products to a very limited extent only, porcine blood plasma (or plasma powder) can be used as an ingredient in various meat products (especially emulsion-type sausages) due to advantageous functional properties and low costs. However, in the European Union, the addition of blood plasma to meat products must be stated in the list of ingredients according to Regulation (EU) No. 1169/2011.
The food control authorities urgently need reliable analytical methods to detect the undeclared addition of porcine blood plasma to meat products to protect the consumer against this type of food fraud. However, the evidence of this type of food fraud has been rather difficult to identify due to the lack of appropriate analytical methods, especially when adding plasma to meat of the same animal species.
The objective of our work was to develop a rapid UHPLC-MS/MS method for the detection of porcine blood plasma in emulsion-type pork sausages. After protein extraction and tryptic digestion in a quick and simple one-pot process, species-specific marker peptides for porcine blood cell proteins (four markers) and plasma proteins (twelve markers) were measured by UHPLC-MS/MS. Regarding the reliable detection of the addition of porcine plasma powder to meat of the same animal species (pork), the purely qualitative detection of plasma peptides does not allow clear conclusions on the addition of blood plasma, because meat and also meat products always contain a certain amount of residual blood and, consequently, plasma proteins. Emulsion-type pork sausages were produced from a variety of raw materials that differed in the age or sex of the slaughtered pigs. Sausages were spiked with 0.5, 1, 1.5, 2, 3 or 5 % meat substitution by one of two commercial plasma powders, or produced as corresponding blank samples, and subjected to different thermal treatments as full or semi-preserves. Four plasma peptides were identified for the overall sample that allowed detection down to 0.7 % meat substitution from the sum of their peak areas, with 5 % error probabilities for both false -positives and negatives.

Keywords: *UHPLC-MS/MS marker peptides porcine blood plasma pork sausages food fraud*

B15

REAL MARKET EXAMPLES - WINE QUALITY, TASTE AND AUTHENTICITY CONTROL

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Wine is a product with thousands years of history. Unfortunately, wine has been traditionally one of the most adulterated food products with a number of scandals standing out in recent years (1985 - diethylene glycol addition in Austria, 2012 - scandal with Rudy Kurniawan in the US counterfeiting premium wines, last years - fake French wines in China, etc.).
On the other hand, wine perception is very subjective and largely driven by marketing. Nevertheless, taste plays obviously a very important role in wine perception. However, it is also subjective and even professional sensory panels might have large variations in their assessments.
Vinoscent is a wine integrity control private company located in the Netherlands. We work with players from the wine industry across the whole supply chain – producers, bottling companies, distributors and retailers. We help to source high quality wines, and monitor stability of wine quality and taste over time.
Our presentation will be dedicated to 3 main parts:
1. Objective approach to monitor wine quality and taste. This is performed with a use of proton-transfer-reaction mass spectrometry (PTR-MS) technique, identification of major flavour compounds and statistical analysis. Over the years we have built a database with 20,000+ wines in it which allows us to compare wine samples to its peers from across the database considering specific vintage, origin and grape variety.
2. Approach to wine authenticity control with a real industry example. This is carried out with a use of PTR-MS, isotope-ratio mass spectrometry (IR-MS) and principal component analysis (PCA).
3. A statistical model to predict taste style of wine based on the laboratory (incl. PTR-MS) measurements. This model was developed for one of the Scandinavian wine markets where strict requirements from governmental monopoly are posed upon wine importers and objective tools are needed to assess wine quality and taste.
To summarize, we will briefly talk about how this approach can be scaled to other geographies (e.g., China) and other food products (herbs&spices, cocoa mass, beer, etc.) to monitor their quality, taste and authenticity.

Keywords: *wine, wine authenticity, wine quality, databases, PTR-MS*

B16

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: *coffee, isotope fingerprints, origin, fraud, traceability*

B17

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, traceability, isotope fingerprints, adulteration, origin*

B18

HOW CLIMATE CHANGE CAN HELP FIGHTING FRAUDULENT DECLARATION OF CEREAL GRAINS

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According to a report by the German Meteorological Service, the year 2018 was one of the driest and warmest since weather records began in 1881. As a result, farmers had to cope with heavy losses in both crop yield and crop quality throughout the country. Such extreme weather events lead to drastic market fluctuations, as they create shortages in high-quality grain supply while simultaneously producing an excess of lower quality grains. To maintain quality parameters like protein content and bulk density, traders are often forced to mix batches from several harvest years, which must be declared. At the same time, more and more customers insist on buying cereal grains from a single harvest year and pay less for mixed batches. Combined with the competitiveness of the market, the risk of mislabelling increases, especially since no method for determination of harvest year is established yet.

Isotope-ratio mass spectrometry (IRMS) could be a suitable approach as plant-based products reflect characteristics of their environment and physiology through the stable isotope ratios of the light elements ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{2}H/^{1}H$), hence also inter-annual changes in e.g. temperature, amount of precipitation and air humidity. IRMS is already a well-established approach in wine authenticity testing, in which it helps verifying the vintage year stated on the label. To test the feasibility of this method for commercial crops from Germany, we analysed 406 cereal grain samples (barley: 219, spelt: 187) from the harvest years 2016-2018 for their carbon, nitrogen and oxygen stable isotope data as well as their element concentrations. In addition, we applied near-infrared spectroscopy (NIRS), a technique with various advantages like affordability, rapid measurements, little to no sample preparation and potential to be used online.

Special attention was paid during sample selection and data evaluation in order to consider and minimize the variability introduced by factors such as genotype or region. Isolated methods showed clear trends for discrimination between harvest years, especially for the year 2018. Separation of the groups was significantly improved when using combined data sets and multivariate statistical approaches with high correct classification rates. Carbon and oxygen natural isotope abundance were the most important variables included into the model.

We are convinced that due to the increased frequency of extreme weather events, this multi-method approach turns out in the future as a valuable and urgently required tool in fighting fraudulent declaration of cereal harvest year.

Keywords: *Stable isotopes, NIRS, Cereal grains, Harvest year, Climate change*

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.*

B19

DIFFERENTIATION OF CEREAL FLOUR SPECIES BY RAMAN SPECTROSCOPY

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Grain is an important staple food and is the 5th most commonly counterfeited food in the world. Due to variation in supply and demand, cereals are subject to substitution and adulteration with cheaper cereals. Therefore, origin and authenticity are becoming increasingly important. In this study, Raman spectroscopy and multivariate data analysis were applied for discrimination of cereal species. Wheat (n = 26), spelt (n = 27), rye (n = 25) and barley (n = 26) flour samples from harvest years 2014 to 2017 were analysed with a confocal Raman microscope. Principal component analysis was used to show qualitative differences in the spectra. The grain species can be differentiated primarily by Raman signals of proteins, pentosanes and starch. Ferulic acid was detected in all samples, however it was not a significant factor. Partial least squares discriminant analysis (PLSDA) was shown to differentiate the species with a cross-validated (cv) true positive rate of 100% for rye, wheat, spelt and 92% for barley. Furthermore, a tendency was observed to separate the species depending on harvest years with cross-validated true positive rates of 45% to 100%. The results show that Raman spectroscopic data in tandem with chemometrics can be applied to differentiate the cereal species wheat, spelt, rye and barley, but that the harvest years have to be taken into account.

Keywords: *Raman spectroscopy, authenticity, cereals, chemometrics*

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.*

B20

DEVELOPMENT OF IMMUNOASSAYS FOR TROPONIN I TO CONTROL A CONTENT AND A SOURCE OF RAW MEAT IN PRODUCTS OF ITS PROCESSING

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Control of food composition and content of main sources of its preparation is essential analytical task of high practical importance. The identification of animals used to prepare meat products allows preventing risks of the presence of ad hoc and low grade components. The common instrumental techniques (PCR, chromatography, microscopy, etc.) should be accomplished by rapid low-cost tests to realize wide and operate monitoring, and immunoanalytical techniques are considered as an efficient solution for this purpose.
The report presents the development of immunoanalytical systems for detection of meat compounds in processed food products. An important issue that determines the applicability of immunodetection in food control is the stability of detected biomarkers after enzymatic and heat treatment in the course of technological processing. In this connection troponins may be recommended as thermostable biomarkers of muscle tissues. The study was based on initial testing monoclonal antibodies against troponin I in terms of their affinity and possibility to recognize troponins from key animals and birds used in the meat industry.
An enzyme-linked immunosorbent assay (ELISA) of troponin I was developed and characterized for analytical purposes. It was found that this ELISA with the chosen completion of immune reactants allowed distinguishing mammalian (beef, pork, lamb, horse) and bird (chicken, turkey, duck) meat sources. Possibility to accelerate the assay by their implementation in kinetic mode was evaluated and as a result the ELISA protocol with a total duration of 30 min was proposed. Lateral flow immunoassay (LFIA) was proposed for rapid (15 min) on-site screening control as well as it could be realized by a simple contact of prepared sample (meat extract) with a test strip containing preliminary applied reactants. Analytical parameters of LFIA were compared for different combination of immune reactants, and regimes for test strips manufacturing were chosen. Methods for sample preparation of meat products before the proposed immunoassays were comparatively tested. Finally, the developed immunotechniques were applied for meat foodstuffs and demonstrated high productivity and accuracy of measurement results.

Keywords: *ELISA, immunochromatography, composition of meat products, biomarkers of muscle tissues, thermostable proteins*

Acknowledgement: *This study was financially supported by the Russian Science Foundation (grant number 19-16-00108).*

B21

MONITORING FOOD AUTHENTICITY USING AN ADVANCED GLYCAN ARRAY PROFILING PLATFORM

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Constant advancements enabling the fraudulent manufacturing of food products have dictated the need for the development of an efficient testing regimen. Public awareness of food fraudulence and the need for robust monitoring notably increased after the 2013 'Horsemeat Scandal'. This, and other episodes, highlighted the need for a structured legislation framework within which authentication technologies can operate. These issues are likely to grow in importance as societies worldwide tackle the food production requirements of a growing population, whilst maintaining quality and nutritional traits.
The work being conducted describes a new analytical platform that enables the polysaccharide profiles of plant-based foods to be created. The method combines the high-throughput capability of microarrays with the specificity of monoclonal antibodies, taking advantage of the uniqueness of polysaccharide profiles in order to target and possibly identify adulterants within food produce, using polysaccharides themselves as markers.
This method adapts a technique that has been widely used for long-range tracking of polysaccharides in complex natural and industrial systems, as shown recently by Fangel et al. in relation to brewing(1). Within this project, the technique has already been applied to whole grain monitoring, as well as looking into the profiling of various herbs and spices.

(1) Fangel JU, Eiken J, Sierksma A, Schols HA, Willats WGT, Harholt J. (2018) Tracking polysaccharides through the brewing process. Carbohydrate Polymers. 15;196:465-473. doi: 10.1016/j.carbpo l.2018.05.053.

Keywords: *Polysaccharide Monoclonal Antibodies Microarray High Throughput*

B22

USE OF THE SPECTROSCOPY-BASED PHASMAFOOD SENSORS FOR THE DETECTION OF MINCED MEAT ADULTERATION

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Food fraud, including among others economically motivated adulteration, has demonstrated an increasing magnitude and significance as a food protection issue the last decade. In this sense, the aim of this study was the assessment of the feasibility of the non-invasive spectroscopy-based PhasmaFOOD sensors as means of detecting minced meat adulteration. The adulteration scenario studied was adulteration of minced pork with chicken and vice versa. Pork and chicken meat, purchased from different butcher shops, were minced and appropriate portions of the two meat types were mixed in order for three adulteration levels to be attained: 25, 50 and 75%. Two levels of pure meat (100% pork, 100% chicken) also were used. Six different samples were prepared for each one of the five abovementioned levels. In total, 120 samples were prepared and subjected to acquisition of near-infrared (NIR), visible reflectance (VIS) and fluorescence (FLUO) spectral data using individual sensors. After proper pre-processing, the collected data were analyzed using a support vector machines (SVM) classification approach, and distinct datasets were used for the purpose of model calibration and validation. Also, different classification schemes were considered: a three-class classification (i.e. adulterated, pure pork, pure chicken) and a five-class classification allowing for prediction of the level of adulteration (i.e. 0, 25, 50, 75 and 100%). According to the SVM classification results for five classes, the VIS spectra resulted in overall correct classification (OCC) of 94 and 77% for calibration and validation, respectively. With regard to the analysis of the FLUO spectral data, the classification results for five classes indicated a very good model performance, with the estimated OCC being 92 and 93% for calibration and validation, respectively. On the other hand, the NIR data resulted in SVM classification with poor performance, demonstrating a limited ability of this type of data to support the development of classification models providing accurate predictions of the adulteration level. Specifically, the OCC in the case of the NIR model and for the five-class scheme was 81 and 20% for calibration and validation, respectively. Moreover, although in the case of the VIS and FLUO models, a clearly better performance was exhibited when considering three classes, this was not the case for the NIR model which still exhibited a poor performance even under this classification scheme. Corresponding model calibration was performed using VIS and FLUO spectral data derived from the first prototype of the PhasmaFOOD integrated device, while the latter data were also used for the external validation of the models previously developed for the individual spectroscopic sensors.

Keywords: *food fraud, adulteration, spectroscopy, support vector machines, classification*

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*

B23

ASSESSMENT OF FRUIT JUICE AUTHENTICATION USING UPLC–QTOF/MS: MASS SPECTROMETRY-BASED METABOLOMICS APPROACHES FOR THE DETECTION OF POMEGRANATE JUICE ADULTERATION

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Pomegranate juice is one of the most popular fruit juices, well-known as a "superfood", and plays an important role in healthy diets. Due to its constantly growing demand and high value, pomegranate juice is often targeted for adulteration, especially with cheaper substitutes such as apple and red grape juice. Conventional analytical techniques can be used to detect severe adulteration practices through the measurement of selected physicochemical indicators (pH, °Brix value, or titratable acidity), but they are often unable to detect small differences that could be indicative of low-level adulteration. In this context, the development of reliable, sensitive, and efficient analytical methodologies to detect pomegranate juice adulteration represents a demanding and challenging task.

In the present study, the potential of applying a metabolomics approach to trace pomegranate juice adulteration was investigated. A novel methodology based on high resolution mass spectrometric analysis was developed using targeted and untargeted screening strategies to discover potential biomarkers for the reliable detection of pomegranate juice adulteration from apple and red grape juice. Robust classification and prediction models were built with the use of unsupervised and supervised techniques (principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA)), which were able to distinguish pomegranate juice adulteration to a level down to 1%. Characteristic m/z markers were detected, indicating pomegranate juice adulteration, and several marker compounds were identified. To the best of our knowledge, this is the first study reporting more than 80 potential m/z markers indicative of the fraudulent addition of apple and/or grape juice in pure pomegranate juices in different portions. The results obtained from this study clearly demonstrate that Mass Spectrometry (MS)-based metabolomics have the potential to be used as a reliable screening tool for the rapid determination of fruit juice adulteration.

Keywords: *fruit juice authenticity, pomegranate juice, adulteration, high-resolution mass spectrometry, biomarkers*

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B24

ISOTHERMAL AMPLIFICATION FOR RAPID IDENTIFICATION OF ANIMAL SPECIES IN MEAT-CONTAINING PRODUCTS

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Food products mislabeling incidents involving substitution of more valuable species with cheaper ones for an economic benefit is becoming an important issue worldwide. In the specific case of meat, the presence of undeclared species in meat containing products has also been reported in meat industries and retail markets, especially after the horse meat scandal in 2013.
Although the presence of species different from those declared in the label might not be directly related to health issues, it evidences the lack of efficient traceability and control systems. Furthermore, ethical issues can arise, such in the case of presence of undeclared pork for Muslim communities.
Consumers´ concerns related to food fraud issues is also growing, involving a stronger demand for controls to verify that the claims made on label do certainly match the contents of food products.
Therefore, there is a need for rapid cost-effective species identification methods that can be employed by control laboratories or retailers willing to control their providers. PCR-based methods are the most widely employed methods for this purpose, requiring highly trained technicians and complex instrumentation. In this sense, Recombinase Polymerase Amplification (RPA), is a technique that allows the development of easy-to-use species verification systems. RPA works at a constant relatively low temperature, avoiding cycling temperatures, enabling the obtention of results quickly (around 15 minutes). Therefore, RPA could be a good candidate to meet this demand of ingredients authenticity control of food products in non-laboratory environments.
We have developed four independent RPA-based systems that allow the detection of pork, beef, horse and lamb. RPA primers and probes were designed targeting species-specific mitochondrial 12S rRNA gene regions. The detection of the different species of interest is performed by means of the RPA exo kit (TwistDx, USA). The specificity of these systems has been evaluated against several unrelated species, showing no cross-reactivity.
In conclusion, the developed RPA-based meat detection systems can be employed to identify meat species in commercial meat products and meat-derived products in a rapid and easy way.

Keywords: *meat, isothermal amplification, fraud, RPA, mislabeling*

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B25

FOOD FORTRESS: THE NEXT PHASE IN SECURING THE DAIRY SUPPLY CHAIN IN NORTHERN IRELAND AND BEYOND

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Biological, chemical or physical contaminants can have a damaging impact on the '*farm to fork*' supply chain, which operates in the dairy industry. The presence of contaminants can result in products spoiling and becoming unfit for consumption, which subsequently leads to significant consequences for both the health of consumers and the industry. The dairy industry in Northern Ireland is vital to the economy with milk and milk products having a turnover of £925 million and exports around £323 million. Therefore, identifying and monitoring potential hazards and contaminants within the supply chain is crucial to ensure continued growth and development of the sector. Previously, '*The Food Fortress*' program was developed in Northern Ireland, with the objective of ensuring the integrity of the animal feed supply chain and reducing the contamination risks which threaten the industry. The program was demonstrated to be successful within the feed industry across Ireland and Great Britain. The program consisted of a risk model using the Rapid Alert System for Food and Feed (RASFF) alerts along with a scientific scoring system for the associated hazards over a five year period. In conjunction with the risk model, an industry-wide program of strategic sampling and testing was implemented identifying potential contaminants including dioxins/PCBs, aflatoxins and heavy metals. The Food Fortress has now been expanded to be deployed into additional industries including the Dairy Sector. In collaboration with 4 dairy processors from Northern Ireland, a new risk model has been constructed for milk, milk products and ingredients. The risk register focused on RASFF alerts from 2013-2017, identifying hazards for different milk commodities and allowed a primary, secondary and tertiary priority testing plan to be compiled. The risk register is to be utilised alongside the industry's testing programs and help in prioritising testing. In addition to hazard identification, initial testing has been carried out in relation to testing for a wider range of antibiotics in milk using the latest multiplex lateral flow device developed by Unisensor.

Keywords: *dairy, supply chain, contaminants*

B26

FACING UP OLIVE OIL FRAUD: FAST DETECTION OF TRADITIONAL AND EMERGENT ADULTERANTS BY SHOTGUN TRIACYLGLYCEROL PROFILE

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Currently, olive oil (OO) tops the list of most adulterated food products due to its high nutritional and hedonic value. The illegal blending of OO with vegetable oils of different botanical origin has become a common fraudulent practice, entailing economic damage and potential food safety concerns related to the non-traceable substances used to commit the fraud. For this reason, it is essential to dispose of fast and high throughput methods that allow an efficient wide-ranging screening analysis in the detection of OO illegal blends. Triacylglycerols (TAGs) seem to be suitable authenticity markers, but its determination by conventional techniques presents some drawbacks such as time-consuming procedures and do not guarantee the detection of the fraud when the adulterants are present at low concentration or when they present compositions similar to that of OO. Thus, TAG profile analysis by flow injection analysis with high-resolution mass spectrometry (FIA-HRMS) allows obtaining, in the same analysis and in short times, the exact masses of many components, including the minor ones.
The present study aims to develop authentication models for detecting OO blends with vegetable oils used in both traditional and emerging frauds, using the TAG profile obtained by shotgun HRMS. The experimental design aimed considering the actual variability of genuine OOs, including two harvest-years, several geographical regions and olive cultivars. The OO blends were made following a Balanced Incomplete Latin Squares, using high linoleic vegetable oils (sunflower and soy bean) at 2 and 5%, and high oleic vegetable oils (high oleic sunflower, hazelnut and avocado) at 2, 5 and 10%; so more than 1,000 samples were analysed.
Two Partial Least Square-Discriminant Analysis (PLS-DA) models were developed according to the type of adulterant, with the 80% of the samples. Prediction results in an internal validation by leave-10%-out cross-validation were successful: a 97% and a 93% of samples were correctly distinguished between genuine OO and illegal blends containing ≥2 % of high-linoleic and ≥2 % of high-oleic adulterants, respectively. The external validation with the remaining 20% of the samples resulted satisfactory: an 81% of correct classification was achieved for the high-linoleic model, while the high-oleic one reached a 74%.
Therefore, this pilot study confirms that the proposed method can be the fit-for-purpose screening tool to support the detection of OO illegal blending with low percentages of different vegetable oils.

Keywords: *olive oil, authenticity, triacylglycerols, fia-hrms, illegal blending*

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B27

AUTHENTICATION OF GINKGO BILOBA HERBAL PRODUCTS USING A SPECIES-SPECIFIC ITS1 MARKER

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Plants have been widely used worldwide for medicinal purposes, leading to the increased consumption of herbal products, such as herbal infusions and plant food supplements. The growing demand of these products leads, inevitably, to the global market growing of herbal products and, consequently, to their adulteration. *Ginkgo biloba* is a Chinese tree whose leaves are extensively used in herbal preparations, being a very popular phytomedicine with high economic value. It has well-established therapeutic indications for the improvement of (age-associated) cognitive impairment and of quality of life in mild dementia, and treatment of problems associated with the peripheral circulation [1,2]. Ginkgo products have been adulteration targets through the addition of pure flavonols/flavonol glycosides or substitution with other botanical species [2]. Therefore, this work aims at proposing a new species-specific PCR approach to detect and quantify *G. biloba* to assess the authenticity of products thereof.
Reference mixtures of known amounts (50% to 0.01%, w/w) of *G. biloba* leaves and *Cammelia sinensis* leaves were prepared. DNA was extracted from referencemixtures and other plant species using the Nucleospin Plant kit. Specific primers targeting the ITS1 region (Genbank: Y16892.1) were designed to amplify a 175 bp fragment of *G. biloba*. A species-specific PCR assay was developed and used to confirm the absence of cross-reactivity with other medicinal plant species. Afterwards, a quantitative real-time PCR assay with EvaGreen dye for *G. biloba* was developed and applied to binary mixtures of *G. biloba* in *C. sinensis.* A normalised calibration model was constructed based on the parallel amplification of the ITS1 region of *G. biloba* and the 18S rRNA gene (reference for eukaryotes). The normalised real-time PCR system allowed establishing a calibration curve covering the dynamic range of 50-0.1% (w/w) of ginkgo in *C. sinensis.* Additionally, a sensitivity down to 2 pg of ginkgo DNA was reached. The method exhibited adequate performance with values of correlation (0.996) and PCR efficiency (84.3%) in agreement with the acceptance criteria for these assays. The results showed that the proposed method could provide a species-specific quantitative tool for the authentication of herbal products containing ginkgo.

Booker, A., Frommenwiler, D., Reich, E., Horsfield, S., & Heinrich, M. (2016). Adulteration and poor quality of *Ginkgo biloba* supplements. *Journal of Herbal Medicine, 6*(2), 79-87.
Gafner, S. (2018). Adulteration of *Ginkgo biloba* leaf extract. *Botanical Adulterants Bulletin*, January 2018.

Keywords: *Plant food supplements, Real-time PCR quantification, Plant authentication, Ginkgo biloba*

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B28

COMPARISON OF TWO COMMERCIAL METHODS FOR SMOOTH-SHELLED MUSSELS (MYTILUS SPP.) SPECIES IDENTIFICATION

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Smooth-shelled mussels (*Mytilus* spp.) account for ~60% of the commercial species of mussels. Chile is the first *Mytilus* mussel exporter, and in this country, the native species (*Mytilus chilensis* Hupé 1854) and the introduced Mediterranean mussel (*M. galloprovincialis* Lamarck 1819), coexist and hybridize. The EU seafood labeling and traceability regulation (EU N° 1379 2013) includes the obligation to declare the commercial and scientific name of the species in foodstuffs. In processed food products, morphological identification is not always possible because the diagnostic traits are usually removed, therefore, to enforce this regulation and to test food authenticity, DNA analysis is used. We tested the performance of two DNA based commercial methods for species identification in smooth-shelled mussels: the HRM PAPM method based on detecting polymorphisms in the *polyphenolic adhesive protein* gene and the FINS *H1C* barcode gene analysis. Analyzing 61 *Mytilus* mussels, HRM PAPM method identified 52 individuals as *M. chilensis,* 9 as *M. galloprovincialis* and no putative hybrids. On the other hand, FINS *H1C* analysis found 43 *M. chilensis*, 7 *M. galloprovincialis*, and 11 putative hybrids. These last ones showed double-peak in chromatograms at five diagnostic sites, which corresponded to the different nucleotides between *M. chilensis* and *M. galloprovincialis*. Cohen's Kappa coefficient (κ = 0.5293, Z = 3.2419, P <0.001) showed only "moderate agreement" between both species identification methods when samples from *M.chilensis* and *M. galloprovincialis* are compared. Considering *M. edulis* (another commercial species), and comparing their published sequencesof *H1C* with *M. galloprovincialis* in these five sites, some individuals were identical, while considering the whole sequence, the percentage of similarity between these two species was not lower than 97.73%. These results showed that an arbitrary threshold value of 98% similarity for FINS *H1C* barcode gene traceability analysis cannot be used as a reliable traceability method to distinguish *M. edulis* and *M. galloproivincialis*, while HRM PAPM can easily separate both species. The lack of agreement between both methods highlights the need for a better standardization of molecular tools, as well as the use of multilocus methods for the traceability of smooth-shelled mussels.

Keywords: *Traceability, H1C barcode gene, HRM PAPM, seafood authenticity*

Acknowledgement: *Sanger sequencing was kindly provided by Eurofins Genomics Europe Applied Genomics GmbH. This research was funded by the Chilean Government with grant FONDEF ID16I10013.*

B29

DETERMINATION OF WILD BOAR AND DOMESTIC PIG MEAT IN COMMERCIAL FOOD PRODUCTS BY REAL-TIME PCR

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Mislabeling of species is a common kind of food adulteration. We aimed to develop real-time PCR assays for the qualitative and quantitative determination of wild boar (*Sus scrofa scrofa)* and domestic pig (*Sus scrofa domesticus*) meat in commercial meat products. Real-time PCR assays for pork have been presented previously, they are, however, only applicable for the differentiation between pork and meat from other domesticated animals, and do not allow distinguishing between pork and meat from wild boar. Differentiation between the two pig subspecies is challenging because the genomes are highly homologous and the number of subspecies-specific bases is very low.

For primer/probe design, we targeted various polymorphisms, including SNP g.299084751C>T (*NR6A1* gene, chromosome 1) and SNP rs81416363 (intergenic, chromosome 9). We pursued different design strategies and located the subspecies-specific base either in the probe or in a primer. We designed a total of 16 forward primers, 56 reverse primers and 9 probes and combined them to 82 primer/probe systems targeting four different gene loci. Finally, we succeeded in developing a duplex real-time PCR assay targeting SNP g.299084751C>T and two singleplex real-time PCR assays targeting SNP rs81416363.

We carefully validated the selectivity of the assays by analyzing muscle meat from 64 domestic pigs (14 different breeds and 6 cross-breeds) and 30 wild boars (from Austria, Germany, Romania, USA and Estonia). We obtained some ambiguous results, by targeting SNP rs81416363 for Mangalica and Krškopolje pig breeds and wild boar individuals from Germany, by targeting SNP g.299084751C>T particularly for the Turopolje pig breed. Selectivity could be substantially increased by taking results obtained for both SNPs into consideration.

The applicability of the assays for processed meat products was investigated by analyzing 35 commercial meat products, including 22 goulash products. Our results suggest that domestic pig DNA is frequently present in products declared to contain 100% wild boar.

Quantification was carried out by relating the concentration of wild boar DNA/domestic pig DNA to the concentration of total meat DNA determined with a

Referencereal-time PCR assay. The accuracy of the results is sufficient to assess whether a commercial meat product is adulterated or meets the legal regulations.

Keywords: *Food authentication, Meat, Wild boar, Domestic pig, real-time PCR*

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B30

VALIDATION OF A DNA (META)-BARCODING ASSAY FOR SPECIES IDENTIFICATION IN FOOD

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Food authenticity is of major importance for consumers and thus requires the availability of analytical methods for species testing, especially to detect meat adulteration. In the last years, the term next-generation sequencing (NGS) was present worldwide in the food research community. In combination with DNA-barcoding this technique is a promising alternative to multiplex real-time polymerase chain reaction (PCR) assays in food analysis.
Starting from previously published primers (Tillmar et al., 2013, *PLoS One*), a DNA (meta)-barcoding method was developed. A duplex PCR assay allows the amplification of DNA from 28 mammalian and 11 poultry species using an additional novel primer pair. Stability and reliability of this system is a prerequisite for routine laboratory use. In this study, we focus on in-house validation of the DNA (meta)-barcoding assay for identification and discrimination of animal species in food (Dobrovolny et al., 2019, *Food Chemistry*). The parameters we were interested in are specificity, selectivity, recovery of DNA from a given species in a background DNA-matrix, limit of detection (LOD) as well as the applicability to processed meat products. The specificity tests include dry-lab (alignment of primer-sequences to reference-genes of target and non-target species) and wet-lab work (applying the complete process to real samples of target and non-target species). To evaluate the method regarding selectivity, recovery, LOD or applicability, the whole workflow was tested repeatedly under optimized/standard conditions with DNA from different sources (individual DNA extracts, DNA extract mixtures in variable combinations, model sausages and processed foods) from various preparations, in different sequencing runs on two MiSeq®-instruments (Illumina). In order to check the developed and optimized analysis pipeline in Galaxy, datasets were re-analyzed with updated analysis-tools and compared to existing older ones.
Pure single muscle-meat resulted in 99.4 to 99.9% of correct assignment based on the number of all reads that passed the Galaxy pipeline. The relative number of reads that mapped correctly to the main (98.0%) and minor (0.1%) components ranged from about 68% to 99% and 0.01% to 3.4%, respectively. The number of assigned reads was not affected significantly by updates in the Galaxy workflow. In model sausages and processed foods, all species were identified.
It can be concluded, that this DNA (meta)-barcoding assay is suitable to identify and distinguish the tested species down to 0.1% even in the presence of an excess of any other species-DNA. Targeting a short region of the mitochondrial 16S DNA reduces run-time from 56 hours (2x 300 cycles) to about 20 hours (2x 125 cycles). Indexing allows parallel analysis of up to 96 samples in one run, which reduces the costs per sample. This whole process is submitted for accreditation.

Keywords: *food authenticity, DNA (meta)-barcoding, amplicon sequencing*

B31

THE FEASIBILITY OF APPLYING HAND-HELD NIR FOR SPECIATION OF BEEF, CHICKEN, MUTTON AND PORK WITH CHEMOMETRICS

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Meat species identification is a significant authenticity issue. NIR is used as a prescreening technology to increase the cost effectiveness of more expensive laboratory methods (like DNA, histology or microscopy). This study evaluates the feasibility of a handheld NIR device (900 – 1700 nm) for speciation of ground meat of mutton, beef, chicken, and pork. Raw meat samples of mutton (28 frontshank and rear shank samples), beef (28 foreshank and hind shank samples) and chicken (31 breast and drumstick samples) were collected from local butchers in Tehran and Yazd cities, IR Iran in 2019. Eighteen pork samples (from different parts of animal body) were collected from other countries in 2019. Each sample was first cleaned, removing the remaining skin and fat that could interfere in the analysis, and it was minced by a Moulinex mincer (1000 W) to homogenize it. Every ground sample was placed in a plastic plate in order to collect the spectral information. For each individual sample, 6 reflectance spectra were acquired (5 at the edges of the sample and 1 at the center) by contacting the probe of the instrument with the sample. Then, NIR spectroscopy was coupled with two different chemometrics models including Partial Least Squares Discriminant Analysis (PLS-DA) and Support Vector Machine (SVM). Spectral datasets were divided into calibration (70%) and prediction (30%) sets with duplex algorithm and pre-processed with SNV and 2nd derivative (Savitzky–Golay) for PLS-DA, and with Gap Segment 2nd derivative for SVM models. In these two models, mutton and pork classes were difficult to separate. But, other classes were very much different and well separated. For PLS-DA model, sensitivity and specificity values in the prediction set were 69% and 86% for mutton, 94% and 99% for beef, 91% and 99% for chicken, and 57% and 89% for pork, respectively. The overall accuracy of the method was 80%. For SVM model, sensitivity and specificity values in the prediction set were 74% and 96% for mutton, 84% and 99% for beef, 91% and 98% for chicken, and 91% and 87% for pork, respectively. SVM model overall accuracy was 84%. The finding presented for the first time the potential of hand-held NIR spectroscopy with chemometrics models for rapid, inexpensive and non-destructive speciation of 4 different types of ground meat samples including mutton, beef, chicken, and pork which could be used as a basis for meat adulteration study.

Keywords: *Hand-held NIR speciation Ground meat Chemometrics IR Iran*

Acknowledgement: *The authors would like to appreciate Dr. MH Shojaei for his kind collaboration in this research.*

B32

TRIACYLGLYCEROLS AS OLIVE OIL AUTHENTICITY MARKERS: A COMPARATIVE STUDY ON THREE ANALYTICAL METHODS FOR DETECTING FRAUDULENT OIL BLENDS

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The high nutritional and economic value of olive oil (OO) makes this food product an objective of fraudulent practices. Among them, illegal blending with other vegetable oils can lead to a food safety problem due to the use of non-traceable products as adulterants. Lipid fingerprints, for instance those based on acylglycerol profiles, are promising candidates to detect olive oil adulteration, and to verify its botanical origin. Indeed, the current
Referencemethod for the control of OO authenticity is based on triacylglycerols (TAGs) analysis (IOC/T.15/Doc No 3/Rev 11 2016 and Reg. (EEC) 2568/91 and its amendments), but as it presents some drawbacks alternative methodologies are being developed. Recently, the analysis of TAG profile by flow injection analysis with high-resolution mass spectrometry (FIA-HRMS) have been proposed as a promising tool to detect illegal blending of OO with low percentages of other vegetable oils, suitable for the screening of a large number of samples. This method allows obtaining, in the same analysis and in short times, the exact masses of many components, including minor TAGs not detected by conventional techniques. However, despite the possibility to detect a high number of TAG species with different elemental formula, the direct injection of the sample precludes the possibility to distinguish TAG isomers, thus missing part of the information. With the aim of overcoming this fact, the introduction of a short liquid chromatography (LC) separation step (<15 min) before HRMS analysis was evaluated. Moreover, to further increase the amount of information obtained by the analysis, an all ion fragmentation experiment was added to the full scan mode in HRMS. Here, a fingerprinting approach was followed, based on the unfolded matrix conformed by ion extracted chromatograms obtained by selecting the exact masses corresponding to possible TAG fragments. In view of the encouraging results obtained by fingerprinting, which is the state-of-the-art in food analysis, the same approach was applied to a more widespread analytical technique, which would be affordable by the routine laboratories, such as high temperature gas chromatography-mass spectrometry (HT-CG-MS).
In order to compare the three above mentioned analytical methods, a sample set of 90 samples was analysed. The set was composed by 30 genuine extra virgin olive oils and their blends at 2% or 5% with 10 different soybean oils and 10 sunflower oils. After performing the proper data pre-treatment, a Partial Least Squares-Discriminant Analysis was performed for each methodology to classify samples as genuine or adulterated.

Keywords: *Olive oil, Triacylglycerols, FIA-HRMS, LC-HRMS, HT-GC-MS*

Acknowledgement: *This project has been funded by ACCIÓ-Generalitat de Catalunya and the European Union through the Programa Operatiu FEDER Catalunya 2014-2020 in the framework of the project Autenfood (Ref COMRDI-15-1-0035) and supported by the Spanish MECD through FPU pre-doctoral program (FPU16/01744) and Ramon y Cajal research program (RYC-2017-23601) from Spanish MINECO.*

B33

CHEMICAL CHARACTERIZATION AND AUTHENTICATION OF CROCUS SATIVUS (SAFFRON) USING LC-Q-TOF-MS AND ADVANCED CHEMOMETRICS

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Saffron is a spice derived from the flower of Crocus sativus plant, commonly known as saffron crocus and it belongs to the Iridaceae family [1, 2]. Saffron is subjected to authenticity studies due to its health benefits (such as antioxidant activity) [3] and high average market value. The authenticity mainly includes the geographical origin discrimination, adulteration control and chemical characterization. High resolution mass spectrometry and metabolomics have opened up new perspective for more complex food authenticity issues such as the effect of environmental conditions on the chemical content of the foods or their production type (organic or conventional) [4]. In this study, Saffron was authenticated based on the processing methods of its stigma (trim of the saffron) and the harvesting year. A comprehensive chemical characterization of the saffron was also conducted using Ultra-High Pressure Liquid Chromatography-Quadruple Time of Flight Mass Spectrometry (UPLC-QToF-MS). 20 samples were collected from Khorosan province in Iran. All the samples were extracted by methanol:water in the proportion of 50:50 and analysed in HRMS. The instrument was operated in both positive and negative electrospray ionisation mode. The MS acquisition was also done in the data dependent (for five most abundant precursor ions) and independent acquisition mode (bbCID and AutoMS) to facilitate data analysis. Different types of Iranian saffron classified into categories based on the harvesting and processing trim, namely Sargol, Negin, Pushal, Bunch, Style and Saffron powder, were chemically characterized and discriminated successfully. Furthermore, different cultivar periods (2017-2019) were compared for the varieties of Sargol, Negin, Pushal and Bunch. The data analysis was performed based on an in-house R package "AutoSuspect". It is concluded that the chemical content of the samples is changed during different harvesting periods. A clear discrimination was observed between Style, Bunch and other red saffron varieties (Pushal, Sargol and Negin) by Principal Component Analysis (PCA). Additionally, Partial Least Square Discriminant Analysis (PLS-DA), useful for biomarker discovery, was used to discriminate among saffron varieties. Surprisingly, the powder variety, which is prone to common adulterant of safflower, had as similar chemical profile as red saffron. Overall, 60 compounds were identified in the samples belonging to amino acids, vitamins, polyphenols, flavonoids, carotenoids, antioxidants, phenolic compounds, cyclohexenones and fatty acids.

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Keywords: *saffron, authenticity, hrms, multivariate analysis*

B34

A SYSTEM CHALLENGE - NON-TARGETED DETECTION OF ADULTERATIONS IN PAPRIKA POWDER WITH FTIR SPECTROSCOPY AND ONE-CLASS CLASSIFICATION

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Non-targeted, so-called fingerprinting, analytical methods have become a powerful tool for the authentication of food and feed [1, 2]. Spectroscopic or spectrometric data with subsequent multivariate data evaluation build the basis for a comprehensive characterization of the analyzed matrix and enable the verification of the geographical or botanical origin of a product and the detection of unknown adulterants. A prerequisite for the application of these non-targeted methods in official food control are among others appropriate validation strategies and the implementation of quality control measures [2]. This includes the assurance of the long-term stability of the models used as well as the determination of detection limits. While in targeted analysis numerous guidelines are already established, in non-targeted analysis there is still need for development.
A typical field of application is the authentication of spices, which are – as a highly priced commodity with a world-wide increasing consumption – very susceptible to food fraud [3]. Therefore, a fingerprinting method, based on Fourier-Transform Infrared (FTIR) spectroscopy in combination with one-class classification was developed to detect adulterations, such as synthetic dyes or bulking agents in paprika powder [4]. The aim of the presented study was to investigate the long-term stability of this one-class model ("system challenge"). The testing of the established model with 50 newly measured commercial samples from 2018 led to a sensitivity of 85%, which confirmed the long-term stability of the model. To increase the sample-specific variability, the new samples were added to the model. Moreover, the limits of detection for selected adulterants (silicon dioxide, starch, Ponceau 4R, tomato powder) were estimated. The extended model showed a good sensitivity for the detection of silicon dioxide in paprika powder (80% for 0.8% (w/w) SiO_2). The decision limit (cc_α) determined for starch was 7.4% (w/w). Here, there is still need for optimization, e.g. in the pre-processing of the data. However, for the detection of the adulterants Ponceau 4R and tomato powder the analytical technique showed some fundamental limitations.

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Keywords: *non-targeted analysis, one-class classification, long-term stability*

B35

METABOLOMICS INVESTIGATION REVEALS 8-C N-ETHYL-2-PYRROLIDINONE SUBSTITUTED FLAVAN-3-OLS ARE POTENTIAL BIOMARKERS OF STORED WHITE TEAS

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Longtime stored white teas are considered having high qualities and high protective health effects, and so the commercial values for the stored white teas are very high in the market. To avoid mislabeling and adulteration of these products for illegal interest, it is essential to develop reliable approach to evaluate them. Here metabolomics approach based on the ultra-high liquid chromatography (UHPLC) coupled with quadrupole time-of-flight mass spectrometry (QTOF/MS) was applied to study the effect of storage duration on the levels of chemical components in the white tea, aiming at seeking the specific biomarkers for evaluating the storage duration of the white teas. Two subtypes of white teas, silver needle and white peony collected under various storage durations, were selected for the investigation. The tea samples were extracted and subjected to analysis using UHPLC-QTOF/MS. The acquired data were then subjected to molecular feature extraction to obtain the whole compounds detectable under the experimental conditions. The resultant compounds were further imported into the chemometric software for statistics analysis. It was found that the metabolite patterns of silver needle and white peony in 1 year, 2-4 years, and >4 years groups could be separated clearly using principle components analysis and partial least square differential analysis. Among the differential metabolites, flavan-3-ols, procyanidins, theasinensins, and aroma primeverosides were identified and found to slightly increase in 2-4 years white tea samples and then decreased in >4 years white tea samples. Other identified different metabolites, including theaflavins, flavonol-*O*-glycosides, flavone-*C*-glycosides, and most of amino acids, were observed reduced during the storage. Surprisingly, 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols, including 7 novel compounds firstly discovered in white teas, were found formed from theanine and flavan-3-ols during the storage and positively correlated with the storage durations. This finding was further confirmed by both linearly increased formations of 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols in standard reaction solution and in white peony stored in environment control cabinet. The result suggests that 8-C N-ethyl-2-pyrrolidinone substituted flavan-3-ols are potential biomarkers for white teas under extended storage duration, may be applied for evaluating the white tea storage duration in the market.

Keywords: *white tea, storage, metabolomics, LC-MS, theanine*

B36

DETERMINATION OF GEOGRAPHICAL ORIGIN BY MULTI-ELEMENTAL PROFILING COMBINED WITH MACHINE LEARNING TECHNIQUES: A STUDY ON CHINESE GEOGRAPHICAL INDICATION (GI) RICE

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The demand for geographical indication (GI) rice has increased amongst Chinese consumers. This demand could create a high risk of adulteration due to limited production and high value of GI rice. This study aimed to develop a novel strategy to determine the geographical origins of Chinese GI rice, based on multi-elemental profiling obtained by inductively coupled plasma mass spectrometry (ICP-MS). One hundred and thirty-one samples of Chinese GI rice from five origins were analyzed. Coupled with feature selection (relief algorithm), two machine learning based classifier, support vector machines (SVM) and random forest (RF) were used to predict the origins of GI rice. The results were validated through repeated grid-search cross-validation. Only four elements (Na, Al, Cd, and Rb) were required for both SVM and RF to enable prediction with 100% accuracy. These results demonstrated that ICP-MS combined with machine learning techniques could be an effective strategy for authentication of GI rice in China.

Keywords: *rice, ICP-MS, geographical origin, machine learning, chemometrics*

B37

WHAT'S IN A WINE? WINE AUTHENTICATION ANALYSIS IN THE EU-CHINA-SAFE PROJECT

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In China, imported wines usually sell at a significantly higher price compared to local ones. Therefore, the wine business can be a profitable target for product counterfeit. For instance, wine labels might untruly claim to originate from famous international wine regions such as traditional European appellations.
One important aspect of addressing wine fraud is to analytically identify false claims on origin, vintage or grape variety, but also to detect other adulterants such as sugar, water or dyes. In the scope of the EU-China-Safe project (part of the Horizon 2020 programme), established and emerging analytical approaches to verify wine authenticity are transferred from the BfR laboratories to several Chinese partner institutions. The applied techniques range from classic targeted chromatographic methods and stable isotope analysis to non-targeted ^{1}HNMR analysis of wines.
The goal of this project is to share technology and experience between China and the EU, but also to enhance communication and cooperation between the partners to improve food quality and battle fraud. Here, we present the framework and setup of this cooperation and the progress we made so far.

Keywords: *wine fraud, analytical authentication, method transfer, laboratory comparison*

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B38

FOOD SAMPLE IDENTIFICATION VIA COATED BLADE SPRAY-HIGH RESOLUTION MASS SPECTROMETRY

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Coated Blade Spray (CBS) is an SPME-based analytical technology that facilitates collection of analytes of interest from a sample and the direct interface to mass spectrometry systems via a substrate spray ionization. The device is comprised of a thin-flat sheet with a pointed tip and consists of a conductive substrate such as stainless steel. As a SPME device, the substrate is partially coated with an extraction phase comprised of polymeric particles and a binder. The function of the polymeric particles is to enrich the analytes of interest from the sample matrix, while collecting the least amount of matrix interferences. As a direct to MS device, this device requires a pre-wetting of the extraction material so to elute the analytes collected on it. Subsequently, a differential potential is applied between the non-coated area of the substrate and the inlet of the MS system, generating an electrospray at the tip of the CBS device. Herein, we demonstrate, as a proof-of-concept, how CBS coupled to High Resolution Mass Spectrometry (HRMS) enables rapid profiling of aqueous (i.e. beer) and solid food matrices (i.e. animal tissue). Unlike other ambient-ionization technologies, CBS allows you to perform sampling remotely, clean-up the sample, and retaining relevant chemical information that facilitates its classification via chemometric tools.
Beer samples from different types/manufacturers/world-regions were purchased at a local store. Samples (300 µL) were deposited on a 96-well plate and analyte collection was performed in high-throughput configuration (i.e. 96-CBS devices concomitantly) for 5 minutes. The discriminant analysis of principal components (DAPC) in combination with Kernel Principal Component Analysis (KPCA) allowed for adequate classification of each of the beer brands under evaluation. Further, when using 60 principal components (PC), the leave-one-out cross validation (LOOCV) and the Support Vector Machine (SVM) unequivocally identified each of the samples. Likewise, CBS was capable of differentiating different types of meat samples (e.g. lamb, beef, chicken, and pork). The DAPC-KPCA plot showed clear distinction of each meat. By using 60 PC, the LOOCV and SVM lead to a predictability of 94 and 96%, respectively. In a third experiment, CBS was used to differentiate diverse fish samples. The DAPC-KPCA plot showed clear distinction of each species. Similar to the meat samples, by using 60 PC, the LOOCV and SVM lead to a predictability of 94 and 96% of the fish samples, respectively.

Keywords: *food safety, mass spectrometry, sampling, sample preparation*

B39

ANALYSIS OF VOLATILE ORGANIC COMPOUNDS BY GC-IMS AND GC-E-NOSE: A POWERFUL APPROACH FOR HONEY DISCRIMINATION

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Introduction: Honey represents one of the most relevant food fraud targets, which is mainly ascribable to its economic value and its worldwide production, but also to the existing problems related to the monitoring and management of bees' farms. To corroborate its authenticity, the assessment of the honey's volatile fraction combined with chemometric techniques could be an innovative and powerful analytical alternative.
The aim of this study was to analyze the volatile organic compounds (VOCs) from Acacia and Millefiori honey samples by using two diverse gas chromatography (GC) instruments (Heracles GC-E-Nose and FlavourSpec GC-IMS), followed by chemometric processing, in order to discriminate honey according its intrinsic characteristics (i.e. botanical origin).
Methodology: The volatile fraction of 54 honey samples (26 Acacia and 28 Millefiori) was analyzed by using two GC instruments: 1) Heracles GC-E-Nose (Ultrafast GC from Alphamos, with 2 capillary columns of different polarity and 2 flame ionization detectors (FID)) used in untargeted mode; 2) FlavourSpec GC-IMS (GC coupled to an ion mobility spectrometer (IMS) from G.A.S. Dortmund) used in both untargeted and target mode. The obtained VOCs raw data were separately subjected to Principal Component Analysis (PCA) statistical analysis.
Results: PCA analysis of raw data was able to similarly discriminate samples into the two main honey classes (Millefiori and Acacia) regardless of the GC instrument used. When PCA was applied to the data obtained with the untargeted mode, the acacia honey samples were classified into conventional and organic ones. Targeted GC-IMS analysis of a non-compliant Acacia sample allowed to identify acetic acid as a "marker" of this type of sample, which is known to be used against Varroasis in the production of organic honey (EU Reg. 2018/848).
Conclusions: The results showed that the two GC instruments were able to generate very similar outputs, when used under the same operating, analytical, and statistical conditions. The untargeted analysis of honeys' volatile fraction associated with chemometric data processing led to sample clustering, thus allowing a preliminary identification of qualitative differences among honey samples. In addition, targeted GC-IMS analysis proved to successfully identify discriminating molecules to legitimize the sample classification obtained with the untargeted GC analysis, being thus useful to distinguish with greater certainty a conventional honey from an organic one.

Keywords: *honey, authenticity, GC, IMS, GC-E-Nose, chemiometry*

B40

EIT FOOD PROJECT: RAPID HANDHELD SPECTROSCOPIC METHODS OF ANALYSIS IN FOOD SUPPLY CHAINS

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EIT Food is a European Knowledge and Innovation Community (KIC), part of the European Institute of Innovation & Technology (EIT), which was set up to transform our food ecosystem. By connecting consumers with industry partners, start-ups, universities and research centres from 13 countries in Europe, EIT Food supports innovative and economically sustainable initiatives which improve our health, our access to quality food, and our environment. EIT Food takes a consumer-centred approach, to empower innovators, entrepreneurs and researchers to develop world-class solutions to societal challenges, accelerate innovation, create jobs and increase Europe's competitiveness. The aim is to develop a highly skilled food sector, which collaborates with consumers to provide products, services and new technologies, which deliver a healthier lifestyle for all European citizens. The need to radically improve ingredient supply management in highly complex supply chains, due to accidental contamination or fraud and food terrorism, has never been greater. Food fraud alone costs an estimated $US49b per year to the global food industry. 'Food Fortress for raw materials and ingredients in Europe – Gaining Consumer trust through transparency of the supply chain' is a project with the objective to identify, manage mitigate and predict risks of chemical contamination and fraud in the food supply chain which included herbs and spices sector.
As part of the ongoing work, spectroscopic techniques, in conjunction with chemometric models, are being developed to screen for several adulteration risks associated with the herb and spice supply chains. An important aspect of this work was to ensure authentic samples of spices were secured by involving a company who could supply these with "AAA" rating direct from the country of origin. Fifteen different spices with high risk of adulteration were identified and 250 samples of each were sent to build up the database of authentic samples which are being used to construct chemometric models of these authentic materials.
At RAFA 2019 data will be presented showing the development of spectroscopic techniques and chemometric models for the detection of herb and spice adulteration.

Keywords: *authenticity, spices, spectroscopy, handheld chemometrics*

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B41

AUTHENTICATION OF OPIUM POPPY (PAPAVER SOMNIFERUM L.) USING DNA ANALYSIS

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Opium poppy (*Papaver somniferum L.*) is an important oilseed using in food and pharmaceutical industry. In the Czech Republic, cultivation of *P. somniferum L.* has a long tradition. Czech poppy seeds have good nutritional and sensory quality and low levels of opiates. Therefore, poppy seeds are an important ingredient in food item not only in the Czech Republic but also in many countries of Central-Eastern Europe. They are widely used for the preparation of pastry, such as cakes or buns. Recently, there have been several cases of poppy seed falsification. Food poppy seeds were mixed with cheaper seeds from pharmaceutical varieties that contain a higher amount of opium alkaloids and have worse sensory properties. In this work, we tested the possibility to differentiate *P. somniferum L.* species and varieties by DNA analysis of genes important for the biosynthetic pathway of opium alkaloids, such as codeinone reductase (COR) or reticuline epimerase (REPI). For DNA analysis, we used polymerase chain reaction (PCR). The selected sequence of the COR gene allows distinguishing *P. somniferum L.* from other tested poppy species. REPI gene seems to be suitable for the differentiation of poppy varieties with low- and high-alkaloid content.

Keywords: *poppy, DNA, Polymerase chain reaction, food fraud*

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USE OF DNA ANALYSIS FOR THE STUDY OF MEAT AND FISH FRAUD

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Food fraud is the current and serious problem on a global scale. Fish, meat and their products are one of the most expensive foods and therefore fall into the category of the adulterated commodities. One common way of deceiving customers is to replace high-quality meat with a less valuable and/or incorrect labeling of the product. Currently, many methods for determining species origin of meat exist. These include molecular-biological methods using DNA analysis, which allow very precise identification of the animal species used in food production.
In this work, the polymerase chain reaction (PCR) was successfully used for identification of fish from the *Scombridae* family (mackerel, tuna, bonito), meat (cattle, pig, horse, chicken, duck, turkey) and products purchased in commercial sources in the Czech Republic. The multiplex PCR method was used for the qualitative determination of meat content in products. The meat of cattle, pigs, poultry, and horses was detected using primers complementary to the gene coding for cytochrome b (mitochondrial DNA). Primers specifically amplifying interleukin II (nuclear DNA) were used to differentiate poultry; species identification of fish was performed by analyzing the sequence of the parvalbumin gene (nuclear DNA). Quantitative multiplex PCR with real-time fluorescence detection (mqPCR) was used to quantify DNA of selected meat species (beef, pork, and chicken) in meat products. In the mqPCR reaction, primers were used in combination with appropriate hydrolysis probes complementary to the DNA encoding the porcine beta-actin, beef cyclic-GMP-phosphodiesterase genes, chicken interleukin II and the gene encoding myostatin, which is a universal marker for mammals and poultry. In some cases, discrepancies between the results of DNA analysis and content of the sample declared by producer were determined.

Keywords: *DNA, fish, meat, polymerase chain reaction, species identification*

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ANALYSIS OF UNDECLARED SYNTHETIC PHOSPHODIESTERASE-5 INHIBITORS IN DIETARY SUPPLEMENTS AND HERBAL MATRICES BY LC–ESI–MS/MS AND BY LC-TOF ON MARKET IN PERIOD 2012-2019

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Erectile dysfunction (ED) is a condition in which man are unable to achieve or maintain an erection firm enough for satisfactory sexual intercourse. Synthetic phosphodiesterase type 5 (PDE-5) inhibitors are widely used for treatment of erectile dysfunction. There are four PDE5 inhibitors approved for treatment in Europe and the United States: avanafil, sildenafil, tadalafil and vardenafil. In order to protect the public from fraudulent practices, adequate methods for dietary monitoring should be enforced. This includes screening methods and strategies for the identification of new and unapproved compounds, while focusing on quantification of adulterants. The aim of this study was to investigate herbal dietary supplements sold on the Croatian market for the presence of PDE-5 inhibitors and their analogues in period 2012-2019. In this study, dietary supplements and herbal matrices of different forms collected from the Croatian market (pharmacies and store) were analyzed by LC–ESI–MS/MS or by HPLC-TOF-MS. Methods have been validated in terms of specificity, precision, accuracy, detection and quantification limits, linearity, stability and recovery. Three different forms of blank samples, pills, capsules and honey were used in the validation process. Validation parameters meet all acceptance criteria. PDE5 inhibitors were analyzed in 94 samples by method LC–ESI–MS/MS 57 samples in the period 2012-2013, 37 samples in the period 2014-2016, and 35 samples were analyzed by HPLC-TOF-MS in the period 2017-2019, of which 11, 15 and 19 were positive, respectively. Using a more sophisticated technique and examination of unknown substances, an increase in positive samples was observed from 19%, 41% and 54% respectively. In the case of positive samples, sildenafil and tadalafil in tablet combination were most commonly detected. As a conclusion adulteration of supplements with PDE-5 inhibitors and their analogues is extremely dangerous for consumers due to the unknown efficacy and toxic effects and should be inspected.

Keywords: *PDE-5 inhibitors, analogues, herbal dietary supplements, High-Performance Liquid Chromatography (HPLC), time of flight mass spectrophotometer (TOF-MS*

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B44

DNA BARCODING APPLIED TO AUTHENTICATION OF FOOD AND FEED PRODUCTS

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Identification of plant ingredients in raw and processed products is a challenge due to the very large variety of plant species. Present methods of molecular biology, as real-time PCR, only allow detection of plant species for which a specific test exists. DNA barcoding combined to High Throughput Sequencing (HTS) is a way to propose untargeted approaches enabling to determine the composition of mixed products or to detect the presence of possible adulterants or contaminants from plant origin.
In our study, we analyzed a large set of samples including 35 mixtures of plant DNA and 13 real-world food and feed samples with three barcodes (ndhJ, UP1-ITS2 and rbcL) on Illumina HiSeq. The bioinformatics pipeline developed to analyse the results was based on a *bash* script combining *Usearch*, *Blastn* and *R* packages.
For most of the samples, results corresponded to what was expected. We however observed differences in sensitivity in the detection of particular plant species in the function of the barcode used. The UP1-ITS2 barcode showed the largest number of identification errors, even if the proposed plant species of the outcome were taxonomically very close to what it should have been. This shows the importance to work with several barcodes tested on a large panel of plant species in order to avoid erroneous determination of the composition of food and feed products.

B45

PHYSICOCHEMICAL ATTRIBUTES AND POLLEN SPECTRUM OF CZECH HONEYS

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Honey is a traditional sweetener with distinctive composition and physicochemical properties according to locality of production. Characterization of Czech honeys is important base for the assessment of anonymous honey samples authenticity and their botanical and geographical origin. Presented work is part of the national project which responds to the necessity to create a database of attributes for the classification and traceability of honeys of the Czech origin. The aim of our research was to characterize authentic Czech honey samples collected in form of comb honey frames in year 2019 from local beekeepers. The determinations of selected physicochemical parameters and quality factors defined by Codex Alimentarius Standard and qualitative pollen analysis were carried out. Set of approx 100 samples included monofloral, honeydew and multifloral honeys from the entire territory of the Czech Republic.

Keywords: *Czech honey, physicochemical parameters, melissopalynological analyses*

Acknowledgement: *Financial support from the MOA project No QK1920344.*

B46

IDENTIFICATION OF CHILEAN EDIBLE CLAM SPECIES USING DNA BARCODING ANALYSIS, PRELIMINARY RESULTS.

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Today, the fisheries and aquaculture sector contribute with the ~20% of the protein consumed in the world food system. Chile is an important seafood exporter, and Clams are the third produced mollusc in the country behind the giant squid and *Mytilus chilensis* mussel. Clams are very appreciated for gastronomy, however to enter to the European market they must comply the labelling and traceability regulation, including in the label the commercial and scientific name of the species (EU.1379 2013). There are at least nine different species of edible clams described in Chile. Among them, *Ameginomya antiqua*, *Protothaca thaca*, *Semele solida*, *Mulinia edulis* and the baby clam *Tawera gayi* are included in international lists of admitted trade names of fishery and aquaculture species (i.e., Res. 9026 2019, Ministerio de Agricultura, Pesca y Alimentación, Spain 2019). However, another clam species also have the potential to be exported (*Gari solida*, *Eurhomalea rufa*, *E. lenticularis* and *E. exalbida*). To give confidence in the quality and safety of seafood products, for example, to identify the presence of potential allergens, is essential to correctly and accurately identify the species. Species identification based on morphological traits is difficult to perform in processed products when the shell is removed. In these cases, "DNA barcoding analysis" is a reliable and cost-effective method for species identification to fulfil and enforce the European Union labelling law (i.e., Regulation EU.1373 2013). This technique is validated and standardized in vertebrates, however, in molluscs is no consensus about the best barcode genes to perform this analysis. In Chile, the lack of official and commercial methods to identify clam species hampers the export of this resource to the European Union, affecting negatively the production chain and the coastal communities employability and incomes. Our goal is to use DNA barcoding to identify these nine edible clam species. Consequently, we amplify the mollusc 18S rRNA mini-barcode, and compare it in NCBI database using BLAST. Only three species gave 18S rRNA mini-barcode successful amplifications, obtaining fragments of 169 pb length in *Gari solida* and *Tawera gayi* , and 170 pb in *Semele solida*. BLAST search revealed a lack of sequences of this small mini-barcode 18S rRNA gene in genebank database for these species, but found some high similarity with other related mollusc species. *S. solida* matches with 100% of identity and 98% of coverage with *S. carnicolor* and *S. zebuensis*. *Tawera gayi* sequence showed a 100% of identity and coverage with the small clam *Irus irus*. In *Gari solida* no published sequence reaches the identity of 98% for a confident species identification. These preliminary results revealed the need of developing a local and taxonomically curated of mini-barcode sequences database, and using more barcode genes to increase the taxonomic coverage of this technique.

Keywords: *18S RNA, mini-barcode, Semele solioda, Tawera gayi, Gari solida*

Acknowledgement: *We thanks to Pesquera Trans Antartic Ltda., Aquagestion S.A. (Abbott Diagnostics Business), Centro de Innovación Acuícola AquaPacífico and Servicio Nacional de Pesca y Acuicultura (SERNAPESCA). This research is funded by the Chilean Government with grant FONDEF ID18I10025.*

B47

FISH SPECIES IDENTIFICATION BY PCR USING PARVALBUMIN GENE AS A PLATFORM

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Food fraud is a significant and growing problem. The increase in international trade, increasing global consumption of fish and different level of supplies and demands for some species, have led to many cases of economic frauds. In this case one type of fish product is illegally replaced by another. This is big economical problem, because mislabeling can result as fraudulent substitution of meat with high value with some less expensive fish. Moreover, proper labeling is also important in terms of the impact on health, certain people can consume only specific fishes, because of allergic sensitivity. The most common determination of fish species is based on morphological traits. This approach faces more and more complications as the level of processing fish flesh into products of food industry and/or complex dishes in gastronomy makes morphological markers less available.
Methods based on the polymerase chain reaction (PCR) are the most spread for control purposes, due to the high level of sensitivity and specificity. The presented analysis is performed as an amplification of nuclear gene encoding important protein of fish muscles parvalbumin, also known as a major fish allergen. Conventional PCR method for the differentiation of the following fish species were developed: black seabream (*Sponyliosoma cantharus*), Atlantic mackerel (*Scomber scombrus*), iridescent shark (*Pangasianodon hypophthalmus*) and angler fish (*Lophius piscatorius*). Specificity was verified on panel of 19 different fish species. This method can be employed as a routine tool for species origin determination irrespectively of morphological traits.

Acknowledgement: *This work was supported by grants from the Ministry of Agriculute of the Czech Republic MZe RO0318 and MZe NAZV QK1910231*

B48

AUTHENTICATION OF DURUM WHEAT, FLOUR AND PASTA USING LC-MS/MS – TARGETED PROTEOMICS APPROACH

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Wheat is one of the most important crop with its worldwide production of 770 million tons in 2019[1]. Durum wheat (*Triticum durum*) is due to its superior rheological properties more preferable in pasta production than common wheat (*Triticum aestivum*). In comparison, durum wheat is almost twice the price of common wheat and significantly lower production (only about 5 % of total wheat production). This is reason enough to adulterate it for economic gain.
An analytical method for detection and quantification of common wheat in flour and pasta samples was established for the purposes of official control. It is based on identification of proteins Puroindoline-A (PIN-A) and Purothionine-A1 (PTH-A1) through their corresponding peptides – specific markers obtained by trypsin digestion. Whereas PIN-A is present in common wheat only, PTH-A1 is present in both cultivars and is used for normalization of wheat content. Quantification is done using external calibration mixtures of known amount of common and durum wheat flours. For this reason is plotted ratio of MRM transition peak area of PIN-A (543.8 -> 657.3) to PTH-A1 (500.2 -> 725.3) against known percentage of common wheat content. The limit of detection (LOD) and limit of quantification (LOQ) were assessed on 0.3 % and 1 % of common wheat content respectively.

[1] FAO forecast (http://www.fao.org/worldfoodsituation/csdb/en/)

B49

APPLICATION OF MID INFRARED SPECTROSCOPY FOR FOOD AND FOOD SUPPLEMENTS AUTHENTICATION

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This work was aimed at the authentication of selected foods and also food supplements. The significant part of the experimental work was focused on plant oils – a recognition of mixtures and evaluation of their stability during a frying process in fast food production chains. During following experiments were compared plant oils based spreads and butters, where the type and fat content were investigated. Also various types of cheeses were compared regarding the recognition of their composition, production technology and ripening period. Finally, fish oils and high-content PUFA food supplements were evaluated regarding the composition of PUFA esters – the distinguishing of natural and processed oils was tested.
For the experimental work attenuated total reflectance – mid – infrared (ATR-FTIR) spectroscopy combined with multivariate data analysis for the profiles changes recognition and comparison using statistical models was employed.
Realized experiments proved the potential of the ATR-FTIR method for the fast recognition of changes of plant oils during a frying process. Especially a pure sunflower oil undergoes to the fast changes, while its mixture with rapeseed oil is significantly more stable similarly as a pure rapeseed oil. Experiments focused on spreads and butters showed the possibility to distinguish products based on the fat and water content. Regarding the cheeses testing, the fat content was the primary parameter for their differentiation. Based on a PLS-DA statistical model cheeses with different ripening periods were recognized. For PUFA food supplements the fast recognition of the form of esters and thus production technology was verified.

Keywords: *mid infrared spectroscopy, food, PUFA, supplements*

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B50

LASER ABLATION–RAPID EVAPORATIVE MASS SPECTROMETRY (LA–REIMS) FOR IDENTIFICATION OF SICILIAN EXTRA VIRGIN OLIVE OILS – STATE OF ART

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Protected Geographical Identification (PGI) is an E.U. program to protect and support the link between the quality or characteristics of a product and the geographical environment it comes from. The increasing demand for quality and traditional products is strictly related to the safeguard of the eco- and agro-systems each territory has, and the benefits of a PGI designation for the Sicilian extra virgin olive oil are immense, not only from an economic point of view, but also for the sustainable development of its agricultural sector.
In this work we present a novel technique: Laser Ablation – Rapid Evaporative Mass Spectrometry (LA–REIMS) to directly vaporise sample with no sample preparation and minimal damage, which combined with chemometric analysis of MS data makes it a powerful tool for discrimination of olive oils origin with results possible in seconds.
Established analytical methods used for discrimination of olive oils geographical origin include GC/LC-MS for volatile/non-volatile components of oils. Both techniques usually require relatively extended sample preparation which can be time and money consuming. Another tool to discriminate olive oils is Fourier transform infrared (FTIR) spectroscopy or near infrared spectroscopy (NIR), which requires minimal sample preparation and is used mainly for detection of adulteration caused by mixing olive oil with low-cost edible oils. Also method of stable isotope ratio can be used for discrimination of origin when mass spectrometry or nuclear magnetic resonance is employed, although these types of analysers which allow to measure isotopes precisely and reliably are very cost consuming.
Most of the analytical techniques used for identification of origin can be combined with chemometric analysis, which enables to extract relevant information based on statistical analysis of the input data. Commonly used untargeted statistical algorithms include principle component analysis (PCA) and supervised linear discriminant analysis (LDA).
Using LA–REIMS combined with chemometric analysis, three Italian PGI extra virgin olive oils (*Val di Mazara, Terra di Bari, Toscano*) were discriminated based on MS fingerprint in the region of 600-1000 m/z. Tentative m/z features responsible for the differences between the PCA clusters were extracted from the input MS spectra and the technique has proven to be highly potential for detection of "unexpected" for food authenticity. Furthermore, its simplicity of operation suggests big potential for automation.

Keywords: *olive oil, chemometrics, authenticity*

B51

CHARACTERIZATION OF QUALITY OF CZECH AND SLOVAK MEADS

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Mead, an alcoholic beverage with specific taste and aroma is typically produced from honey, water, spices, herbal and fruit extracts by fermentation process. The composition of mead is strongly affected by honey content, added ingredients and processing conditions. The aim of this research was to evaluate chemical, physical and microbial characteristics of 17 mead samples originating in the Czech Republic and Slovakia. This set of samples included meads with and without added sugar and alcohol, flavoured and natural, pasteurized and no-heat meads. Following qualitative parameters were analyzed: acidity, yeast assimilable nitrogen, soluble solids, total phenolic content, hydroxymethylfurfural, alcohol by volume, electrical conductivity, colour, sugar (glucose, fructose and sucrose) and organic acids composition, and profile of volatile compounds. Microbiological analysis was also performed. Collected data were statistically analyzed to classify samples according to used recipe and processing technology.

Keywords: *meads, honey wine, food analysis, food composition*

Acknowledgement: *Financial support from specific university research (MEYS No 21-SVV/2019).*

B52

EIT FOOD PROJECT: CAPABILITIES OF RAPID EVAPORATIVE IONIZATION MASS SPECTROMETRY (REIMS) AS A DETECTION METHOD FOR ORGANIC FRAUD IN BEEF

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This European Institute of Innovation and Technology (EIT) project is aimed at protecting the European organic brand by determining industry applicable methods to detect organic fraud in beef. As organic beef is a prime product which consumers associate with high animal welfare standards, greater sustainability, superior production practices, and more nutritious products. Consequently, consumers are willing to pay premium prices for these products. Due to its premium market value, organic beef is highly susceptible to food fraud. This is supported by large amounts of anecdotal data found in media, yet official reports of this type of fraud are relatively scarce. It is likely that this discrepancy is due to a lack of science based detection methods for organic beef fraud. Although there has been attempts to develop methods which can distinguish organic and conventional meat such as gas chromatography (GC) and stable isotope ratio analysis (SIRA), these techniques can struggle to robustly identify organic beef as they largely focus on a single variable associated with organic production. While organic beef is not defined by one specific measure but by multiple conditions, such as, consumption of 100% organic feed, very limited routine antibiotic use, and cattle housing. In order to protect the organic brand, the beef industry needs analytical techniques able to verify the claim. Furthermore, these techniques need to be rapid and easily used by non-specialised personal in order to be utilised in industry. This study aims to show the potential of rapid evaporative ionization mass spectrometry (REIMS) to distinguish organic and conventional beef. 137 samples consisting of neck, shin, and rump, muscles from 31 organic and 36 conventional Angus steer carcases sourced from ABP Cahir, Country Tipperary have been taken and analysed using a PCA-LDA model. The model produced to date has an 85% classification accuracy in distinguishing organic and conventional meat and this will likely increase as sample numbers added to the model also increase. REIMS shows promise as a rapid screening method to distinguish between conventional and organic beef.

Keywords: *fraud, beef, organic, REIMS*

Acknowledgement: *This EIT Food activity has received funding from the European Institute of Innovation and Technology (EIT), a body of the European Union, under Horizon 2020, the EU Framework Programme for Research and Innovation. Funding has also been received from ABP Food Group.*

B53

A NOVEL SENSITIVE LC-MS/MS METHOD FOR PORCINE GELATIN DETECTION IN COSMETIC AND CONFECTIONERY PRODUCTS

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Gelatin is a mixture of polypeptides derived from hydrolysis of collagen extracted from skins, bones and connective tissues of animals. Gelatin has been widely used in food, cosmetic and pharmaceutical industries. Nearly 80% of gelatin are made from pork because of the cost-effectiveness and availability. However, consumption of pork and/or pork-based byproducts is strictly forbidden in certain religions. Thus, it is necessary to develop a detection method that can identify and quantify the biomarkers specific for porcine gelatin. In this work, we present a novel and robust LC-MSMS method for sensitive and specific detection of porcine gelatin in cosmetic and confectionary products. The established method can be easily applicable for routine laboratory testing to verify halal authenticity of gelatin.
Eleven peptides were found to be specific to porcine gelatin and they were not detected in bovine Referencematerials. Among the eleven peptides, the five most sensitive and robust peptides were employed for the SRM method development. The SRM transitions for each peptide marker were optimized to predict the SRM transitions and respective collision energies (CE) using the porcine Referencematerial. The method was then tested on hair cream and facial gel specimens spiked with known amount of porcine gelatin. All the porcine-specific markers were successfully detected in the spiked hair cream and facial gel specimens with good repeatability (CV < 20 %) and linearity (R^2 > 0.97). The method was capable of detecting 0.01 % (LOQ) of porcine gelatin in the hair cream specimen, and 0.02 % (LOQ) of porcine gelatin in the facial gel specimen. The results of the extracts conducted on different days also showed good inter-day precision. In-house prepared marshmallow, made by porcine gelatin, was also tested to assess the applicability of this method for detection of porcine gelatin in confectionery products. All the SRM transitions for the porcine-specific markers were detected in the in-house prepared marshmallow, demonstrating excellent specificity of the established method. A broad range of commercially available cosmetic and confectionery products, such as fruit gum candies, collagen drinks, lipstick, facial masks, etc., were also analyzed by the established method.

Keywords: *halal, authenticity, adulteration, porcine, gelatin*

B54

AUTHENTICITY OF SWEET RESERVE – DETECTION OF BEET SUGAR IN WINE USING KRUEGER FORMATES

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Today's common oenological practice to produce semi-dry wines consists of a complete fermentation first and then adding back "sugar", the so called sweet reserve. For the purpose of sweetening wines after fermentation only the addition of grape must or concentrated grape must (also rectified) is permitted. The addition of foreign sugar like sucrose from beet is illegal. This should not be confused with chaptalization which is the enrichment of the must prior fermentation to enhance the alcohol content. This is allowed under certain conditions.
To check for an illegal addition of sugar to wine, it is useless to look for the presence of sucrose since a "smart" fraudster will use full invert syrups which show the natural sugar composition of grapes (glucose/fructose : 1/1). In such cases, the residual sugar can be evaluated by stable isotope ratio analysis. The available detection method implies the fermentation of the isolated sugar, quantitative distillation and NMR analysis of the ethanol. Using SNIF-NMR (site-specific natural isotopic fractionation nuclear magnetic resonance) the addition of C3-sugars like beet can be verified. In principal this concept works but there are major disadvantages which are first and foremost the need of an expensive NMR instrument. Furthermore, it is very time-consuming because of the fermentation and fairly large sample volumes are needed which can limit the possibility to carry out an analysis.
We present a novel method to proof the illegal addition of beet invert sugar to wine using so-called Krueger Formates. The principal of the method is to convert the residual sugars to formic acid followed by a steam distillation and precipitation as calcium salt. Now the hydrogen isotope ratios of the calcium formate can be determined. They display only the non-exchangeable hydrogen of the sugar, a requisite for a successful method. The $\delta^2H_{V\text{-}SMOW}$-values of beet sugar with about -80‰ differ significantly from those of the wine (grape sugar) with about 10‰ and usually more which enables the detection of a foreign sugar addition. Compared to the SNIF-NMR concept the Krueger Formate method can be done in a much shorter time, needs much less sample and uses IRMS or CRDS for the detection which are much more common and less costly than NMR. First measurements already convicted a counterfeiter so to speak in flagranti.

Keywords: *wine fraud, foreign sugar addition, full invert syrup, sweet reserve, SIRA*

Acknowledgement: *The authors would like to thank Dana Krueger, KFL Inc., Massachusetts, USA for the idea and support and Dr. Norbert Christoph from LGL the Bavarian Health and Food Safety Authority, Germany for samples in course of the validation.*

B55

THE USE OF ISOTOPE RATIO INFRARED SPECTROMETRY (IRIS) TO DETECT WINE FRAUD – OXYGEN ISOTOPES FOR WATER ADDITION AND CARBON ISOTOPE RATIOS TO AUTHENTICATE CARBON DIOXIDE IN SPARKLING WINE

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In vino veritas people say. The ancient germanics drank wine in their council meetings because they knew nobody was able to lie effectively when they were drunk. But what if the wine was diluted with water? The people would not get drunk, were able to lie and the saying might have never been created. To prevent such and other practices, there are clearly defined standards and provisions in the oenological practice of wine making. An addition of water is generally forbidden, also in the course of chaptalization via the in water dissolved sugar. The oenological practices also include the addition of carbon dioxide to make sparkling wine. The CO_2 must originate from the (second) fermentation. An addition of industrial CO_2 made by the combustion of fossil fuels or the thermal treatment of carbonates is not permitted unless labelled in accordance with European legislation.
The proof for an addition of foreign water to wine is made by the determination of the oxygen isotope ratios of the wine water. During ripening on the vine heavy 18O isotopes are enriched in the grape water (ca. $\delta^{18}O_{V\text{-}SMOW}$=3‰). This enables a differentiation towards regular tap water with $\delta^{18}O_{V\text{-}SMOW}$ values of ca. -7‰. For the detection of an addition of CO_2 to sparkling wine the determination of its carbon isotope ratios is used. The CO_2 from fermentation has $\delta^{13}C_{V\text{-}PDB}$ values in the range of -26‰ to -17‰ and can be distinguished from industrial CO_2. Depending of the source of the added CO_2 sparkling wines with $\delta^{13}C_{V\text{-}PDB}$ values lower -29‰ and higher -10‰ can be considered falsified.
We present a novel approach for the determination of the oxygen isotope ratios of water in matrices like wine and the determination of carbon isotope ratios of CO_2 in champagne or other sparkling products. The methods combine the classical CO_2 equilibration technique respectively a direct carbon isotope ratio determination of CO_2 via gasbench to an Isotope Ratio Infrared Spectrometer (IRIS). This setup brings various advantages such as precise, robust and cost-effective results as well as an easy to handle isotope ratio measurement system. For the validation, repeatability (r) and internal reproducibility (R) were determined. They fulfill the requirements of the Referencemethods OIV-MA-AS2-12 and OIV-MA-AS314-03 (International Organisation of Vine and Wine). Accuracy has been verified by CRM and successful PT participations.

Keywords: *wine fraud, dilution, fake champagne, IRIS*

B56

IDENTIFICATION OF ADULTERATION IN HIGH QUALITY STYRIAN PUMPKIN SEED OIL USING UNTARGETED ANALYSIS VIA LC-QTOF FOLLOWED BY ANALYSIS OF SPECIFIC ENTITIES VIA LC-QQQMS

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Food fraud and adulteration are critical issues, that are surging globally and have reached the forefront of the industry. Recently, nontargeted chemometric approaches are increasingly gaining in importance to find robust identifiers for the characterization of authentic food. Both powerful mass spectrometers, able to measure accurate mass, and modern statistical software tools are of pivotal importance to address food fraud.
Pumpkin seed oil is a genuine specialty of the province of Styria in southern Austria, listed in the European Commission's Database of Origin & Registration (DOOR) as product of protected geographical indication (PGI). The oil is a premium product of high value that is likely a target for economically motivated adulteration, leading to loss of quality and causing significant loss to consumers. Most of the resolved fraudulent cases either used cheaper seeds from other regions (China, Ukraine…), or blended the products with other, cheap vegetable oils such as canola-, or sunflower oil.
The work presented herein addresses the problem of adulteration carried out with cheaper vegetable oils. It follows an approach starting with untargeted analysis to identify specific chemical entities, robust identifiers, for each of the tested oils, which is then followed by "targeted" analysis using a UHPLC QQQ system to acquire MRM transitions of the identified oil specific entities. Pumpkin seed oils were mixed with canola- and sunflower oil in different ratios, extracted and analyzed by UHPLC Q-TOF system. In the first phase of the data analysis the Agilent Profinder Software was used to create entity lists by applying the "Batch Recursive Feature Extraction" workflow. Differential analysis was then performed with the aim to find unique entities for each of the oils using Agilent Mass Profiler Professional software. These entity lists were used to create a prediction model and were also exported and further reviewed in Agilent MassHunter Quantitative Analysis Software. Entities which showed reasonable and robust signals and specificity were subjected to fragmentation experiments. With information obtained from the fragmentation experiments, MRM measurements were conducted using an UHPLC QQQ system. Entities with a linear relationship between the percentage of added sunflower-, or canola oil and signal height were identified, and some oil test samples were "quantified" using these calibration curves.
Untargeted analysis using a UHPLC Q-TOF system turned out to be a practical and valuable tool to identify specific entities in the tested oils and allowed the identification of oil adulteration down to 5%. However, with the "targeted" approach our initial experiments showed the possibility to detect even lower percentages of blended vegetable oils.

Keywords: *food authenticity, adulteration, pumpkin seed oil, protected geographical indication, untargeted analysis*

Acknowledgement: *This project was supported by the Federal Ministery for Sustainability and Tourism Austria, the European Agricultural Fund for Rural Development and the Verein für regionale Kulinarik Austria*

B57

INVESTIGATING THE AUTHENTICITY OF LOCALLY-GROWN STRAWBERRIES WITH ISOTOPE-RATIO MASS SPECTROMETRY

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Locally-grown food is popular and has additional economical value. Thus, the risk of food fraud by selling non-local fruits and vegetables as locally grown is present. Among other fruits and vegetables, strawberries are grown in the state of Geneva and sold on local markets and grocery stores at relatively high prices. In order to detect potential fraud concerning the geographical origin of strawberries, a method using the stable oxygen isotope ratio ($^{18}O/^{16}O$) via isotope-ratio mass spectrometry (IRMS) was developed and used.
Swiss and European strawberries were collected at local food stores. Local field-grown and soil-free cultivated
Referencestrawberries samples were sampled at the production site and at a local agricultural training center. Exact information about strawberries variety, water-supply during culture and cultivation method was also collected. Finally, commercially "local grown" strawberries were collected at local stores and markets. The berries were mixed, centrifuged, and 300 µl of the aqueous supernatant were added into a gas-tight vial. Then, the $^{18}O/^{16}O$ ratio was measured using a headspace equilibrium method. For this, CO_2 was injected and equilibrated at 40°C for 8 hours using an Isoflow system (Elementar, UK). After equilibration, the CO_2 was injected into a PrecisION isotope-ratio mass spectrometer (Elementar, UK). The $d^{18}O$ values were calculated against laboratory-used standards calibrated with the VSMOW reference.
First, aqueous oxygen isotope ratios from strawberries grown in various regions and countries in Europe were measured. These preliminary measures indicated that Swiss-grown berries differ by -3 to -6 ppm units when compared to Spanish ones. In average, French, Dutch and Belgian berries had also higher $d^{18}O$ values compared to Swiss ones, although by only two or three ppm units. In some cases, overlapping isotopic values could be observed. Berries grown in Swiss alpine valleys had the most negative $d^{18}O$ values. Second, a
Referencedatabase of Geneva-grown strawberries was created. Global trends regarding the influence of berries sizes, ripeness and water-supply could be identified. Finally, Strawberries sold as "Geneva grown" or "Swiss grown" were collected at local markets (n = 12) and retail stores (n=12) by official food inspectors and analyzed. Among these samples, three differed significantly from the expected $d^{18}O$ value. After investigation and document review, they could all be traced as not locally grown.
These results show that IRMS combined with a high-quality database can be used to verify geographical origin claims of locally grown strawberries. Proven cases of food fraud were subsequently identified. Official controls regarding "local grown" claims might increase consumer's confidence in such labels, prevent further frauds, and protect the local farming economy.

Keywords: *authenticity, isotope ratio, mass spectrometry, geographical origin*

Acknowledgement: *We like to thank Vanessa Incarbone, Paul Garnier, Léticia Luczinski (Official Food Control Authority of Geneva), Stéphane Papis, Victor Fell (Lullier Professional Training Center for Nature and Environment, Geneva, Switzerland) and Olivier Rouquette (Union Maraîchère de Genève) for their precious help on this work.*

B58

DETERMINATION OF PHLORIDZIN AS A BIOMARKER FOR ADULTERATION OF WINES BY LC–MS/MS QTRAP® USING DIFFERENT ACQUISITION MODES

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The adulteration of expensive wines particularly sparkling wines and champagnes with other fruit juices, like apple juice or cider, is strictly controlled. Usually, phloridzin ($C_{21}H_{24}O_{10}$), a dihydrochalcone characteristic of the *Rosaceae* family, which is found mainly in apple trees up to a concentration of 50 mg kg^{-1}, but it has not been described in the genus *Vitis* is used as a marker of the presence of apple juice or its derivatives in wines. Phloridzin is extracted from the fruit during the juice making process. Nowadays, the official wine analysis protocol to detect phloridzin is based on HPLC-DAD determination, but its sensitivity is low and the results can be argued, especially at levels close to 0.1mg kg^{-1}.
In the present work, the absence of phloridzin in wines has been challenged, using high sensitivity analytical techniques such as LC-MS/MS. The LC-QTRAP® configuration is particular useful for the task, as it provides, not only high sensitivity but also the possibility of performing MS^2 determinations, in order to obtain the finger print of the molecule. All instrumental parameters were optimized for detection of phloridzin by multiple reaction monitoring (MRM) and enhanced product ion monitoring (EPI), using electrospray ionization in negative mode (ESI^-). This operational sequence allows us to obtain the MS^2 spectrum, where the spectrum from the $[M-H]^-$ fragmented ion has an identification value. After adjusting spectroscopic parameters, and developing the ideal chromatographic conditions, the final method was validated. Sample treatment was performed by simple methanol dilution of wine. The optimized LC conditions were a gradient of water with 0.1% HCOOH and MeOH with a flow of 400 µL min^{-1}, and two transitions (435/273; 435/167) were monitored. Under the conditions described above, a limit of quantification of 5 µg L^{-1} (expressed as phloridzin dihydrate) was achieved. Linearity in methanol was determined, showing a linear range of 5 to 100 µg L^{-1} that was verified by visual inspection, correlation coefficient and residue analysis.
The protocol was applied to 8 commercial red and white wines samples. The presence of phloridzin in all samples in a range of 71.2 to 172.9 µg L^{-1} was assesed. These preliminary results confirm the presence of this natural product in wines at concentrations 100 times lower than those found in apple ciders. The data could be useful from a regulatory point of view, setting realistic maximum limits for the presence of phloridzin in wines in order to ascertain the genuininess of the product.

Keywords: *phloridzin, biomarker, wine, adulteration*

Acknowledgement: *DT-UDELAR*

B59

STEPS TOWARD HARMONIZATION IN NON-TARGETED ANALYSIS - COMPARISON OF MEASURING INSTRUMENTS

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Harmonized and valid analytical methods are required to ensure the quality, safety and authenticity of products along the worldwide supply chains. Accordingly, non-targeted analysis (so called fingerprinting), for which spectroscopic methods such as Fourier Transform Infrared Spectroscopy (FT-IR) are often used, gain risen scientific interest. These analytical approaches are based on the acquisition of a chemical fingerprint – spectroscopic data – of the respective food or feed and allow a comprehensive characterization of the product. By the use of chemometric analysis of the obtained data various non-targeted authentication challenges can be investigated.
Although these approaches seem to have a high potential to authentication purposes, their routine use is currently restricted to certain products, such as wine, fruit juice and honey. A number of prerequisites regarding the application in routine analysis have to be addressed, e. g. (i) accepted strategies for method validation and quality assurance measures, (ii) reliable databases of representative samples, and (iii) jointly usable databases with uniform data exchange formats.
In order to fulfil these prerequisites, approaches towards comparability of FT-IR fingerprinting spectra acquired on different instruments were investigated. For this, a sample set of edible seed oils, especially rapeseed and pumpkin seed oils, was analyzed by three FT-IR spectrometers (two identical devices from the same vendor and one from a different vendor) following the same Standard Operating Procedure. The generated data by one instrument is used as a reference. Several approaches to improve the comparability, like a specific correction factor or combinations of different preprocessing steps and Piecewise Direct Standardization (PDS) were applied. In addition, prediction results of the model 'rapeseed vs. pumpkin seed oil' (before and after mathematical correction) using Partial Least Squares Discriminant Analysis (PLS-DA) and quantitative analysis by Partial Least Squares (PLS) are compared and illustrated.

Keywords: *standardization, spectroscopy, fingerprint*

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B60

DATA SHARING PLATFORMS FOR THE FOOD INDUSTRY – IS IT A NEED, REALITY OR FICTION?

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Food fraud is estimated to be up to 40 bn USD on annual basis and growing. It has negative consequences for both consumers and food industry: consumers are turning away from certain food products and brands, suffer health issues. The food industry consequently suffers from lower revenues.
There have been multiple ways to address food fraud: audits and controls, analytical checks, early warning systems, etc. We would like to discuss, in particular, within-the-industry data-sharing platforms to mitigate food fraud, their pros and cons and maybe their future.
The concept is a platform through which food industry companies can share analytical and other data and findings on food authenticity (and integrity) control in an anonymous way with each other. It has been widely discussed in the food industry. Such, extended research has been perform to understand the potential of this idea and potential hurdles (1). Few initiatives have been actually realized in the industry with food industry intelligence network (FIIN) being one of the most successful ones. However, it has been focusing primarily only on the UK market.
We will discuss further the potential of such platforms, hurdles and the feedback we received from our conversations with the food industry partners.

(1) Food Supply Chain Stakeholders' Perspectives on Sharing Information to Detect and Prevent Food Integrity Issues, Foods 2019, 8, 225; doi:10.3390/foods8060225.

Keywords: *food fraud, data sharing platform, FIIN, consumer trust*

B61

COMPLEMENTARY MASS SPECTROMETRY TECHNIQUES TO DETERMINE GEOGRAPHICAL ORIGIN OF MEAT

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According to OECD/FAO report, the global meat production per year for the period of 2016-2018 amounted to 317-330 Mt. Over the same time, meat production in the Russian Federation amounted to 9.90-10.64 Mt, while an additional 2.53 Mt of meat worth $ 6.45 billion were imported for domestic needs. Considering that total meat production is projected to increase, reaching nearly 367 Mt by 2027 (OECD/FAO2018), meat will remain one of the most economically important products for a decade at least. Thus the information about its origin might become a weighty argument, which may affect the decision on import from certain states. The importance of such a tool that provides reliable information about the origin of the product can hardly be underestimated. From an economic and biosafety standpoint, this makes it possible to monitor compliance with the regime of restrictions on the import of products from countries with an unfavorable environmental, sanitary, or epidemiological situation. One way or another, knowledge of product origin serves as a solid guarantee of quality and safety and ultimately determines the price and demand of that product.

Isotope ratios mass spectrometry (IRMS) is considered as the most powerful method that is able to establish a link between the isotopic composition of some object and it's the geographical origin. In this study, a combination of three methods such as elemental analyzer-isotope ratio mass spectrometry (EA-IRMS), inductively coupled mass spectrometry (ICP-MS) and chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS), is proposed.

The analysis of 218 raw meat samples of different species derived from 24 countries (North, Central, South America, Europe, and Oceania) was undertaken to assess the feasibility of applying single and combined mass spectrometry techniques to ascertain the geographical origin of meat.

In total, the following three data sets consisting of four isotope ratios ($\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{14}N$), 25 elements, and $\delta^{13}C$ values of 13 proteogenic amino acids were acquired by EA-IRMS, ICP-MS and GC-C-IRMS respectively. The method capability of different combinations was assessed by an advanced chemometrics approach, which involves different supervised classification algorithms: random forest, gradient boosting machines, support vector machines, fully connected multilayer artificial neural network and linear discriminant analysis. Classification accuracy was used as a measure for model performance. The best accuracy was achieved within 80-95 %. On the whole, the combination of three techniques provides 2.5-5% greater performance score than any single method. The classification results can be characterized by positioning – accuracy trade off – the more precise location is set the less accurate classification outcome is produced and vice versa. The variable importance analysis revealed that the top significant factors are $\delta^{2}H$, Cs, $\delta^{13}C$, Pro, Met, Ala, Rb, Arg, Co, Se.

Keywords: *mass spectrometry, classification, geographical origin, chemometrics*

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TRACING THE GEOGRAPHICAL ORIGIN OF MILK BY STABLE ISOTOPE AND MULTI-ELEMENTAL ANALYSIS

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This work was carried out under Coordinated Research Project (CRP) with the aim to facilitate developing and creating a Control system to trace geographical origin of dairy products. Through the cooperation with the participants of the CRP the key research steps as well as the main methodology have been established. The isotope analysis, including its various combination with other analytical techniques has been used in tracing the origin of food and foodstuff for years. It is also recognized that isotope and elemental analysis are the main and most widely used instrumental techniques in this research field. The essential part of the project was to create a Data base of the authentic milk samples so that unknown samples might be compared with and assigned to a geographical area with some degree of probability. Collecting samples from farms in different countries is however an expensive, laborious and time-consuming step. As an alternative, creating local data bases, and then sharing them with other scientific groups seems more rational. To fulfill that concept, a well-established, harmonized standard operating procedures is required. The objective of this particular study was to establish a method based on stable isotope and elemental analysis, and to assess its applicability for confirmation of geographical origin of milk produced in Russia. As an outcome a method of isotope ratio mass spectrometry combined with inductively coupled mass spectrometry has been developed to determine isotope ratios ($\delta^{13}C$, $\delta^{18}O$, $\delta^{2}H$, $\delta^{14}N$) and concentration of 28 elements in milk. The applicability of method was evaluated on 400 milk samples collected in different parts of Russian Federation. A quantitative assessment was carried out by performing several classification test, such as linear discriminate analysis, random forest, support vector machines, artificial neural network and stochastic gradient boosting. Non-linear models were optimized by repeated k-fold cross-validation. The highest classification accuracy obtained on independent test set (100 samples) has reached 96%, with overall precision within 93-96% for all non-linear models.
The results of the study showed that isotopic ratios of light elements in combination with the elemental composition can effectively discriminate milk samples on a scale as large as the territory of Russian Federation. An essential role in that result is addressed to the bulk isotopic $\delta^{2}H$, $\delta^{14}N$, $\delta^{13}C$ and trace elements composition, e.g. Rb, Sr, Co, Cu, Ba, Mn. To increase accuracy of the classification unknown samples and hence to differentiate more closely located geographical points, we propose in the future to involve extra factors (variables), for instance obtained from the compound specific isotopic analysis (CSIA) or strontium isotope analysis, which might extend the capabilities of the method and data base so that more precise and reliable results can be achieved.

Keywords: *mass spectrometry, geografical origin, ICP-MS, IRMS, classification*

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B63

FAUTHENT: AN OPEN SOURCE PLATFORM TO SHARE SCIENTIFIC DATA

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In the field of food fraud, non-targeted analysis, e.g. by Nuclear Magnetic Resonance Spectroscopy (NMR), can be used to generate so-called chemical fingerprints of food samples, to distinguish between authentic and non-authentic products. These data need to be stored combined with its metadata (e.g. food product data), processed and analyzed. Reliable sharing of these collected data between different parties, for example, other companies or federal institutions, can be difficult.
The FoodAuthent project is currently finalizing the implementation of a comprehensive solution called fAuthent-framework. The system serves as an in-house database to collect/manage data and its metadata as well as allowing automatically data processing up to the level of obtaining predictions.
The sharing capabilities among different fAuthent-systems are another prominent feature of the fAuthent-framework. First of all, the framework provides a restrictive rights management to protect valuable information from misuse. The data owner can precisely define what data can be accessed by other users.
In addition, a so-called Discovery-service is providing the option to find out if other fAuthent-systems have data about a specific food item and to ask for permission to access these data.
The fAuthent-framework and a demonstrator have been developed and tested. The system is supposed to be widely accepted and to improve the collaboration between different fAuthent-partners.
In addition, it is planned to design a user interface and to support further methods.

Keywords: *Food Authenticity, NMR spectroscopy, predictive modelling, software framework, metadata*

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B64

NOVEL APPLICATION OF HAND-HELD NIR SPECTROMETRY AND CHEMOMETRICS APPROACH FOR RAPID DETECTION OF LIME JUICE ADULTERATION

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The aim of this study is to investigate the novel application of a hand-held NIR spectrometry coupled with classification methodologies as a screening approach in the rapid detection of adulterated lime juices. For this purpose, a miniaturized near infra-red spectrophotometer (Tellspec®) in the spectral range of 900–1700 nm was used. Three diffuse reflectance spectra of 31 genuine lime juice samples (collected from Jahrom city, IR Iran) and 25 adulterated samples were acquired. The principal component analysis was almost able to generate two clusters. Partial least square discriminant analysis and k-nearest neighbor algorithms with different spectral preprocessing techniques were applied as predictive models. In the partial least squares discriminant analysis, the most accurate prediction was obtained with SNV transforming. The generated model was able to classify juices with an accuracy of 88% and the Matthew's correlation coefficient value of 0.75 in the external validation set. In the k-NN model, the highest accuracy and Matthew's correlation coefficient in the test set (88% and 0.76, respectively) was obtained with multiplicative signal correction followed by 2nd-order derivative and 5th nearest neighbor. The results of this preliminary study provided promising evidence of the potential of the hand-held NIR spectrometry and machine learning methods for the rapid detection of lime juice adulteration. Since a limited number of samples were used in the current study, more lime juice samples from a wider range of variability need to be analyzed in order to increase the robustness of the generated models and to confirm the promising results achieved in this study.

Keywords: *Hand-held NIR Chemometrics lime juice adulteration IR Iran*

Acknowledgement: *The authors would like to appreciate the Food and Drug Reference Laboratory, Food and Drug Administration, IR Iran for providing adulterated lime juice samples.*

B65

PTR-TOF/MS COMBINED WITH CHEMOMETRICS AS A NOVEL NON-TARGETED APPROACH IN THE DETECTION OF LIME JUICE ADULTERATION

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As the lime juice adulteration is an ongoing problem, suitable analytical methods are needed for rapid detection of lime juice adulteration. Aroma profile of the volatile fraction of lime juice is a useful marker for its quality control. As far as we know, there is no method reported in the literature for evaluation of VOCs of lime juice using PTR-ToF-MS. Therefore, the aim of this study is to develop for the first time a PTR-ToF-MS method combined with chemometrics approach as a non-targeted technique for detection of adulterated lime juice samples. Fifteen Iranian genuine lime juice samples and 28 adulterated samples with the citric acid to iso-citric acid ratio over 300 (based on our previous study applying LC-MS/MS) were used in this study. For PTR-ToF-MS measurement, 500 µl of each sample was diluted with 9500 µl of purified water, and 500 µl of the mixture was introduced to PTR-ToF-MS machine. For each sample, mass spectral data was collected in duplicate over 70 cycles in the mass range from 15 to 506 m/z. Principal component analysis (PCA) was performed on 290 variables in the whole averaged spectrum of genuine and adulterated samples. Partial Least Squares Discriminant Analysis (PLS-DA) following different data pre-processing was performed to make a predictive model. In all genuine lime juice samples, ions with the m/z values of 81 and 137 had the highest concentrations, and were identified as cyclohexadiene and limonene, respectively. By performing PCA on log10 transformed and then mean-centered data, almost all genuine samples were separated from adulterated ones. Regarding PLS-DA analysis, the best result was achieved with mean-centering and then log10 transformation. A predictive model of 10 genuine and 20 adulterated samples (training set; 70% of samples) delivered an accuracy of 100% on hold-out samples (test set; 30% of samples) using 4 factors for each group. Furthermore, propionic acid/methyl acetate, cycloheptan, furfural, p-alpha-Dimethyl styrene, 2-Isopropyl-N, 2,3-trimethylbutyramide, and 2-Octylfuran were identified as classifier mass peaks in adulterated samples. This is the first study focusing on the application of PTR-ToF-MS technique as a rapid technique for detection of lime juice adulteration based on the VOCs profile. The result of this study holds a great promise for future application of PTR-ToF-MS and classification methodology in detection of lime juice adulteration. Further model validation using lime juice samples from a wider range of variability is required to confirm the promising results of this study.

Keywords: *PTR-ToF/MS Chemometrics lime juice adulteration IR Iran*

Acknowledgement: *The authors would like to appreciate the Food and Drug Reference Laboratory, Food and Drug Administration, IR Iran for providing adulterated lime juice samples.*

B66

LC-MS WITH SELEXION® DIFFERENTIAL MOBILITY SEPARATION TECHNOLOGY AS A SENSITIVE AND SELECTIVE TOOL TO VERIFY QUALITY OF OLIVE OIL

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Nowadays food quality is in focus and more and more seen as a matter of course by consumers. Talking about food quality is typically directly related to food safety and authentication. Authentication of olive oil has been the focus worldwide. From the chemical point of view, olive oils are majorly triglycerides and it contains up to 77 % the highest amount of 18:1 fatty acids of all vegetable oils. There are different homologues of this fatty acid: oleic acid, a *cis*-isomer, and its *trans*-isomer, the elaidic acid. The latter is transformed from oleic acid. Here we like to present a fast, simple, sensitive and robust LC-MS/MS method for the qualitative and quantitative detection of oleic and elaidic acid isomers.
Methods: A SCIEX QTRAP® 5500 LC-MS/MS system, attached with SelexION® differential mobility separation (DMS) technology was coupled to the Shimadzu Nexera system. Oleic acid and elaidic acid standards were diluted in isopropanol and used for optimizing the MS parameters. SelexION DMS device is placed after ionization and before the entrance of the MS system. Compared to classical ion mobility, where the different shapes of isomers are separated by flight time, DMS is separating the isomers by a voltage, the compensation voltage (CoV). The specific DMS-MS/MS parameters for the two isomers were optimized using infusion mode. For on-column analysis, the total LC runtime was 10 minutes and the LC separation was done on Phenomenex Evo column.
Preliminary Data: In contrast to MS systems, in DMS the precursors of identical mass were separated just by their specific shape. In negative ionization mode, both the fatty acid isomers gave monoisotopic mass of 281.2. Using CoV of -29 V for oleic acid and -26 V for elaidic acid, a distinct base-line separation in DMS device could be achieved. SelexION DMS device was working in 'low' DMS resolution enhancement mode, DR. DMS temperature, DT, was set to 'low' and DMS Offset, DMO, to -3 V. A separation voltage, SV, was found to match best at 4100 V. The DMS modifier was added by custom settings using 'low' modifier composition, MDC. Olive oil samples were also analysed by diluting 1:1000 using *iso*-propanol. The chromatographic run with MRMs with exactly the same MS parameters but at two distinct CoVs of -29V and -26V showed the separation of the two fatty acid isomers on-column as well. The analysis of two olive oil samples showed higher level of oleic acid in the fresh oil samples and a higher level of elaidic acid in the stored oil samples.
Novel Aspect: Unique qualitative and quantitative approach using SelexION Technology to separate cis and trans isomer of fatty acids

Keywords: *differential mobility, ion mobility, olive oil, quality, authenticity*

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EIT-FOOD PROJECT: BEEF CRIMES RISK ASSESSMENT

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This European Institute of Innovation and Technology-Food (EIT-Food) project focuses on proactive risk management in the beef supply chain. There are numerous guidance documents available to the food industry describing food fraud as well as general mitigation strategies to protect the authenticity of products. Generally, this guideline revolves around supply chain mapping practices and creating supplier trust. These tactics are necessary in risk mitigation, however, they are not enough. In order to better protect authenticity, targeted risk management practices and guidance specific to each supply chain needed. Therefore, to better mitigate risk in the beef supply chain, hazards must first be identified. In this study hazards have been determined through media mining of online publications, news articles, and academic literature. This search resulted in the identification of 22 types of hazards referred to as beef crimes. Following the identification of these beef crimes, this project aims to risk assess and rank each crime by its commercial risk. Commercial risk referring to the overall damage a given crime could potentially cause a defrauded company has been calculated by identifying and quantifying the relevant factors including: human health risk, consumer perception of risk, business impact, number of documented historical incidents, awareness of threat, likelihood of detection and complexity of detection. In order to make the risk assessment as accurate and applicable as possible we are seeking the input via a survey of individuals who work in the beef supply chain and those with expert knowledge in food fraud analysis. Through this we aim to gain a better understanding of risk associated with beef crimes and how these hazards are perceived by industry professionals and the scientific community.

Keywords: *food fraud, beef, risk assessment*

Acknowledgement: *This EIT Food activity has received funding from the European Institute of Innovation and Technology (EIT), a body of the European Union, under Horizon 2020, the EU Framework Programme for Research and Innovation. Funding was also received from ABP Food Group.*

B68

NEW CHALLENGE FOR NATURAL VANILLA FLAVOUR AUTHENTICATION DUE TO NOVEL PRODUCTION PATHWAYS OF BIOVANILLIN

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Vanilla flavour is one of the most expensive, popular and widespread flavors in the food industry. The price level in 2017 reached 500 US$ per kg vanilla pods or even higher for premium quality. Vanillin, the most characteristic compound in vanilla flavour can be produced by much cheaper methods like chemical and biotechnological synthesis. The willingness of the consumer to pay a higher price for natural products in combination with the disparity of prices has led to many cases of economically motivated fraud. For more than 40 years efforts have been made to authenticate the origin of vanillin, whereas the assessment of the stable isotope carbon ratio is widely used. However, with the invention of new biosynthetic pathways, vanillin overlapping with the characteristic isotopic carbon ratio range reported for vanillin from vanilla pods can be produced. We present site-specific analysis by GC-IRMS of stable carbon and hydrogen isotope ratios of vanillin derived from glucose. This is the first time a value for the isotopic carbon ratio for biovanillin that is higher compared to vanillin from vanilla pods is reported. The possibility to simulate the isotopic carbon ratio range of vanillin from vanilla pods by combining vanillin derived from inexpensive sources constitutes an increased risk for fraud being perpetrated while remaining unnoticed. It must be expected that these findings will influence and encourage the extension of databases and the development of new methods.

Keywords: *fraud, vanilla flavour, stable isotope analysis, authenticity, biovanillin*

Acknowledgement: *The authors would like to thank Professor Birger Lindberg Møller, Plant Biochemistry Laboratory at the University of Copenhagen for supplying vanillin derived from glucose for this project.*

B69

CHARACTERIZATION OF THERMALLY TREATED POPPY SEEDS BASED ON METABOLOMIC FINGERPRINTS

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In recent years, many cases of poppy seeds adulteration based on dilution with significantly cheaper seeds from pharmaceutical cultivars have been reported. As this waste product typically has a high content of opium alkaloids (hundreds mg/kg), it cannot be used for human consumption. To overcome this problem, counterfeiters have found a way how to decontaminate seeds by a process called thermostabilization (treatment by hot steam). This process, indeed, inactivates lipolytic enzymes (causing hydrolytic rancidity) and, at the same time, opium alkaloids located on the seeds surface are fairly removed. However, it can be assumed that high temperatures, to which seeds are exposed, reduce their oxidation stability.
The aim of this study was to describe the effect of thermostabilization on the chemical composition of poppy seeds to create a methodology for revealing of undeclared thermostabilization process. Two instrumentations for the metabolomic fingerprinting (i) ultra-high performance liquid chromatography coupled with high-resolution tandem mass spectrometric detection (U-HPLC–HRMS/MS) and (ii) Fourier transform infrared spectroscopy (FTIR), were used. The obtained data were chemometrically processed (PCA, PLS-DA, PLSR). Within the first part of the study, the change in the chemical composition of treated and untreated poppy seeds during storage (38 days experiment) was studied. An authentic sample of blue poppy seeds (Major variety), which was thermostabilised under laboratory conditions (also applied to the following experiments), was used for this experiment. For untreated seeds, a significant increase in free fatty acids (lipase activity) was observed after milling. The thermostabilization process caused increased lipid oxidation, so reduced oxidation stability of treated poppy seeds was confirmed. The next step was to evaluate the effect of the thermostabilization process on lipase activity. In order to ensure the greatest variability of the sample set, 5 poppy seed varieties, which were grown in 3 different localities of the Czech Republic and harvested in the years 2015-2018, were used. For this experiment, lipase activity was expressed as an increase in acid value (AV) 24 hours after sample grinding. AV was measured using FTIR instrumentation (PLSR model enabling quick quantification of acid value directly from milled poppy seeds was created a validated). In untreated poppy seed samples (n = 20), an increase in AV of 34-163% was observed. On the other hand, in the case of thermostabilized poppy seed samples (n=10), changes observed in the AV value corresponding to the relative standard deviation of the assay (RSD = 12%).

Keywords: *poppy seed, metabolomic profiling, mass spectrometry, infrared spectroscopy, thermostabilization*

Acknowledgement: *This work was supported by OPPC (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), NPU I (LO1601 - No.: MSMT-43760/2015) and by National Agency for Agriculture Research (Project no. NAZV-QK1720263).*

B70

AUTHENTICATION OF PANAX GINSENG BASED NUTRACEUTICALS USING 'CHEMICAL MARKERS' STRATEGY

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Nutraceuticals include a wide range of products containing bioactive components usually of natural origin which are expected to have a beneficial effect on a human organism. Nowadays, simultaneously with a growing demand for these products, various economically motivated fraudulent practices can be encountered.
This study is focused on the development of a method for authentication of nutraceuticals containing high value ginseng, *Panax ginseng*. For this purpose, analytical strategy based on metabolomic fingerprinting using ultra high performance liquid chromatography – high resolution mass spectrometry (UHPLC-HRMS) was applied. Worth to notice, that several representatives of *Panax* genus exist differing in content in biologically active substances such as ginsenosides, gypenosides, majorosides etc.; moreover, there are other plants which contain them, thus they can be used as undeclared substitutes.
Application of ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) with data processing using advanced statistical methods (principal component analysis and partial least square discriminant analysis) enabled to find 105 specific markers for *Panax ginseng,* 65 for *Panax notoginseng*, 120 for *Withania somnifera*, 99 for *Eleutherococcus senticosus* and 109 for *Gynostemma pentaphyllum* in aqueous methanolic (MeOH:water, 80:20) extracts of these herbs. In the next step, extracts of the most common ingredients (green tea, ginger, peppermint, rooibos, ...) contained in herbal teas were analyzed and the specificity of these markers was verified. It was found that only 5 markers of *Panax ginseng* (ESI+: *m/z* 974.5323 (retention time 4.57 min), *m/z* 1109.6108 (RT 4.62 min); ESI-: *m/z* 793.435 (RT 5.11 min), *m/z* 955.4903 (RT 4.59 min), *m/z* 1077.585 (RT 4.67 min)), 2 markers of *Panax notoginseng*, 30 markers of *Withania somnifera*, 6 markers of *Eleutherococcus senticosus* and 20 markers of *Gynostemma pentaphyllum* are specific for them.
The analysis of these specific markers allowed authentication of food supplements containing *Panax ginseng*, especially herbal teas with *Panax ginseng* content. The presence of *Panax ginseng* was confirmed in 7 of 15 analyzed food supplements with its declared content. Characteristic markers of *Panax notoginseng, Withania somnifera, Eleutherococcus senticosus and Gynostemma pentaphyllum* were not found in these food supplements.

Keywords: *ginseng, authenticity, Panax ginseng, food supplements, UHPLC-HRMS*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, 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 and by the Czech Republic National Agency for Agricultural Research (Project no.QJ1530272).*

B71

HOW MANY DIMENSIONS FOR A SEPARATION? SPICES ADULTERATION CASE STUDY

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Currently herbs and spices market is worth US$4 billion. EU imports three times the amount of herbs and spices than it produces already with third of them and with the market still expanding, the supply chain will be unable to meet the demand in the future. The fact that a third of imported materials are crushed and ground, makes some of the products indistinguishable. Those factors combined with the complexity of the supply chain art thought to contribute to the expected rise in herbs and spice adulteration. Current study focused on developing a reliable testing regime based on mass spectrometric (MS), fingerprinting approach allowing for detecting substitution of three spices i.e. cumin, fennel and coriander which adulteration has already been reported. Different analytical approaches have been studied to assess how the length of the chromatographic (LC) separation (10min, 2min, ambient MS - DART), analytical column and mass analyser (Waters QToF G2XS, Agilent QToF 6545, Waters QDa) influence the reliability of the final analytical strategy. The number of detected features and their selectivity was shown to be heavily dependent on the length of the LC gradient. Even though, all of the supervised statistical models (PLS-DA with 5 components) yielded accuracy of 100% and fit R2 values higher than 99%, the predictive value of the models (all higher than 93%) were shown to be dependent on the chromatographic separation employed. Due to shortest time of analysis, promising performance of the statistical model and ease of use, DART-QDa will be further assessed as a rapid MS based alternative for spices adulteration detection.

Keywords: *spices, adulteration, ion mobility*

Acknowledgement: *Presented research was funded by the ChemJet fellowship (4008 323).*

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ADDED SELECTIVITY OF ION MOBILITY MASS SPECTROMETRY IN FOOD ADULTERATION: ALGAE SUPPLEMENTS CASE STUDY

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Due to high nutritional value, both Spirulina (filamentous cyanobacteria) and Chlorella (single-cell green algae) have become very popular food/feed supplements, consequently dominating the global microalgae market over the years. Even though those products are regarded as safe, cases of adulteration, including mislabeling and dilution, have been reported. Presented study aimed at utilizing liquid chromatography/high resolution mass spectrometry to address the problem of algae products adulteration at the same time assessing the advantages of additional selectivity offered by ion mobility separation (IMS). Principal component analysis (PCA) based on over 3,500 features detected revealed excellent data quality with visible separation between the groups and five components explaining over 75% of data variability for both models assessed. Over 300 fully exclusive markers have been selected with subsequent PCA models explaining over 99.8% data variability, yielding excellent tools for adulteration detection. As much as IMS have not improved quality of the statistical models massively, it definitely offers another layer of information describing differences between samples in more detail which may be helpful in more challenging authentication studies such as geographical origin assessment.

Keywords: *Spirulina, Chlorella, adulteration, fingerprinting*

Acknowledgement: *Presented research was funded by the ChemJet fellowship (4008 323).*

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NON-TARGETED FINGERPRINTING TECHNOLOGY TO VERIFY THE AUTHENTICITY OF GEOGRAPHICAL ORIGIN OF CHINESE WINE

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Wine samples from three distinctive geographical origins (Shacheng, Changli and Xinjiang) in China including red wines and white wines were measured by non-targeted ^{1}H NMR spectroscopy fingerprinting technology. A segment-wise peak alignment was employed to handle peak misalignments of recorded ^{1}H NMR spectra. Binning of the aligned ^{1}H NMR spectra was performed for data reduction. Principal component analysis (PCA) combined with linear discriminant analysis (LDA) using internal leave one-out cross validation were employed to establish the identification model of the three geographical origins. Accurate classification for the wine samples of Shacheng, Changli, and Xinjiang were 92%, 73%, 95%, respectively. The average correct classification of Shacheng, Changli and Xinjiang wine samples were determined to 87%. In order to avoid the overfitting, repeated double random cross-validation methods were used to verify the validity of the PCA/LDA model. The results demonstrated that non-targeted ^{1}H NMR spectroscopy combined with multivariate analysis is an effective tool to verify the authenticity of the geographical origin of Chinese wine. The unique Chinese wine ^{1}H NMR database is of great importance for industrial conventional quality control and regulatory enforcement.

Keywords: *non-targeted fingerprinting, 1H NMR spectroscopy, geographical origin, chinese wine authentication, multivariate analysis*

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B74

13C/12 C ISOTOPE RATIOS OF CITRIC ACID DETERMINED BY HPLC-CO-IRMS FOR JUICES AUTHENTICITY

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Citric acid is one of the most widely used food additives, especially in beverages. However, in both China and Europe, under GB/T 21731-2008 and EC Directive 2001/112/EC relating to fruit juices and certain similar products, its use is strictly limited and must be mentioned in the ingredients list. As China and EU are both exporter and importer in terms of fruit juices consumption, there is a need for an analytical tool capable of detecting and quantifying citric acid addition to be able to enforce this legislation and fight against food fraud. Stable isotope ratio technique is a powerful tool for food adulteration detection as isotope ratios are related to plant growth localization, and the carbon isotope ratio characteristic of citric acid has therefore been proposed as a means of detecting the addition of citric acid from C4 plant sources (cane or maize) in C3 fruits such as citrus and lemon, however, the pioneering method is rather cumbersome, due to the pure citric acid required for the analysis and the complicated preparation procedure of isolation comprised three chromatographic clean-up steps and a subsequent preparative liquid chromatographic separation, meanwhile, the isotope fraction risk was increased. Finally, these purification steps and one by one sample preparation limited the use of this characteristic in authenticity control routine applications.

High performance liquid chromatography linked to isotope ratio mass spectrometry via an interface allowing the chemical oxidation of organic matter (HPLC-co-IRMS) was used to online determine carbon 13 isotope ratio (δ^{13} C) of organic acids, glucose, fructose, ethanol, and glycerol while equipped a H+ ion exchange column, but limit for citric acid direct analysis due to the existence of sucrose molecule in the sample. As sucrose can be degraded into glucose and fructose, the pretreatment conditions of amount of sulfuric acid solution addition and incubation time were optimized to remove sucrose molecules, therefore the samples can be injected into HPLC-co-IRMS system directly. Upon the experiences obtained, the elimination of sucrose on δ^{13} C analysis of citric acid were discussed with regard to mix critic acid with different sucrose regent (with δ^{13} C values from -10‰ to -28‰). The measured δ^{13} C value of citric acid from different samples after pretreatment kept consistent with pure critic acid regent, which allows a set of juice samples can be pretreated at the same time. Then, analysis was performed on 19 orange fruits from various geographical origins and squeezed in the laboratory with the standard deviation better than 0.25‰, and a variation of δ^{13} C values of -28.35‰ to -25.04 was found for critic acid.

Keywords: *food fraud, citiric acid, orange juice, stable carbon isotope ratio, Lc-co-IRMS*

Acknowledgement: *This research was supported by the EU-China-Safe Horizon 2020 Project (Grant No. 2017YFE0110800 and 727864), the National Natural Science Foundation of China (Grant No. 31601553 and 31601580), the International S&T Cooperation Program of China (Grant No. 2016YFE0113200),the Czech-Chinese mobility project (Grant No. 43-12), the National Standardization Management Committee of China (Grant No. 20101051-T-607) and the National Key Research and Development Programme on key techniques development of food safety(2018YFC1603203).*

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INTRODUCING UNIQUE GRAPE VARIETIES FROM ALBANIA FOR RED AND WHITE WINES PRODUCTION

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Albania is characterized by high variability of local grape populations. Located in Southern Europe, this country has a millennial viticulture tradition. Among local grape varieties *Kallmet* and *Sheshi i Zi*, are distinguished for the production of red wines, while *Sheshi i Bardhë* is distinguished for producing the white wine. Other local grape cultivars to be mention are *Vlosh*, *Pulsi*, *Serinë e Zezë,* and *Debinë e Bardhë*. Although these grape varieties are of high value in terms of wines' yield and quality, and therefore might compete with many introduced grape varieties, however, their metabolome was not examined in detail yet.
For this reason, an untargeted analytical approach employing ultra-high-performance liquid chromatography coupled to high-resolution tandem mass spectrometry was used to acquire metabolic fingerprints of unique Albanian wine varieties.
Monovarietal commercial wines (43 samples) were provided by the different local wineries, both from local varieties as well two introduced grape varieties. The wine samples belonging to red wines group (n=26) were: *Kallmet* (n=10), *Shesh i Zi* (n=7), *Serinë e Zezë* (n=2), *Vlosh* (n=2) and *Merlot* (n=5); and wine samples belonging to white wines group (n=16): *Shesh i Bardhë* (n=6), *Pulsi* (n=2), *Kallmet i Bardhë* (n=3), *Debinë e Bardhë* (n=3) and *Chardonnay* (n=2), for producing seasons 2015-2017. Aliquots of wine samples (3 mL) were stored at -20 °C. Wine metabolomics analysis were conducted by using UHPLC–HRMS in two ESI modes (positive and negative), as well as employed two extraction methods (direct injection and EtOAC extraction).
In addition, chemometric analysis (PCA) was used for the first view on the acquired data structure. In this way, similarities and differences among analyzed wine samples were observed.

Keywords: *wine authentication, Shesh i Zi, Kallmet, Vlosh, Albania*

Acknowledgement: *FOODINTEGRITY European Project*

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METABOLOMICS ON APPLE CUTICLE: ASSESSMENT OF DATA OBTAINED BY SFC AND LC COUPLED WITH HRMS/MS

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Apples are one of the most consumed fruit globally and also the most important fruit commodity in the Czech Republic; however, their domestic production is threatened by cheaper imports. To date, only a few methods for apple authentication have been published, and new, reliable approaches are thus highly demanded. Our study was focused on apple cuticle, which is very rich in various metabolites (often with significant bioactivity) and reflects directly the growing conditions of apple trees. The analysis of extracted cuticle components was performed by two different separation techniques, supercritical fluid chromatography (SFC) and liquid chromatography (LC), both coupled with high-resolution tandem mass spectrometry (HRMS/MS). Surface of whole apples was extracted by five different solvents or mixtures, with a mixture of dichloromethane/methanol (1:1, *v/v*) giving the best results with respect to the number of detected ´features´ and obtained chromatogram patterns. A wide range of compounds (metabolites) with a potential for apple authentication was identified (e.g. phenolics, sugars, acylglycerols, triterpenic acids and their esters, and fatty acid esters with aliphatic alcohols or alcohols characteristic for cuticular layer, such as farnesol, p-coumaryl alcohol or oct-5-ene-1,3-diol) based on the detected accurate *m/z* values, isotopic patterns and fragmentation spectra, and their retention and peak shapes in both techniques were compared. Currently, a large set of various samples is being collected to find the markers of different geographical origin by using multivariate statistical methods.

Keywords: *cuticular waxes, fingerprinting, geographical origin, malus domestica, terpenoids*

Acknowledgement: *This work was supported by the specific university research (MSMT No 21-SVV/2019) and Project "Research of metabolomic methods for laboratory authentication of apples geographicity", supported by QK - Applied Research Program of the Ministry of Agriculture 2017-2025, Czech Republic (QK1910104).*

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SEMI QUANTIFICATION OF ANIMAL DNA IN FOOD USING REAL TIME PCR

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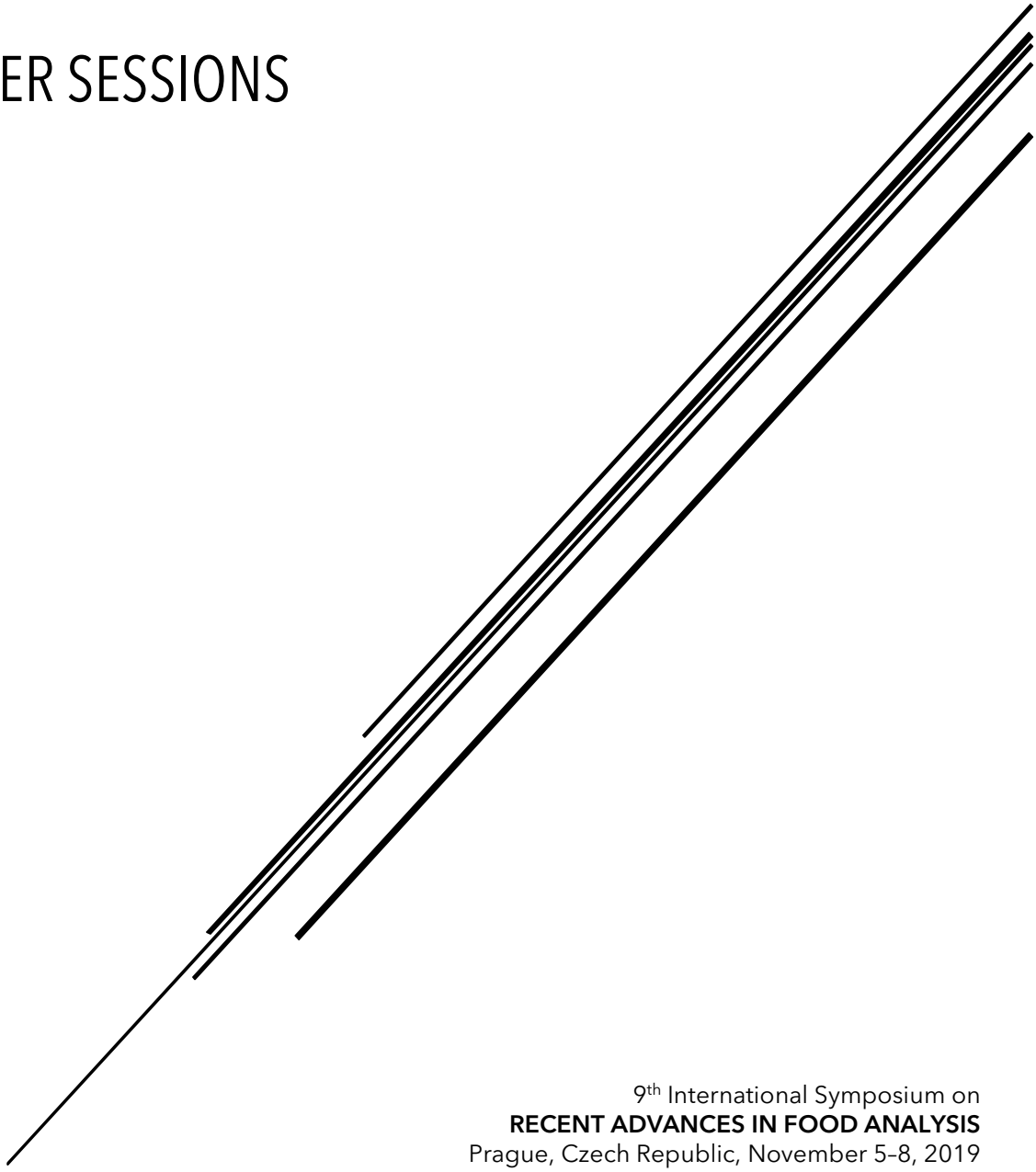
There is an increased demand for animal DNA quantification in various food products. This is required to establish potential fraud, contamination or verify claims based on religious and ethical or lifestyle choices. Quantifying DNA is a difficult process which carries a lot of uncertainty due to the nature of the target analyte. This becomes even more complicated in food products which have been through various temperature based treatment and can also contain ingredients which can cause inhibition. The study design was based on the cut off procedure set up by the EURL regarding PCR of animal species.
The project objective was to establish cycle threshold (Ct) values to use as a cut-off threshods for semi-quantitative determination of meat content in food samples. In order to determine the Ct brackets equivalent to particular range of percentage meat content, the so-called cut-off Ct values will be generated by testing known as percentage of various meat concentrations using multiple replicates of DNA mixes. The species validated were horse (*equus caballus*), beed (*bos taurus*), sheep (*ovis aries*), goat (*capra hircus*), pork (*sus scrofa*), chicken (*gallus gallus*), turkey (*meleagris gallopavo*). The samples were extracted several times under repeatability conditions. Each cut off was calculated based on 12 individual PCR measurements obtained from independent DNA extracts and dilutions. The data were analysed by using several different threshold setting in order to choose the most optimal method: automatic, manual, fixed.
The results obtained enabled us to set up quantification brackets that clearly distinguish between contamination and substitution. The method doesn't require individual calibration curves and is applicable to several food products that contain meat.

Keywords: *PCR, meat, DNA, authenticity, speciation*

BIOANALYTICAL METHODS FOR FOOD CONTROL

POSTER SESSIONS



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C1

A DNA-BASED FAST METHOD FOR THE DETECTION OF SPOILAGE FUNGI IN FRUIT PREPARATIONS

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Spoilage of food products due to fungal contamination is still of great concern due to the vast food waste and economical losses for the food industry [1]. Traditional culture-based techniques to detect spoilage fungi require up to seven days, rendering them unsuitable for products with short shelf-lives. Furthermore, these techniques can only be used retrospectively being of limited value for quality and process control [2]. As a consequence there is a need for faster, and more sensitive, methods for fungal detection that will reduce the overall costs of the food industry due to product recalls and long analyses [3,4].
In this sense, DNA-based techniques, such as PCR and qPCR, can result in decrease of costs for the food industry, due to reduction of analysis time and higher sensitivity [5]. Furthermore, to improve the detection by amplification techniques an efficient DNA extraction and purification has to be performed to obtain enough concentration of DNA and eliminate possible inhibitors present in the food sample or produced by the microorganism's growth [6]. Differential centrifugation and combination of enzymatic, chemical and physical methods seem to provide efficient lysis of most of the microorganisms.
With the objective of reducing the time of analysis, at INL we are working on the development and optimization of a qPCR technique for the detection of spoilage yeasts and moulds in fruit preparations. In this sense, various primers were compared in order to find the most suitable set of primers for universal fungal detection. In addition, different strategies were evaluated, such as matrix lysis and short enrichment protocols, to reduce enrichment time of yeast and moulds, since this step is frequently considered as the major bottleneck when developing faster methods, without losing sensitivity. Finally, different DNA extraction and purification protocols were compared to allow reliable amplification results. In this way, we were able to develop a fast and sensitive method that requires less than 48 hours for the detection of spoilage fungi.

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Keywords: *spoilage fungi, DNA analysis, fast molecular methods*

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C2

MOLECULAR IMAGING ANALYSIS OF PESTICIDES AND ADDITIVES IN FOODS USING SURFACE-ASSISTED LASER DESORPTION/IONIZATION MASS SPECTROMETRY WITH METAL FILM

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Introduction: Movement of pesticides in plants and dispersion of additives in foods has been evaluated using radioisotope imaging (Grossmann, K. and Ehrhardt, T. 2007) and liquid chromatography (LC)/mass spectrometry (MS), respectively. However, expensive and exclusive facilities are needed for the radioisotope experiments, and LC/MS is not suitable to localization analysis for molecular imaging. Simple and inexpensive novel analytical methods for direct imaging are required in food chemistry fields to examine the movement of pesticides in plants and dispersion of additives in food.

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI) MS imaging (MSI) has been used for visualizing the distribution of molecular ions in a dissected biological tissue on a target plate (Garrett, T. J. 2007). MALDI with UV-absorbing organic compounds as a matrix of an ionizing agent has been widely used for analysis of biomolecules. However, characterization of small molecules by MALDI/MS is often difficult because of interference originated from ionized excess matrices in detection. As a new approach to MALDI-MS for detection of small molecules, surface-assisted laser desorption/ionization (SALDI)/MS has been investigated. SALDI is a method using UV-absorbing nanomaterials as SALDI matrix. In this study, we demonstrated SALDI/MSI with platinum film and zirconium (Zr) film as new SALDI matrices for imaging analyses of pesticides in plants and dispersion of additives in foods.

Results and discussions: In the SALDI/MS experiments, Pt and Zr films are coated on surface of samples by sputter deposition. Then, the samples are irradiated by UV pulse laser and detected. It seems to obstruct ionization of sample molecules for the metal film coating. However, the SALDI gave higher ion yields of standard samples such as pesticides and additives. It is conceivable that the ionized samples under the film could be detected through the pores in thin metal films. The ion yields of standard pesticides acephate and acetamiprid on leaves were evaluated using MALDI/ and SALDI/MSI. These compounds could not be adequately detected by MALDI/MS because of the charge-up effect on the surface of the leaf. On the other hand, Pt- and Zr-SALDI gave higher S/N values for these compounds than MALDI because of the conductivity. This means SALDI with metal film allows imaging analysis of non-conductive samples.

Next, Distribution of pesticide in plant and additives in food were investigated using MSI method. In the experiment, Non-conductive surface of leaves picked from a plant grown in soil mixed with the same solution were prepared and analyzed directly by SALDI/MSI without using radioisotope. In the results, the chemicals in leaves and additives in foods could be detected and visualized by localization analysis. It is conceivable that SALDI with metal films allows for the MS imaging analysis on various samples such as plant, food, biological tissue, and polymer film.

Keywords: *pestisides, additives, mass spectrometry, SALDI, metal film*

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C3

UPLC ANALYSIS OF BIOGENIC AMINES IN DIFFERENT CHEESE VARIETIES RETAILED IN AUSTRIA

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High concentrations of biogenic amines (BA) can be found due to microbial activity intrinsic to typical fermented foods such as wine, fermented meat and especially cheese. During cheese ripening, accumulated free amino acids may act as precursors for the conversion into BA mostly affected by bacterial *decarboxylases* of a contaminating microflora. High levels of biogenic amines can result in food poisoning, and cases of histamine/tyramine intoxication have occurred subsequent to the consumption of cheese.
Considering the toxicological implications of these amines, and the general interest in occurrence data for "risk assessment" of fermented foods, the objective of this study was to analyze BA concentrations in various commercial cheese samples (n = 151) representing most common cheese varieties. AccQ-Fluor derivatizing reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) was used to analyze primary and secondary biogenic amines by *Ultra-Performance Liquid Chromatography* (UPLC™).
In general, cumulative levels of biogenic amines varied to a great extent with exceptional samples having amounts up to 150-300mg/100g cheese (e.g., Tiroler Graukäse with 313mg/100g or Tiroler Almkäse with 185g/100g), whereas only 5% of the analyzed cheeses showed total concentrations higher than 90mg/100g (median 5.7 mg/100g). Regarding the most relevant biogenic amines, histamine was found in 79% of all samples, with maximum concentrations for Tiroler Almkäse (116 and 82mg/100g), but only 5% of the cheeses had a histamine level above 17mg/100g (median 0.9mg/100g). For tyramine (72% occurrence; 5% > 37mg/100g), highest values were found for Algunder Graukäse (160mg/100g), Tiroler Almkäse, French raw milk cheese, Olmützer Quargel or Harzer cheese (each ~50 mg/100g; median 1.0mg/100g). Putrescine was detected in 70% of the cheeses (up to 80mg/100g for some acid-curd cheeses; median 0.6mg/100g; 5% > 26mg/100g). Cadaverine was found in 47% of the samples (5% > 22mg/100g), with highest concentrations for Harzer cheese and Olmützer Quargel (126 and 75mg/100g, median 0.2mg/100g). Tryptamine had the lowest occurrence (15%; 5% > 8mg/100g) and a median concentration of 0.3mg/100g.
In conclusion, high (and toxicologically critical) levels of biogenic amines are definitely not associated with a certain type of cheese (as it is sometimes reported in former literature), but may vary depending on a large number of different incalculable factors (e.g., hygiene during the whole cheese production process, number and class of contaminants, degree of proteolysis in cheese, uncontrolled technological aspects). For all analyzed cheeses, both the individual and the total amounts varied greatly, making it virtually impossible/inadequate to pinpoint certain cheese types as more potent sources for intrinsically high biogenic amine levels. Thus, obligatory monitoring of biogenic amines should be considered to ensure high quality and safety of cheese products in future.

Keywords: *UPLC, biogenic amines, cheese varieties, histamine, tyramine*

C4

SIMULTANEOUS QUANTIFICATION OF MAJOR FOOD ALLERGENS USING A MULTIPLEX IMMUNOASSAY

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Overview: Quantification of food allergens is increasingly important for dose assessments of food preparations used in oral food challenges (OFC), food allergy prevention, and monitoring safety in the food industry.
Introduction: Generic immunoassays for 'total protein' do not measure specific allergens. Our aim was to use a molecular approach to food allergy to develop a multiplex immunoassay capable of simultaneously measuring specific allergens, the 'active ingredients', from peanut, cow's milk, shellfish, egg, cashew, soy and hazelnut.
Methods: The multiplex array was developed on the Luminex xMAP system. Microspheres coupled to specific monoclonal antibodies were used for allergen capture. Biotinylated specific monoclonal or polyclonal antibodies were used for detection.
Referencestandards were formulated from natural or recombinant allergens, with purity established by mass spectrometry. Full method validations were performed to determine parameters of linearity, range, limits of quantification and detection, accuracy and precision of the multiplex food immunoassay.
Results: Method validations were completed for the major food allergens. Standard curves for all analytes allow for quantification over a broad dynamic range. Limits of detection were as low as 0.01ng/ml. Intra- and inter- assay accuracy and precision of three samples assayed in triplicate on four occasions passed acceptance criteria within the range of 70-130% recovery and a coefficient of variation of <15%. Food products and the NIST SRM 2387
ReferenceStandard were analyzed using the multiplex immunoassay.
Conclusions: A quantitative, accurate and precise multiplex immunoassay was validated for the simultaneous detection of major food allergens. The multiplex array provides a sensitive and efficient tool for measuring specific food allergens, as opposed to generic food source proteins, with potential applications for risk assessment in the food industry and standardization of clinical OFC.

Keywords: *multiplex, food allergen analysis, immunoassay, Luminex xMAP*

C5

QUANTITATIVE IDENTIFICATION OF MUSCLE TISSUE BY MEANS OF BIOMARKER PEPTIDES FOR MEAT PRODUCT AUTHENTICITY CONFIRMATION

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Insufficient quality and food fraud are current problems today. Previously, using proteomic technologies in meat samples and in specially developed sausages; several tissue-specific proteins were identified by two-dimensional electrophoresis (2-DE). Specific soy and chicken proteins were also identified, and assigned to production falsification markers. However, a new method of identifying species-specific molecular markers, based on a combination of two very effective methods in the field of food analysis: liquid chromatography (LC) in combination with mass spectrometry (MS) used for the detection of peptides, is gaining momentum.
The aim of the work was to develop a fast, highly specific and reliable method for the identification and quantitative determination of peptides. The paper presents the LC-MS method adapted for the detection and quantification of two different types of meat (beef and pork) in a complex biological matrix, such as structureless minced meat. After isolating the proteins and digesting them with trypsin, species-specific peptide markers were selected for each type of animal for quantitative determination.
Model samples of minced meat, prepared from several species of animals (beef and pork) in the ratio of 75% and 25%, 50% and 50%, 25% and 75%, 5% and 95%. There were also standard Referencematrices for each species. Then the meat proteins were extracted and trypsin digested, and the peptide mixtures were analyzed by LC in combination with high-resolution tandem mass spectrometry. Protein analysis was performed using biomodelling in the Skyline program, which is able to carry out theoretical protein cleavage and display a list of selected reaction monitoring (SRM) for each peptide. The major proteins with biomarkers selected for authenticity of meat products are: myoglobin (HPSDFGADAQAAMSK; HPGDFGADAQGAMSK) and lactate dehydrogenase (SNVSDAVAQSAR; THVSEAVAQSTR).
The selected peptide markers were used to construct calibration curves with good linearity (R ^ 2≥0.964) and high reliability (in the range of 80–120%), which allows to obtain a quantitative estimation of the meat from animal species presented. A good sensitivity was established (LOD 0.2–3.27%). Proteomic strategies are one of the main steps to obtaining high quality animal products [1]. Obviously, similar approaches are applicable to all meat products, which contain proteins of muscular origin. Thus, the proposed methodology can find quite a wide use with respect to the various tasks facing the scientific community regarding the practical implementation of the results of their activities with obtaining a specific result.

Vostrikova N.L., Chernukha I.M., Khvostov D.V. Methodological aspects of identification of tissue-specific proteins and peptides forming the corrective properties of innovative meat products. Theory and practice of meat processing, 2018, vol. 3, no. 3, pp. 36-55. https://doi.org/10.21323/2414-438X-2018-3-3-36-55

Keywords: *biomarker, species identification, HPLC/MS*

Acknowledgement: *The work was supported by the Russian Science Foundation, project no. 16-16-10073 П.*

C6

AUTOMATED MULTIPLEXED ELECTROCHEMICAL IMMUNOSENSING PLATFORM FOR ANTIBIOTIC RESIDUE ANALYSIS IN MILK SAMPLES

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Increasingly research efforts are focused on the development of multi-analyte residue analysis using portable devices for the *on-site* evaluation of food-quality, and specifically in milk. According to the directive 37/2010/EU, the EU establishes maximum residue limits (MRL) for each family of antibiotics (ATB) used in the veterinary field. The risk of ATB overuse in animals raises serious concerns for human health and the threat of antibiotic-resistant microorganisms.[1] In this regard, we developed an automatized electrochemical platform for the multiplexed determination of the most commonly used antibiotics families in cow milk.

The different immunoreagents involved in the determination of the targeted antibiotics (fluoroquinolones, streptomycin, sulfonamides and chloramphenicol) were characterized by indirect competitive enzyme-linked immunosorbent assay (ELISA). Each assay was based on the immobilization of haptenized proteins and the use of broad specificity antibodies, following a previous strategy developed by our group.[2],[3] Once the parameters of the assay were optimized, the matrix effect of full-fat commercial milk was assessed at different dilutions factors, ranging from 1:1 to 1:5. In all cases, the ELISAs reached a IC_{50} values lower than the corresponding EU MRL.

Afterwards, the same immunoreagents were implemented in an automated amperometric immunosensing platform provided of gold screen-printed electrode arrays formed by eight 3-electrode electrochemical cells. The device worked using a cocktail of antibodies for the simultaneous determination of all targeted antibiotics. The LOD and IC_{50} values achieved are in the same range as the microplate ELISA. Matrix effect studies demonstrated that samples could be measured without any pretreatment. Moreover, a regeneration protocol was established for the consecutive measurements of real samples using the same electrodes.

Altogether, our results suggest that the proposed system has a great potential for the *on-site* semicontinous analysis of a broad range of antibiotics residues in milk samples.

[1] S. N. Garcia et al., One Health, 7 (2019), 100086.
[2] J. Adrian, et al., Journal of Agricultural and Food Chemistry, 57/2 (2009), 385–94.
[3] Adrian J. et al., Analytical and Bioanalytical Chemistry, 391/5 (2008), 1703–12.

Keywords: *food safety, electrochemical determination, milk analysis, multi-analyte sensing, antibiotics*

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C7

LIGHT SCATTERING PHENOTYPING FROM BACTERIAL COLONIES BY SUPERCONTINUUM LASER

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Background: Optical techniques in food safety have shown great potential in detecting hazardous contaminants in food to prevent customers from serious illness. BARDOT (Bacteria RApid Detection using Optical Technology) is one of the optical techniques, which discriminates microbial organisms using elastic light scattering pattern produced as a coherent light source transmit through a bacterial colony. BARDOT utilizes a diode laser to generate the forward scattering pattern, which contains highly informative morphological characteristics of the colony. BARDOT offers a label-free and non-destructive measurement that the same samples can be further analyzed if necessary, showing excellence in pathogen screening that can effectively save time and cost. Based on the successful achievements, we have implemented more wavelengths in the system and developed a hyperspectral elastic scatter phenotyping instrument (HESPI) for the detection of foodborne pathogens to increase the ability to discriminate among sub-species of pathogens.
Method: The new instrument consists of a supercontinuum laser and an acousto-optical tunable filter (AOTF) to provides coherent light source in every wavelength within 470 – 720 nm. As result of integrating an AOTF, the wavelength of incident light can be switched very rapidly so that the overall acquisition time stays under a minute. Due to wavelength-dependent characteristic of AOTF, a pair of optical lenses was utilized to compensate beam spot movement. To capture the hyperspectral patterns, a CMOS sensor was placed under a sample plate.
Result: The average FWHM of the wavelength band was 2.84 nm, and total of 20 bands were required to sweep the spectral range without the bands overlapping each other. For each wavelength of interest, the pattern collection time was about 0.5 seconds and took 10, 20, and 40 seconds for 20, 40, and 80 spectral patterns respectively. Four bacteria collected from lettuce, *Arthrobacter*, *Curtobacterium*, *Massilia*, and *Microbacterium*, were measured under the newly developed instrument. The wavelength-dependent characteristics were clearly observed in the hyperspectral patterns, particularly in the shape. The size of pattern and the number of diffraction rings within the pattern were increased while the wavelength was decreasing.

Keywords: *bacterial colony, phenotype, light scattering, supercontinuum laser*

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C8

STRATEGY TO ASSESS ECOTOXICOLOGICAL RISK AND SEAFOOD RISK OF SEDIMENTS WITH BIOASSAYS

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The Hangzhou bay region has the fastest growing economic development and is the most contaminated region of China. Recent research reveals high levels of toxic compounds in the sediments in this region. This implies many potential adverse consequences for the benthic ecosystem health and services it can provide, benthic eco-toxicity, seafood safety and whole benthic ecosystem stability. This study aims to study the eco-toxicological effects in the field, with application of novel bioassays for monitoring the toxic potency of the sediment and toxicological risks assessment for seafood originating from this region.
Using collected historic monitoring data about marine sediment quality and benthic animals' species variety, arsenic, copper, chromium and cadmium is identified as the key benthic pollutants. Meanwhile, more than 60% of benthic species are disappeared in last 20 years. Crustacean group animals are mostly survived compare to other species and have big concern for contamination accumulation in the seafood crustacean species like swimming crabs.
Furthermore, novel in-vitro bioassays were applied to quantify genotoxicity potencies of toxic compounds in marine sediments and benthic animals. The novel assay employs a multiplex of 38 bacterial reporter strains (key proteins) covering seven recognized DNA damage repair pathways such as double strand break, DNA lesions and so on. Via GFP fusion proteins this genomics assay enables quantification of the potency to activate multiple DNA damage mechanisms by compounds extracted from sediments and from benthic animals. Chemical analysis was performed for calibration of the bioassay. Results illustrated swimming crab samples showed very high response to umuD and lexA genes and it can strongly relate to genotoxicity effects. Late chemical analysis proves crabs' samples showed gene response does contain high PAHs levels compare to safety level.
Based on historical data and current collected & analyzed samples, assessment of sediment showed moderate ecological risk mainly caused by multiple heavy metals, while food risk assessment illustrated Cadmium and Naphthalenefrom swimming crab has very high food risk to human in acute and chronic period.
All in all, the study found Hangzhou bay region does have many ecological and seafood concerns in benthic ecosystem. Arsenic,chromium and cadmium are exceeding safety levels and more than half benthic animal species are disappeared in last twenty years. Genotoxicity are found in seafood samples and moderate risk are assessed from sediment. Meanwhile, in vitro bioassays and risk assessment were proved as alternative tools in the region to find out the hidden hazards and support quantitative risk study.

Keywords: *seafood, genotoxcitiy, in-vitro bioassay, Hangzhou Bay*

Acknowledgement: *National Key R&D Program of China (2018YFC1603300) and consigned program from CFSA*

C9

RAPID AND SENSITIVE MALACHITE GREEN TOTAL ELISA

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Triphenylemethane dyes such as malachite green (MG) and crystal violet (CV) are organic dyes widely used in industry for dying purposes. They have been also used in aquacultures to treat and prevent fungal and parasitic infections in fish and shrimp. However, due to their genotoxic and carcinogenic effects, they have never been authorised to be used in aquaculture products for human consumption. These dyes are extensively absorbed and metabolised to the reduced leuco forms: leucomalachite green (LMG) and leucocrystal violet (LCV) that can persist in tissues for a long time. The detection of MG, CV and their metabolites in fish or shrimp indicates illegal use of these dyes in aquacultures. In the European Union the minimum required performance limit for a method for the detection of a sum of MG and LMG is 2 µg/kg (Commission Decision 2002/657/EC).
Malachite Green Total (MGT) ELISA was developed to provide a new method for simultaneous detection of residues of both MG and CV. The new test is based on a polyclonal antibody against MG and cross-reactivity with CV. The test is applicable for analysis of fish and shrimp samples. The development of a simple extraction method giving high recoveries in ELISA was an analytical challenge due to the complexity of the samples used, matrix effects and possible degradation of the analytes during sample processing. A novel sample preparation method was developed and combined with fast oxidation step in which LMG and LCV are converted to their parent compounds. The ELISA test is calibrated with MG standard curve and the results are expressed as total MG concentration: a sum of MG, CV, LMG and LCV. The method was validated for fish and shrimp and the detection capability was determined to be 0.3 µg/kg for all 4 analytes. Mean recoveries determined in fish and shrimp samples over different days at 3 spiking levels: 0.3 µg/kg, 0.5 µg/kg and 1 µg/kg were 69%, 110%, 75% and 79% for MG, LMG, CV and LCV, respectively. The performance of the test was evaluated in Food Analysis Performance Assessment Scheme (FAPAS) scheme and the z-score was -0.8 for a fish sample containing both MG and LMG (concentration determined by MGT ELISA was 6.15 µg/kg while assigned value was 7.42 µg/kg). MGT ELISA can be used as a fast, simple and cost-effective screening tool for the detection of illegal use of both MG and CV in aquacultures.

Keywords: *malachite green, crystal violet, ELISA, fish, shrimp*

C10

DETECTION AND IDENTIFICATION OF FOOD ENZYME PRODUCING MICRO-ORGANISMS IN FOOD ENZYME PREPARATIONS

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The use of food enzymes (FE) in commercial food applications is growing continuously. Recent European regulations require safety assessments of food enzymes (FE) before their commercialization on the European market. One of these EU requirements concerns the mandatory absence of the FE producing micro-organism or it's associated DNA in the final product. Nowadays, the food industry is responsible for the safety of the FE's introduced on the food market. However, accidental contaminations of such impurities have previously already been observed. As currently no strategy exists for an efficient and accurate control and monitoring of such contaminants in FE preparations, we therefore propose a generic strategy of first line screening to detect and identify the potential presence of FE producing micro-organisms, including bacteria, fungi and yeast, in FE preparations.
First, the list of 304 FE dossiers, submitted to EFSA for safety evaluation, was analysed. This displayed that 87% of the FE's are produced by micro-organisms, including fungi (53%), yeast (2%) and bacteria (32%), resulting in 71 different microbial species. Then, in order to identify these micro-organisms, a straightforward strategy, based on PCR amplification and Sanger sequencing, was developed and applied on the 71 identified microbial strains. More precisely, the 16S-rRNA region and ITS region were amplified by PCR for respectively the bacterial and fungal or yeast strains. The obtained amplicons, sequenced by Sanger sequencing, were subsequently characterized through a blast analysis against an in-house constructed database, containing
Reference16S-rRNA and ITS sequences for most of these identified microbial strains producing FE, collected from public databases (UNITE and NCBI). The proposed strategy allows the identification of the FE-producing micro-organisms down to the genus level and, for most of them, down to the species level. The relevance of the sequence analysis using the in-house database was verified through a consensus tree analysis. Afterwards, the sensitivity of the proposed strategy was assessed using an artificially spiked FE preparation. Finally, the applicability of the proposed strategy was confirmed using commercial FE preparations.

Keywords: *screening, identification, PCR technology, food enzymes, producing micro-organisms*

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 17/5 SPECENZYM]"*

C11

STRATEGY TO ASSESS ECOTOXICOLOGICAL RISK AND SEAFOOD RISK OF SEDIMENTS WITH BIOASSAYS

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Keywords: *seafood, genotoxicity, in-vitro bioassay, Hangzhou Bay*

Acknowledgement: *National Key R&D Program of China (2018YFC1603300) and consigned program from CFSA*

C12

RAPID COLORIMETRIC ASSAY USING ENZYMES AND NANOPARTICLES FOR MULTIPLEX DETECTION OF BIOGENIC AMINES

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Biogenic amines are low-molecular weight substances that can be produced during storage by bacterial enzymatic decarboxylation of free amino acids. The most prominent biogenic amine is histamine. Histamine poisoning often results from the ingestion of food containing unusually high levels of histamine. Its toxicity is potentiated by other biogenic amines (e.g. putrescine, tyramine, cadaverine, spermine). Biogenic amine levels can be directly correlated with food freshness.
For accurate detection and quantification cost-effective and time-consuming chromatographic methods (HPLC, GC) are mostly used. On the other hand, for rapid quality control of raw materials for food production a rapid screening method is urgently needed. The low immunogenicity of biogenic amines makes it difficult to produce high specificity and affinity antibodies for ELISA development. Enzymatic detection therefore represents an attractive alternative.
Herein we present first results of the development of a multiplex colorimetric bioassay consisting of several cross-reactive enzymes coupled with a pattern recognition algorithm to detect histamine, putrescine, cadaverine and other biogenic amines. The single enzyme does not identify a specific biogenic amine, but crossreacts with many biogenic amines to a certain extent.
We have isolated diamine oxidase (DAO) from various types of plants including peas, maize, and beans using roots and shoots of seedlings. DAO metabolises biogenic amines under consumption of water and oxygen to aldehyde, hydrogen peroxide and ammonia. DAO isolates from pea mainly degraded cadaverine and putrescine, while isolates from maize responded essentially to spermidin and only slightly to spermine. Isolates from beans were not effective. The amount of degraded biogenic amine was proportional to the amount of produced hydrogen peroxide, which was either quantified with substrates like 4-aminoantipyrine (AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS) using horseradish peroxidase (HRP) or Ag nanoparticles biosynthesized with extracts from leaves of various trees and black tea. Extract preparation was varied as well as reaction conditions (temperature, concentration) with AgNO3 solution. The Ag nanoparticles were characterized by UV-VIS spectroscopy and DLS measurements and tested as HRP mimic in the assay. Next, the two assays will be technically evaluated with real samples to estimate the potential of the colorimetric assay as a rapid and reliable screening method for biogenic amines.

Keywords: *biogenic amines, diamine oxidase from pea, Ag nanoparticles, colorimetric enzyme assay*

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C13

RAPID SEPARATION OF TRANS/CIS FATTY ACID METHYL ESTERS WITH AGILENT DB-FASTFAME GC COLUMN

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The GC analysis of fatty acids as their methyl esters derivatives (FAMEs) is an important tool in the characterization of fats in the determination of total fat and trans-fat content in foods. Traditionally, the detailed separation of complex FAMEs requires the use of a long (100 meters or more) capillary column coated with a high polarity cyanopropyl stationary phase to differentiate between the multiple FAME isomers. However, some of the carbon chain lengths usually overlap on the high polarity phase, causing problems in peak identification. Therefore, long analysis time (more than 70 minutes) is required to achieve good FAME separations.
The Agilent J&W DB-FastFAME GC column was specifically engineered for the fast separation of FAME mixtures, including some key cis-trans separations. This application shows that the 90m DB-FastFAME GC column can effectively separate 57-component FAME mixture including 37 representative FAMEs and some representative trans FAMEs within 40 minutes, and most of cis-trans isomers can be baselined separated. Analysis of 63-component FAME mixture on DB-FastFAME GC column also demonstrates that DB-FastFAME capillary GC columns can provide rapid analysis and the necessary selectivity to resolve cis-trans pairs in food samples to ensure the food conforms to label requirements.

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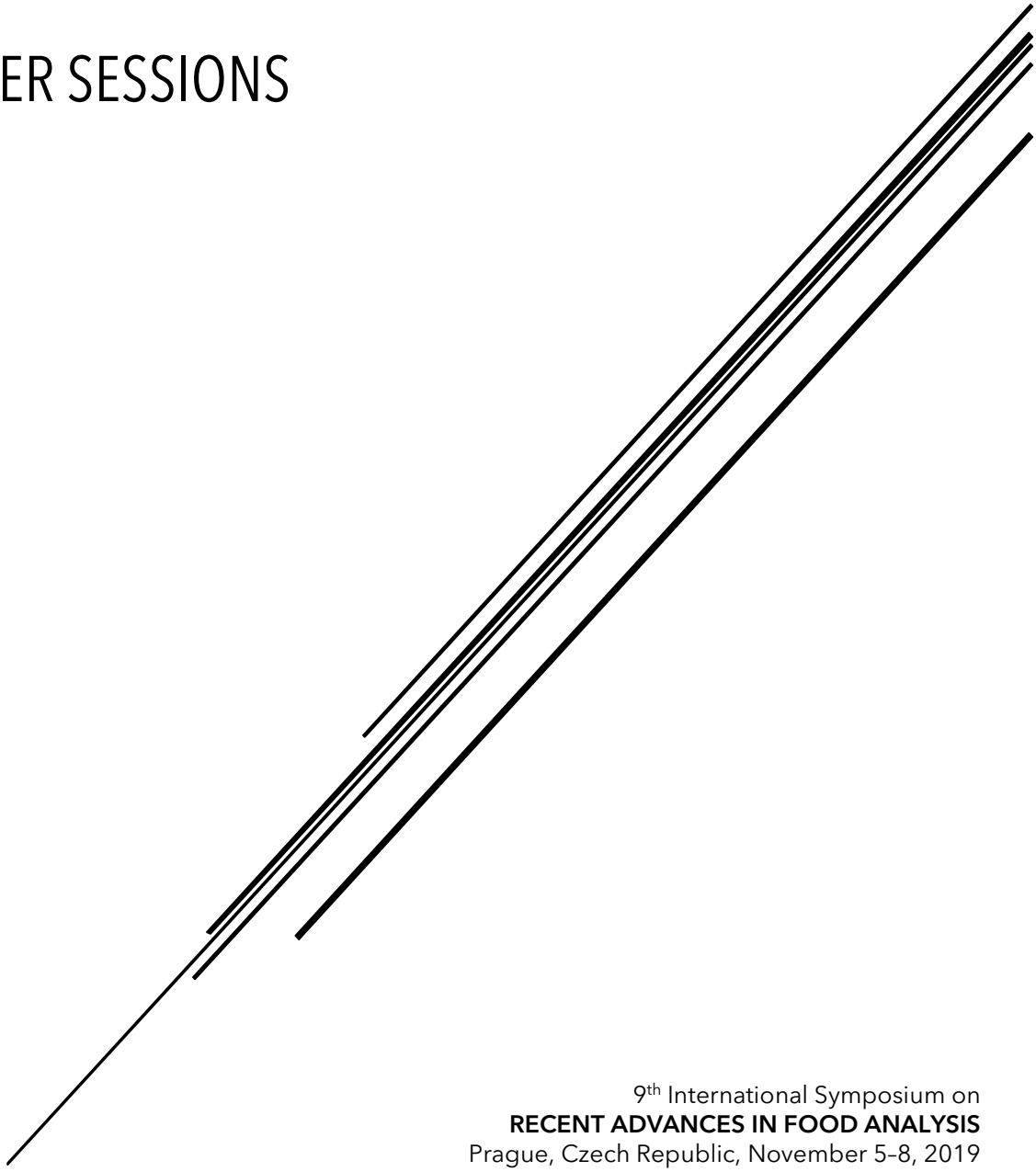
Keywords: *FAMEs, trans-fat, FastFAME, FoodLabelling, isomers*

Acknowledgement: *Gustavo Serrano. GC columns Product Manager at Agilent Technologies. Phil Stremple, Chemistries Division Marketing Manager. Yun Zou, Application Chemist*

BIOLOGICALLY ACTIVE, HEALTH PROMOTING FOOD COMPONENTS

POSTER SESSIONS



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D1

ELETROFORESIS SDS-PAGE AND TRIS / TRICINE FOR IDENTIFICATION OF PROTEIN-PEPTIDE FINGERPRINT IN BLACK SOYBEANS

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Black soybeans (*Glycine max* (L.) Merril) have been used over millennia in oriental countries, as an infusion for medicine purposes and, also, as a well-appreciated dish. Its attributed health benefits include anti-diabetic, anti-obesity, anti-cancer, anti-inflammatory properties. Most of these physiological actions are due to the anthocyanins present in the black seed coat. Recently, some of these health benefits have been associated to bioactive peptides (PBAs). As proteins are macromolecules that are found in higher amount in beans (35-40%), this makes of black soybean a potential source of PBAs. This work aims to characterise the protein-peptide profile of black soybean samples. A specific method of sample preparation that is adequate for solubilize and purify the soybean proteins extracts was developed using electrophoresis, with the final proposal of simultaneous analysis of protein and peptides. For the extraction of proteins from soybean samples was used the electrophoretic buffer under vigorous agitation. In the purification step, fat was removed using sample/acetone proportion of 1/10 (w / v). Four soybean samples were analyzed: 1) Yellow soybean cultivar PF 122105, 2) Black soybean line BRM09-50901, 3) Two commercial black soybean flours acquired in the retail market. Three electrophoretic variations (SDS-PAGE 10% and 12% and TRIS / TRICINE) were required to identify and quantify marked differences between yellow soybean (PF 122105 cultivar) and black soybean (two commercial flours and line BRM09-50901). The comparison of the yellow and black soybeans allowed the identification of a protein of 100kDa and a peptide of 11kDa, both present only in black soybeans samples. Finally, it was demonstrated that this methodology is effective to obtain a specific fingerprint for each line or cultivar. Evaluation of larger numbers of genotypes of black will be accomplished in the future, to screen molecular markers with potential new PBAs. Therefore, it is concluded that the method employed is suitable for the analysis of proteins and peptides of both yellow and black soybeans. The identification of differentiated protein and peptide only in the black soybean samples suggests that it is a potential good source for new bioactive peptide.

D2

MARINE CAROTENOID FUCOXANTHIN AS BIOLOGICALLY ACTIVE FOOD COMPONENT FROM AMPHORA CAPITELLATA AND NANOFRUSTULUM SHILOI

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Microalgae have been one of the most important natural sources of bioactive products. Among these, carotenoids play a protective role for many diseases. Fucoxanthin, a yellowish-brown carotenoid is found in brown macroalgae and unicellular microalgae such as diatoms. It attracts much attention because of its beneficial effects on human health. It has been reported to have several therapeutic activities, including anticancer, anti-hypertensive, anti-inflammatory, and anti-obesity effects. Therefore, its large-scale production with efficient and feasible ways is of important concern as it can be used as a biologically active compound.

Most of these studies including the industrial production were conducted with fucoxanthin isolated from macroalgae. However, in the present study, we investigated extraction of fucoxanthin under various conditions and suggest its production from novel brown microalgae, *Amphora capitellata* and *Nanofrustulum shiloi.* Optimization of extraction parameters is the key step in green extraction technologies since shorter extraction times, less solvent, and having higher extraction yield play an important role for obtaining food bioactives.

For this reason, this study aims to find out the optimum solvent extraction conditions using ultrasound assisted extraction (UAE) method for fucoxanthin in order to develop new systems for its efficient production at industrial scale. Briefly, the effect of extraction solvents, extraction time, extraction temperature and repetitive extraction on the amount of fucoxanthin obtained were investigated. The experiments were performed using ultrasonic bath.

External standard calibration method was applied for the determination of fucoxanthin content in extracts. Analyses were performed by using HPLC-DAD at 450.0 nm with a flow rate of 1.0 mL/min. Separation was achieved on a YMC Carotenoid column (5µm 4.6x250mm) at 25.0^{0}C applying isocratic elution with methanol and acetonitrile, each containing 0.10% TEA. Fucoxanthin was then purified by column chromatography on silica using gradient elution using acetone and hexane mixture. Identification was carried out comparing the spectral data obtained with DAD with the Reference standard. Moreover, the confirmation of fucoxanthin was provided by LC-APCI-MS analyses of the extracts.

The results have demonstrated that pure ethanol extraction provides the highest fucoxanthin yield within 15.0 minutes for *Amphora capitellata* whereas 20.0 minutes for *Nanofrustulum shiloi* at 40.0^{o}C. Fucoxanthin content was found to be as 6.36 mg/g and 13.22 mg/g in *Amphora capitellata* and *Nanofrustulum shiloi,* respectively.

As a conclusion, it can be said that both species have great potential for large-scale extraction of fucoxanthin and the proposed study is very encouraging for further uses of fucoxanthin as a biologically active and health promoting food component.

Keywords: *carotenoids, fucoxanthin, brown microalgae, extraction, bioactive compound*

Acknowledgement: *The authors would like to thank and acknowledge The Scientific and Technological Research Council of Turkey (TÜBİTAK) for the financial support of this work throughout the project 216Z167 and also EGE MATAL for the facilities (SEM and HPLC-DAD analyses).*

D3

BREWERS SPENT GRAIN AS A POTENTIAL SOURCE OF BIOACTIVE MOLECULES

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Large amounts of underexploited wastes are generated constantly in all the sectors of food industry, the disposal of these by-products representing a real environmental problem for which an effective management solution is imperative to be developed and implemented. Recent advances in biotechnology ensure that brewer's spent grain (BSG), the major by-product of the brewing industry, is no longer regarded as a waste but rather a rich source of bioactive compounds.
Due to the increasing interest in finding new sources of bioactive compounds, the brewers spent grain by-products resulted from different types of beer were analyzed regarding the phenolic compounds and screened *in vitro* for their antioxidant and antimicrobial activities.
The quantification of total phenolic compounds was achieved by Folin-Ciocalteu method, while the antioxidant activity was assessed by evaluating their radical scavenging activity on DPPH radical. All the samples were screened *in vitro* for their antimicrobial activities against different strains of bacteria, *Staphylococcus aureus* ATCC 49444, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Listeria monocytogenes* ATCC 19114, according to the guidelines of the National Committee for Clinical Laboratory Standards. The minimum inhibitory concentration (MIC) was determined using the resazurin microtiter plate-based antibacterial assay.
According to the obtained results, the high content of phenolic compounds gives these by-products antimicrobial properties against the tested strains. The administration of polyphenols in different concentrations inhibit the bacterial growth in a dose dependent manner. Regarding de antioxidant activity, the scavenging efficiency of BSG extracts against the DPPH radical is strongly correlated with the phenolic content of each tested sample.
The obtained results provide further information to the current knowledge regarding the brewers spent grain composition, with perspectives in the development of novel pharmaceutical, cosmetic and functional food products.

Keywords: *bioactive compounds, brewers, spent, grain, waste management*

Acknowledgement: *Acknowledgement. This work was supported by a grant of Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2016-0869 and by a mobility grant of the Romanian Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-MC-2019-0519, within PNCDI III.*

D4

PINE BARK (PINUS PINASTER AITON SUBSP. ATLANTICA) - TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY BY MICROWAVE ASSISTED EXTRACTION (MAE)

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Pine bark is a residue from the timber industry and a great source of phenolic compounds. Polyphenols are natural occurring compounds that have received great attention from the food industry due to its several beneficial properties such as antioxidant, antimicrobial, anti-inflammatory, among others. The reuse of agro-residues, like pine bark, by its addition to food products, using environmentally clean processes, will play a central role within this purpose.
The objective of this study was the extraction of phenolic compounds from pine bark (*Pinus pinaster* Aiton subsp. *atlantica*) by two techniques, Soxhlet and microwave assisted extraction (MAE), using an hydroethanolic mixture (50% water + 50% ethanol). Classical Soxhlet equipment (4 hours under reflux) and a Microwave ETHOS X (Milestone, Italy) (operating conditions: 1600 W, at 110 °C for 30 minutes) were used in this study. The pine bark was dried to reach equilibrium humidity at 40 °C for 72 hours and milled to a particle's size of 200-850 µm. For each technique, the mass extracted (g extract/g bark), total phenolic compounds (by Folin-Ciocalteu), and antioxidant activity (diphenylpicrylhydrazyl, DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS) were quantified.
The extraction technique significantly influenced the analysed parameters ($p<0.05$). The extraction yield was 21.22±0.20 and 17.55±0.16% w/w for MAE and Soxhlet, respectively. MAE also showed great ability in extracting phenolic compounds, 88.92±3.93 mg GAE/g sample versus 54.88±2.58 mg GAE/g obtained by Soxhlet. In relation to antioxidant activity with DPPH, 654.16±21.52 and 438.68±22.02 µmol TEAC/g sample were obtained for MAE and Soxhlet, respectively. Regarding the determination with ABTS, 853.96±21.93 and 464.80±21.20 µmol TEAC/g sample were obtained for MAE and Soxhlet, respectively. Hence, as MAE technique performed better and it is a simple and rapid method, it can be considered for extraction of polyphenols from *P. pinaster* Aiton subsp. *atlantica* bark.

Keywords: *bark, pinus pinaster aiton subsp. atlantica, phenolic compounds, antioxidants, microwave assisted extraction*

D5

ALGAE AS FUNCTIONAL INGREDIENT

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In countries like China, Japan, South Korea and many other edible algae are an important food or food ingredients. There are more than 1000 species of algae in the world and are divided into brown, red and green according to color. For human consumption only about 50 species of algae are available. The main cultivated and consumed varieties are *Ulva lactuca*, *Laminaria japonica*, *Undaria pinnatifida* and *Porphyra purpurea*.
Edible algae contain protein, carbohydrates and minerals. Among them, brown algae such as *Undaria pinnatifida* and *Porphyra purpurea* have a high protein content. According to many studies the protein content in edible algae can be as high as 45.7%. Edible algae are also a type of low-calorie and low-fat food.
Extracts from the most common edible seaweeds contained appreciable polyphenol content. Differences in yield and the extractability of phenolic compounds from seaweeds are well documented and genus *Ulva* has been found to have low polyphenol content compared to species of red and brown algae. Marine algae present a good antioxidant activity.
In conclusion edible algae are an important source of much needed substances necessary to our health and can be used as functional ingredients.

Keywords: *algae, extract,s functional polyphenol*

D6

ADVANCEMENT IN THE DETERMINATION OF BIOACTIVE OLIVE OIL PHENOLS HOSTED UNDER THE EFSA HEALTH CLAIM. STARTING POINT THE COI/T.20/DOC. NO. 29 METHOD

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The almost universal interest in the phenolic compounds that are transferred from olive drupe to olive oil due to the presence of certain bioactive compounds, derivatives of oleuropein and ligstroside, led to numerous publications on their analysis since the '60s. In 2009, the International Olive Council (IOC) adopted an HPLC method for the 'determination of biophenols in olive oil by HPLC' that was based on the isolation of polar fraction with methanol:water, 80:20, v/v, separation of phenolic compounds on an ODS2 (5μm) column and quantification at 280 nm via converting the sum of the areas of the related chromatographic peaks to tyrosol equivalents. The latter is achieved using the corresponding relative response factor of tyrosol to that of syringic acid used as internal standard [1]. Approval of the health claim on 'olive oil polyphenols' regarding 'protection of blood lipids from oxidative stress' [2] raised analytical concerns for the determination of the 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil using this IOC protocol due to poor phenol separation and, thus, difficulties in identification and quantification, the latter being based on the assumption that the same correction factor may be used for all determined compounds. This ambiguity was recognized by IOC that seeks for an appropriate protocol to support market demand. Because the many efforts of different groups continue to different directions in terms of analytical strategies, there is a need for consensus among all interested parties that will lead in a harmonized and standardized protocol suitable for commercial needs.

In the present study, the same virgin olive oils were analysed using both the existing IOC procedure using MS for identification and diode array detection for quantification, and by a recently in house validated UHPLC –diode array procedure. Data comparison in absolute values and statistical treatment substantiates the pros of the 2nd one that is currently on the way for a formal interlabolatory testing and highlights the sources of error using the IOC protocol

[1] IOC, COI/T.20/Doc. no. 29, 2009; [2] EC Regulation No. 432/2012, *Off. J. Eur. Comm*. 2012, *L136*, 1, [3] Tsimidou et al. (2019), *Molecules*, 24, 1044.

Keywords: *olive oil, phenols, EFSA health claim, UHPLC-DAD, International Olive Council*

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D7

BIOACTIVE NON-EXTRACTABLE POLYPHENOLS: RESPONSE SURFACE METHODOLOGY TO OPTIMIZE THEIR ENZYME-ASSISTED EXTRACTION FROM CHERRY POMACE

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Agro-food production chain sustainability, which refers to waste reduction and the exploration of innovative ways to increase resource efficiency, is of increasing importance. Cherries generate great amount of by-products which are mainly constituted by cherry pomace. It has been shown that cherry pomace may be an interesting source of bioactive polyphenols. However, during the extraction process, an important fraction of polyphenols called non-extractable polyphenols (NEPs) remains retained in the extraction residue. They are either polymeric polyphenols or single polyphenols linked to macromolecular food constituents; they are not extracted by common aqueous-organic procedures. Therefore, the aim of this work was to optimize the extraction of NEPs from cherry pomace based on enzyme-assisted extraction (EAE) employing three different enzymes (Depol, Promod and Pectinase).
A response surface methodology was used to study the influence of four independent variables on the NEPs extraction from cherry pomace with each enzyme: temperature (60-80 °C), extraction time (5-40 min), enzyme concentration (0.5-140 µL/g), and pH (6-10). Optimal extraction conditions employing EAE to recover NEPs from cherry pomace were compared with conventional, alkaline and acidic extractions. The total proanthocyanin content in the extracts was measured from three different methods, DMAC, vanillin, and HCl/butanol assays, whereas Folin-Ciocalteau method displayed the total phenolic compounds. In addition, antioxidant and antihypertensive capacities were evaluated with ABTS, DPPH, and hydroxyl radicals scavenging assays and angiotensin converting enzyme inhibition capacity, respectively. The results indicated that EAE was a suitable extraction method to obtain NEPs from cherry pomace having the extracts a higher content of proanthocyanins and bioactivity than those obtained by conventional extraction and alkaline and acid hydrolysis. Size-exclusion chromatography profiles showed that EAE recovered NEPs with higher molecular mass than conventional extraction and acid hydrolysis. Nevertheless, alkaline hydrolysis extracts presented the highest amount of high molecular mass compounds although this method is less selective and other compounds may be extracted.

Keywords: *non-extractable polyphenols, enzyme assisted-extraction, cherry pomace, response surface methodology, bioactivity*

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D8

PRESSURIZED LIQUID EXTRACTION AND DEEP EUTECTIC SOLVENTS, TWO GREEN METHODOLOGIES TO EXTRACT BIOACTIVE COMPOUNDS FROM POMEGRANATE PEEL (PUNICA GRANATUM L.)

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Food processing results in large amounts of byproducts, which could contain bioactive compounds. Pomegranate peel is a byproduct that presents polyphenols and proteins, among others, and that it is usually discarded. The exploitation of this byproduct requires the optimization of methodologies for the extraction of those polyphenols and proteins. Methodologies using inexpensive, non-toxic, and biodegradable solvents are more sustainable than conventional techniques that usually require high amounts of polluting solvents. The main aim of this work was to develop green analytical methodologies using pressurized liquid extraction (PLE) and deep eutectic solvents (DES) for the extraction of bioactives and proteins from pomegranate peel and to characterize these extract.
Pomegranate peel proteins and bioactives were extracted using pressurized liquid extraction (PLE) with different ethanol:water mixtures and high intensity focused ultrasounds (HIFU) with different deep eutectic solvents (DES). The extracts with the highest protein and polyphenols contents were digested using two different enzymes (Alcalase and Thermolysin). Antioxidant, hypocholesterolemic, and antihypertensive capacities of hydrolysates were evaluated. The highest antioxidant capacity was observed in hydrolysates obtained from PLE extracts and the most significant antihypertensive capacity was detected in the hydrolysates from the DES extracts. Extracts were analyzed using high performance liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometry (HPLC-ESI-Q-TOF/MS) for the identification of molecules responsible of bioactivity. While PLE extracts showed a higher ratio in polyphenols than in peptides, activity in DES extracts seemed to be mainly due to peptides and scarcely to polyphenols.

Keywords: *deep eutectic solvents, pressurized liquid extraction, boactive peptides, polyphenols, pomegranate peel*

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D9

AUTOMATED EXTRACTION AND ANALYSIS OF PHENOLIC ACIDS AND FLAVONOIDS IN HUMAN PLASMA VIA MINIATURIZED SPE

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The analysis of phenolic acids in plant material and biological fluids has become increasingly important in the nutritional and food sciences. In addition to their presence in many plants and food like parsley, berries and coffee, phenolic acids result from the metabolism of various phenolic compounds, e.g., flavonoids, by the gut microbiota in the human intestinal tract. For an accurate assessment of the adsorption, distribution, metabolism and excretion of bioactive substances, not only the phase II metabolites such as sulfates, glucuronides and methyl conjugates need to be considered, but also the metabolites produced by the colonic microbiota should be included to achieve a comprehensive evaluation. A simple, fast, automated high-throughput method for the extraction and quantification of phenolic acids and flavonoid aglycones using 24 selected substances in human plasma has been developed. The solid-phase extraction was performed using extra small cartridges filled with only 10 mg of hydrophilic-lipophilic balanced copolymer. The quantification of the analytes was carried out using a LC-ESI-LIT-MS system that was optimized with respect to chromatographic separation and sensitivity. The whole method was validated concerning linearity, limit of detection and limit of quantification, precision of the measuring system and precision of the method, and recoveries. The miniaturized cartridges used in this study are superior to the more common larger cartridges because they allow shorter extraction times, reduced solvent consumption, and smaller sample volumes. The method developed here is suitable for the simultaneous analysis of phenolic acids and flavonoid aglycones in human plasma, especially when only small volumes are available.

Keywords: *human metabolites, phenolic acids, flavonoids, miniaturized SPE, LC-MS/MS*

D10

DETERMINATION OF CANNABINOIDS IN PLANT MATERIALS, OILS AND CONCENTRATES USING UHPLC–DAD/MS: SINGLE LABORATORY VALIDATION FOR AOAC FIRST ACTION OFFICIAL METHOD CONSIDERATION

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This study describes a single-laboratory validation (SLV) of a liquid chromatography–diode array detection (LC–DAD) method for quantification of twelve major cannabinoids in *Cannabis* dried plant materials, oils and concentrates. The method met AOAC Standard Method Performance Requirements (SMPRs) for quantitative analysis of cannabinoids in *Cannabis* concentrates and *Cannabis* dried plant materials. The limits of quantification (LOQs) were in the range 0.003 - 0.10 % (w/w), depending on the analyte and matrix. Spike recoveries were between 96.7 - 101.3% with relative standard deviations (RSDs) ≤ 2.3%. Precision expressed as repeatability (RSD_r) and intermediate precision (RSD^{INT}) was within 0.3 - 4.8 % and 1.1 - 5.1 %, respectively. The chromatographic separation conditions used in this versatile method are compatible with both DAD-UV and mass spectrometric detection. During method validation, high-resolution quadrupole-time-of-flight mass spectrometer (Q-TOFMS) was employed as a secondary detector (connected in series to the LC-DAD instrument) to provide high confidence identification of target analytes. The obtained results demonstrate excellent performance of the method in quantitative analysis of important cannabinoids in dried plants, concentrates and oils.

Keywords: *cannabinoids, dried plants, oils, concentrates, UHPLC-DAD/MS*

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D11

ANALYSIS OF FOODS WITH FUNCTION CLAIMS CONTAINING ENZYMATICALLY MODIFIED HESPERIDIN

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In Japan, the "Foods with Function Claims" system was introduced in April 2015 in order to make more products available clearly labeled with certain nutritional or health functions and to enable consumers to make more informed choices. Food business operators have to submit the necessary information including scientific evidence on food safety and effectiveness to the Secretary-General of the Consumer Affairs Agency according to the Food Labelling Act as well as the "Guidelines on Notification of Foods with Function Claims". The most important thing for safety and effectiveness of Foods with Function Claims is that a third party can qualitatively and quantitatively analyze functional substances and evaluate the labeling value. From this point of view, the guidelines were revised in April 2018 and food business operators are expected to disclose the analytical method of functional substances.

Various functional substances have been developed by a Japanese company and especially, flavonoid compounds or their derivatives, such as anthocyanins, isoflavones, and monoglucosyl hesperidin are frequently used in foods. Monoglucosyl hesperidin is a derivative of hesperidin and one molecule of glucose is enzymatically connected to beta-rutinose moiety of hesperidin. This is used in foods for the purpose of improving the peripheral circulation. To date, forty-two products containing monoglucosyl hesperidin have been submitted and four products of them were chosen for the analysis. Test samples and standard samples were prepared by the identical procedure disclosed by each company and subjected to reversed-phase HPLC analysis under the following conditions: YMC-Pack ODS-A (5 μm), 6.0 mm x 150mm (YMC, Japan); 30 °C; 2.5% aq. acetic acid / acetonitrile / methanol (35:7:5); 1.0 mL/min; 280 nm; 20 min of run-time; 10 μL injected. The results showed that three of four products contain a sffiecient amount of monoglucosyl hesperidin.

Due to the further rapid analysis and less cost, we considered the run-time for analysis and amount of mobile phase should be improved. Various columns, compositions of mobile phase, and flow rates were examined and the better conditions were found as follows: 1) YMC-Pack ODS-A (3 μm), 4.6 mm x 150mm (YMC, Japan); 30 °C; 0.5% aq. formic acid / acetonitrile (70:30); 0.5 mL/min; 284 nm; 9 min of run-time; 5 μL injected, and 2) J-Pack Core ASB C18 (2.7 μm), 2.1 mm x 75mm (JASCO, Japan); 30 °C; 0.5% aq. formic acid / acetonitrile (80:20); 1.0 mL/min; 280 nm; 5 min of run-time; 5 μL injected. Both conditions shorten the run-time and need less mobile phase and injection volume. These HPLC conditions will give the rapid routine analysis in quality analysis of products.

Keywords: *flavonoid, quality control, LC conditions, enzymatically modified hesperidin*

D12

DETERMINATION OF CANNABINOIDS IN FOOD BY LC-MS/MS

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Cannabis sativa has been cultivated by man for at least 8000 years. Its glandular hairs produce a resin consisting of 80-90% cannabinoids, the remainder being a mixture of essential oils, phenols, terpenes and waxes. Today, more than 100 cannabinoids are known, most of them being present in minor concentrations. Some of them are known to be psychoactive, most notably Δ9-THC. Recently, the importance of cannabinoid analytics increased as more and more hemp containing products are being introduced into the market.
In 2000 the former German Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV) published Reference values for total THC, i.e., the sum of THC and THC-acid, contents in food. These reference values have been confirmed by the Federal Institute for Risk Assessment (BfR) recently. As a consequence, most laboratories used to determine total THC contents, but did not differentiate between THC and its precursor THC-acid.
In 2015 a scientific opinion by EFSA derived an ARfD value for Δ9THC and called for methods which are able to differentiate between psychoactive compounds and not psychoactive compounds. Also in 2015 the European Commission published a recommendation for a monitoring program on several cannabinoids which emphasized the need for a method which allows separate determination of different cannabinoids.
In order to keep up with these analytical requests, we developed a sensitive and robust LC-MS/MS method employing an Agilent 6470 TripleQuad. The method allows specific quantification of several cannabinoids. As of now, its scope encompasses Δ9-THC, Δ8-THC, Δ9-tetrahydrocannabinolic acid, cannabinol, cannabidiol, cannabigerol, cannabidivarin and tetrahydrocannabivarin. This scope can of course be extended further. Δ9-THC, Δ9-THC-acid, cannabinol and cannabidiol, whose quantification is frequently requested, are quantified using 2H-labeled standards.
Depending on the matrix to be analyzed, two different, validated sample preparation methods are applied in our laboratory. The work-up of oily samples is based on a method previously published in the German Official Collection of Methods of Analysis (ASU). It employs a liquid-liquid extraction using iso-octane and methanol as immiscible solvents.
Other food samples containing high amounts of fat are extracted according to a QuEChERS-like protocol followed by solid phase extraction clean-up by the Agilent EMR (Enhanced Matrix Removal) SPE material.
The limit of detection for the proposed method is about 10 µg of each cannabinoid / kg of sample.
Our affiliation to the §64 LFGB (German Food and Feed Act) committee for plant toxins (which is working on standardization of cannabinoid analytics) facilitates a regular professional exchange with other laboratories working on the analytics of cannabinoids and enables us to use a state of the art method.

Keywords: *cannabinoids, hemp, tetrahydrocannabinol, THC, LC-MS/MS*

Acknowledgement: *Many thanks to Agilent Technologies for the excellent cooperation and support.*

D13

ATTENUATION POTENTIAL OF INTESTINAL GLUCOSE TRANSPORTER AND ENHANCEMENT OF METHYL DONOR COMPOUNDS UPTAKE BY IN VITRO DIGESTED RAW AND FERMENTED WHEAT BRAN IN CACO-2 ABSORPTION MODEL

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The valorization of nutrient-rich cereal milling by-products (bran and germ) is nowadays an interesting research area alongside with the promotion of a healthier diet. In fact, whole grain consumption is associated with several health benefits, such as a reduced risk of type 2 diabetes [1], mainly related to bioactive compounds present in the outermost grain fractions. The objectives of this study were to study the potential inhibition of intestinal glucose transporter (IGT) and to monitor the uptake of methyl donor compounds such as betaine and choline of raw (RB) and fermented wheat bran (FB) after an *in vitro* simulated gastro-intestinal digestion, using a CaCo-2 cell-based absorption model.
Cell culture were seeded on polycarbonate inserts (pore size of 0.4 µm) and grown up to 21 days [2] and treated with different concentration of digested RB, FB (1 mg/mL in phosphate buffer solution), and IGT inhibitors (phloretin and phlorizin) under a medium glucose concentration condition (25 mM). Then, samples were collected from the basolateral and apical chambers at different times (10-120 minutes) for the determination of glucose (glucose oxidase assay) and betaine and choline quantification (LC-MS/MS). Transepithelial electrical resistance (TEER) and the effect of the treatment on the epithelial barrier function (Lucifer yellow assay) were also assessed.
Phlorizin and phloretin arrested the glucose uptake in CaCo-2 cells via inhibition of sodium glucose linked transporter (SGLT1) and sodium independent glucose transporter 2 (GLUT2), respectively [3]. RB inhibited the glucose uptake at the initial times (10-30 min), however the FB inhibition effect was significantly higher ($p<0.05$). This probably means that during the fermentation novel or modified compounds with IGT inhibition potential were created and subsisted to the digestion phase. Moreover, compounds like feruloylated arabinoxylan mono- and oligosaccharides, which are present wheat bran, can also perform as IGT inhibitors [4]. Nevertheless, the high glucose concentration led to a saturation during the late uptake period (60-120 min). In addition, the initial uptake of betaine and choline was slightly higher in cells treated with FB in respect to the ones treated with RB. These results affirmed that RB and FB can effectively modulate the activity of the key intestinal glucose transporters and fermentation could further enhance this property. Moreover, both RB and FB are a good source of important methyl donor compounds which are largely absorbed by the enterocyte under the studied conditions.

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Keywords: *bran fermentation, CaCo-2 cells, glucose transporters, in vitro simulated gastro-intestinal digestion, methyl donor compounds*

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D14

NEW TECHNOLOGIES AND PLANT-DERIVED INGREDIENTS FOR THE PRODUCTION OF INNOVATIVE PROCESSED MEAT PRODUCTS FOR CONSUMER'S HEALTH

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Meat represents an important ingredient in human diet, being a dietary source of high-value proteins, important minerals (iron and zinc), and essential B vitamins. It is generally consumed as fresh cooked meat or as processed or preserved meat, such as salami, raw and cooked ham, etc. Despite its nutritional benefits, an excessive consumption of meat, in particular of red, processed and preserved meat, has been associated with the insurgence of the colorectal cancer (CRC) in human [1]. CRC could be caused by the presence of different carcinogens formed during meat processing and/or during the gastro-intestinal digestion process, mainly originated by the chemical cocktail formed by heme-Fe, nitrates and nitrites, and lipids and their peroxidation products. For this reason, in the last years, several researches were devoted to identify strategies to partially or totally replace the use of nitrites and nitrates as meat preservatives. In this context, plant extracts naturally rich in polyphenols, well known antioxidant compounds, have been investigated as alternative preservatives for processed meat products [2, 3]. They are particularly interesting both for their technological effects on the products as well as for their potential beneficial effects for human health.
The aim of the present work was to evaluate innovative technologies and the use of vegetable extracts rich in polyphenolic compounds, as possible substitutes of nitrites and nitrates, in the formulation of pork meat sausages and to verify the potential beneficial effects both on product quality as well as on the reduction of the risk factors associated with the consumption of cured meat. In particular, extracts obtained from grape and blueberry skin, as from olive, green tea and oregano plants (checked for the absence of naturally occurring nitrates) were used for the preparations of salami. At this purpose, both processed meat preparations and samples derived from the simulated gastro-intestinal digestion of the products were analysed and characterized in order to identify the effect of the natural antioxidants on meat quality (Fe-heme content, antioxidant activity, lipid peroxidation markers, polyphenol content, bioactive compounds, etc.). In addition, samples obtained by in vitro gastro-intestinal digestion were used to test the differential response of human intestinal cell lines to evaluate the toxicity and/or protective effects of the different formulations and related components. Data obtained indicated that the innovative formulations are characterized by a significant reduction of several risk factors, thus envisaging new strategies for innovative production of healthier processed meat products.

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Keywords: *processed meat products, in vitro simulated gastro-intestinal digestion, polyphenols, colorectal cancer, intestinal cells*

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D15

THE STUDY OF CAMELLIA SINENSIS PHENOLIC COMPOUNDS EFFECT ON QUALITY OF GOAT MILK YOGURT

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White tea is obtained from the the bud or first leaves of *Camellia Sinensis* that are plucked and dried with minimal processing giving it a light and delicate flavor that is more accepted in Europe than that of green tea. Currently there is a great public interest for white tea due to sensory and health benefits (high levels of phenolic compounds of which the most important are catechins and strong anti-mutagenicity action).White tea also exhibits some antimicrobial properties therefore its extracts can be use as natural preservatives for stabilizing food products.The purpose of the present study was to determine the effect of the addition of the *Camellia Sinensis* extracts to goat milk at the beginning of fermentation with *Lactobacillus bulgaricus and Streptococcus thermophilus* as yogurt starter culture on pH, total titrable acidity, viable cell counts and sensory properties. The White tea–goat milk yogurt samples were produced by adding the white tea aqueous extract in pasteurised milks at 0, 2.5, 5.0 and 10% (*v/v*) concentration and incubated at 42-44°C. The yogurt samples were then storage at 4-6°C for 21 days for further analysis (the antioxidant capacity by the "diphenyl picrylhydrazyl" (DPPH) and total phenolic content (TPC)). The results of this study show that the viable cell counts of lactic bacteria increased with the increase concentration of White Tea extract. The 10% addition of White Tea extract lead to a 2-folds higher TPC on day 0 and 7 in goat milk yogurt than in control samples but reduced the lactic acid production during the whole storage period. On the other hand the pH values were higher in all white tea–goat milk yogurt samples compared to respective plain-yogurts.Regarding the sensory testing analysis the results showed that all goat milk yogurts fortified with Camellia sinensis water extracts obtained better sensory profile compared to the classical yogurt.

Keywords: *Camellia sinensis (white tea), yogurt, phenolic compounds, viable lactic bacteria cells*

D16

ANALYSIS OF BIOACTIVE COMPOUNDS IN SEA BUCKTHORN SYRUP WITH THE AIM TO ASSESS IMPACT OF CONVENTIONAL AND MILD PRESERVATION TECHNOLOGIES

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Sea buckthorn (*Hippophae rhamnoides*) (SBT) is a dioecious shrub of the family *Elaeagnaceae* originated from Asia and East Europe. Various parts of SBT, especially berries, are used as raw materials for nutraceuticals and therapeutic drugs. Recently, the nutritional importance of the berries in the form of healthy drinks has been increasing in Europe and North America. Over 200 bioactive components have been found in SBT, of which many are investigated in berries. Berries are rich in ascorbic acid, carotenoids, tocopherols, flavonoids, essential fatty acids, minerals and organic acids. These compounds have biological and therapeutic activity, such as antioxidant, antibiotic, hepatoprotective, anti-tumoral and immunomodulatory activity.
Increasing consumers´ demand for nutrient-rich food and a "fresh-like" taste, has led to the development of new, mild processing technologies to enhance or substitute conventional ones using thermal processing for food preservation. Several non-thermal pasteurization methods, including application of High Hydrostatic Pressure (HPP) or Pulsed Electric Fields (PEF), have been developed to achieve microbiological reduction and, concurrently, maintaining the nutritional and sensorial value of food.
In this study, two varieties of sea buckthorn (Botanica and Leicora) were used to prepare fruit syrup. This product was treated by PEF, HPP, ohmic heating and conventional heating. The treated samples were then stored for 8 weeks in the refrigerator, in order to find differences caused by the preservation technologies or storage time. Contents of vitamin C, carotenoids and tocopherols were determined by high-performance liquid chromatography coupled to a diod array detector and fluorescent detector (HPLC-DAD/FLD). Metabolomic fingerprints of methanolic extracts of fruit syrups were obtained using ultra-high performance liquid chromatography coupled to tandem high-resolution mass spectrometry (U-HPLC-HRMS/MS). Neither preservation nor storage time had any apparent effect on the content of vitamin C, carotenoids and tocopherols, but significant differences were observed in case of metabolomics fingerprints. Moreover, all products, suitable for consumption, were sensorially evaluated by a test panel, where the HPP treated SBT syrup was rated as the most plausible.

Keywords: *sea buckthorn, pulsed electric field, ohmic heating, high pressure processing, metabolomic fingerprinting*

Acknowledgement: *Project "Volumetric preservation technologies for food quality improvement by retention of sensitive and mitigation of neoformed compounds (volTECH)", supported by CORNET (Collective Research Networking) and funded in the Czech Republic within the national Operational program Enterprise and Innovations for Competitiveness, support programme Cooperation - Clusters (CZ.01.1.02/0.0/0.0/16_079/0008876).*

D17

MONITORING OF FROZEN VEGETABLE QUALITY: CHANGES IN BIOLOGICALLY ACTIVE COMPOUNDS DURING LONG STORAGE OF CARROT

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The quality changes of processed and frozen vegetable were monitored using the metabolomic fingerprinting technique. Frozen vegetable products are becoming more and more attractive and available for consumers. Gentle and fast processing will lead to less loss of health related compounds as well as better sensory properties and texture. The study was focussed on the assessment of fresh, processed and frozen carrot (*Daucus carota*). Changes in the content of biologically active compounds were monitored in carrot processed by various technological processes, frozen by different technologies, and subsequently stored for a long time (up to 18 months).
Metabolomic fingerprints of methanolic extracts of freeze-dried carrot samples were studied using the ultra-high performance liquid chromatography coupled with quadrupole/time-of-flight tandem mass spectrometry (U-HPLC QTOF MS/MS). The obtained data were statistically processed, markers important for the separation of individual groups, representing different processing or storage, were selected and some of them identified. The identified metabolites belonged mainly to groups of organic acids, polyphenols, lipids (phospholipids and fatty acid), and saccharides. Processing such as cooking and steamblanching has the most significant impact on metabolome, changes during freezing are not so significant. Freezing and subsequent storage of frozen samples is suitable for long-term vegetable storage.

Keywords: *carrot, metabolomics, mass spectrometry, long term storage, fingerprinting*

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D18

DEVELOPMENT OF METHOD FOR ANALYSIS OF PHYTOCANNABINOIDS IN PLASMA OF LABORATORY ANIMALS: TOXICOKINETIC STUDY

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Although positive effects of phytocannabinoids on the human body, their fate in the organism has not been thoroughly described. The aim of the study was developed, optimize and validate based on ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC MS/MS) for the target analysis of 17 phytocannabinoids and three Δ9-THC metabolites in rat blood plasma. After addition acetonitrile to plasma sample and removing of precipitate by centrifugation, the samples were ready for analysis. The recoveries of phytocannabinoids ranged from 82-to111% and in case of THC metabolites from 113 to 121%. The repeat abilities of measurements expressed as RSD were in range 3% - 9%. The linear range was from 0.5 ng / ml to 250 ng / ml of plasma. The method was applied for analysis of 219 blood plasma samples of rats that were given pure cannabidiol (CBD) in the form of six different carriers' formulations to determine the bioavailability of CBD. The concentration profile of CBD after its administration was recorded. The lowest availability was observed for pure CBD. Using high-resolution mass spectrometry (HRMS), further screening of other phytocannabinoids and their metabolites was performed. Interestingly, number of phytocannabinoids and various phytocannabinoids metabolites was detected in rats´ plasma although pure CBD was administered.

Keywords: *cannabinoids, metabolites, rat plasma, UHPLC-MS/MS*

Acknowledgement: *Financial support from specific university research (MSMT No 21-SVV/2018) and BIOCIRTECH (TN01000048) project.*

D19

INFLUENCE OF UV-C TREATMENT OF SEEDLINGS ON ORGANOLEPTIC AND NUTRITIONAL PARAMETERS OF WATERMELON (CITRULLUS LANATUS)

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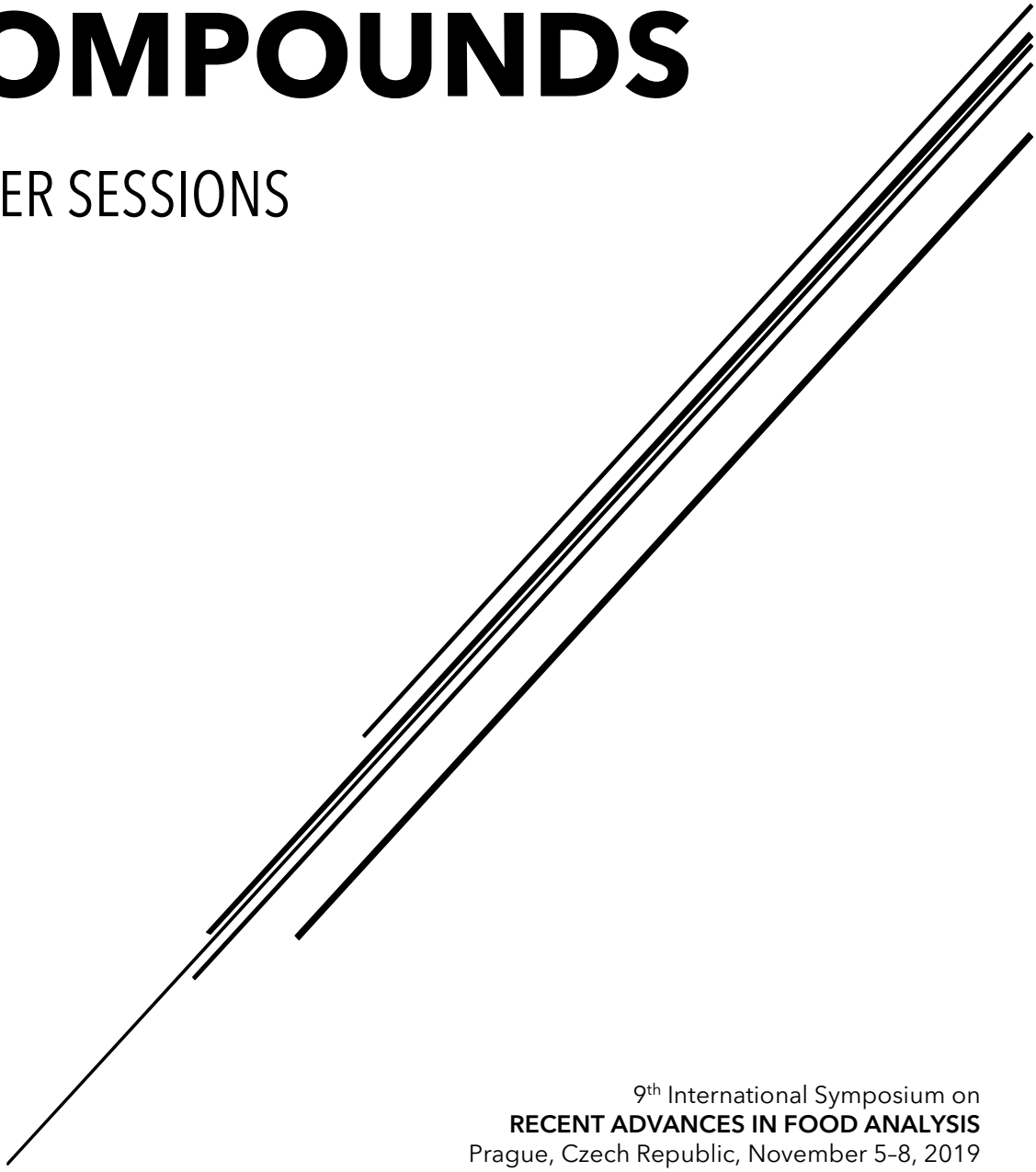
Watermelon (*Citrullus lanatus*) is an important horticultural crop highly cultivated around the word. Nowadays, this fruit has gained increasing popularity among consumers as it is a rich natural source of functional compounds. It has been shown that the treatment of food, including vegetables, with UV-C light may affect the concentration of some compounds and can catalyze oxidative changes, causing variation in secondary metabolism and on the synthesis of phytochemicals with nutraceutical activity. Little information is available on the effect of UV-C light on organoleptic and nutraceutical quality in watermelon. The objective of this work was to analyse the effect on the sensory-physical parameters and the nutritional quality of watermelon fruits obtained from seedlings treated with different doses of UV-C light. Watermelon seedlings were treated with six UV-C doses (control, 100, 200, 300, 400 and 500 Jm^{-2}). The color, texture, titratable acidity and the pericarp thickness were measured. After that, watermelon samples were extracted using appropriate extraction solvents. Hydrophilic extracts were analyzed for polyphenols and sugars by using HPLC-HRMS and HPLC-RI, respectively. Additionally, the antioxidant activities on the hydrophilic and lypophilic extracts were determined by ORAC, ABTS and DPPH assays. The total carotenoid content was also measured due to the importance of carotenoids in watermelon. The treatment of seedling with UV-C light did not affect significantly the titratable acidity, texture and pericarp thickness of the watermelon fruits. However, these UV-C treatments have a great impact on the watermelon color showing that samples from the seedling treated with the highest UV-C lights (400 and 500 Jm^{-2}) presented a colorful red tone. Regarding the antioxidant activity, the results shown that watermelon fruits from seedling treated with all the UV-C light presented high antioxidant capacity by the three methods comparing with the control. This increase in the antioxidant capacity was more pronounced when watermelon seedlings were treated with a dose of 100 Jm^{-2}. A similar trend was observed with the total carotenoid content and in the analysis of the individual sugar, being the watermelon fruits from seedling treated with 100 Jm^{-2}, the fruits that presented the highest concentration in glucose and sucrose levels. Moreover, the UV-C doses of 100 and 200 Jm^{-2} produced watermelon with increased quantities of hydroxybenzoic acids, hydroxycinnamic acids and flavonoids compared with the control. Our results highlighted the used of lower doses of UV-C light (between 100 and 200 Jm^{-2}) applied to watermelon seedling, could be a success treatment to obtain watermelons with increase nutritional properties by increasing its antioxidant activity with an increase on polyphenols and with a more sweetness pleasure due to the high concentration on sugars, all by preserving the rest of the sensory attributes

Keywords: *UV-C irradation, UHPLC-HRMS analysis, antioxidant activity, sugar profile, watermelon*

Acknowledgement: *To the project RTC-2016-5802-2 granted by the Spanish Ministry of Science, Innovation and Universities (National Agency of Research) and FEDER funds.*

FLAVOUR SIGNIFICANT COMPOUNDS

POSTER SESSIONS



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E1

CHARACTERIZATION OF THE VOLATILE PROFILES OF BEER USING IN-TUBE EXTRACTION–GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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Hops are one of the basic raw materials employed in brewing, which provide both bitterness and "hoppy" flavor to beer. The predominant classes of volatile compounds in beer are the various alcohols, esters, aldehydes, ketones, hydrocarbons and organic acids. Esters, especially the group of fatty acid methyl esters represent the flavor compounds which contribute to the fruity-flowery aroma of beers.
In this study, a chromatographic method was developed for the identification of volatile compounds from beer during the fermentation process. The study was performed on the comparative evaluation of beer volatile profiles, in traditional and flavored beers with Hüller Bitterer essential oil, by the identification of volatile markers. The volatile compounds were isolated using the in-tube extraction (ITEX) technique followed by identification and quantification through gas-chromatography–mass spectrometry (GC-MS).
The main volatile compounds identified in traditional beer were process markers, including aromatic compounds, aldehydes and alcohol esters. The most relevant volatiles from the flavored beer were classified as authentication marker compounds, especially terpenoids and acid esters.The ITEX-GC-MS analytical method used in this study gives good specificity and sensitivity and is suitable for characterizing the volatile fingerprint of different beers during the production process. Beers produced using the aromatization with essential oil are perceived as hoppier than traditionally hopped beer, by a sensory panel with fruity-flowery aroma.

Keywords: *beer essential oil volatile compounds in-tube extraction (ITEX)*

E2

EXPLORING THE PERFORMANCE OF VACUUM-HS-SPME FOR VOLATILE PROFILING OF OLIVE OIL

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Headspace solid-phase microextraction (HS-SPME) is an easy, effective and selective technique for the analysis of volatiles and semi-volatiles compounds. For the latter, long equilibration times are needed, which are typically shortened by e.g. applying agitation or heating the sample. A recent and less explored way to improve the extraction kinetics of analytes with a low-affinity for the headspace is to sample the headspace under vacuum conditions. The methodology was termed vacuum-assisted HS-SPME (Vac-HS-SPME) and was always found higher extraction efficiencies in much shorter sampling times. This sampling strategy has been formulated and thoroughly investigated for water-based samples. However, the applicability and behavior of Vac-HS-SPME sampling of the non-aqueous sample have never been studied before.
The aim of this work was to compare Vac-HS-SPME and regular HS-SPME sampling from non-aqueous samples. Extra virgin olive oil was selected as being an important ingredient of the Mediterranean diet with peculiar health benefits and sensory qualities. Extra virgin olive oil has a complex volatile aroma profile, which depends on several parameters (i.e., cultivar, geographical origin, fruit ripeness, processing practices, and storage). Past and current research efforts focus on unraveling the composition of this informative volatile fraction, so as to understand correlations with quality attributes. In the present work, the effects of extraction temperature and sampling time were investigated using traditional one variable at a time approach. Then, to better optimize the final extraction conditions and evaluate the temperature-time interaction, a two-variable central component design (CCD) was applied. A polydimethylsiloxane/carboxen/divinylbenzene (DVB/CAR/PDMS) fiber was used followed by gas chromatography-mass spectrometer (GC-MS). The results showed a great improvement in the extraction of semi-volatile compounds using Vac-HS-SPME leading to an enhancement in the information gained by the olive oil aroma fingerprint. The inherent complexity of the system requests further research to correlate thermodynamic conditions to the significant improvement in extraction conditions under non-equilibrium conditions.

Keywords: *vacuum assisted-solid-phase microextraction (Vac-SPME), volatiles, extra virgin olive oil, gas chromatography-mass spectrometer (GC-MS)*

E3

COMPARISON OF VOLATILE PROFILES OF DIFFERENT VARIANTS OF TOMATO-BASED HOMEMADE SOFRITO BY USING A NOVEL OPTIMIZED PROCEDURE

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Sofrito has been recently included in a reduced list of recommended foods for a healthiest Mediterranean diet. During the cooking process, new volatiles are generated mainly as products of oxidized carotenoids and Maillard reaction products whereas others are lost. The acceptability of consumers is strongly dependent of the aroma profile of food products. However, the volatiles extraction step is a complex procedure affected by different factors. In this study, six different *sofrito* formulations were analysed and compared with the raw recipe for their volatile profile. Thus, a novel headspace solid phase microextraction-gas chromatography mass spectrometry method was optimized (HS-SPME/GC–MS) by using a Box-Behnken design. The selected conditions that maximize the sum of areas of hexanal, trans-2-decenal, octanal and trans-2-heptenal were 0.5 grams extracted at 60 °C during 60 minutes without the addition of NaCl. Significant differences were observed in the profile of volatile compounds identified in the studied samples. In general, the cooking process improved the release of active and interesting compounds from the matrix reducing the degradation of *sofrito* sauce with an increase in their shelf-life. In addition, *sofrito* with rosemary presented values of hexanal and sum of aldehydes lower in comparison with another *sofritos*.

Keywords: *sofrito, volatile compounds, SPME*

E4

A HOLISTIC APPROACH FOR THE ANALYSIS OF FREE AND TOTAL COUMARIN IN MAHALEB AND TRADITIONAL FINE BAKERY WARES

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Mahaleb is a spice, coming from mahaleb cherry trees (*Prunus mahaleb)*, that owes its flavor to coumarin. Coumarin, a well-known phytochemical, is categorised as "active principle" (Council of Europe, 1981) and according to Regulation (EC) 1334/2008 is limited to 15 mg kg^{-1} in bakery ware, because of possible hepatotoxicity. To our knowledge, no previous research till now has investigated coumarin content in raw mahaleb and in bakery products containing mahaleb. A fit-for-purpose analytical method for quantitation of coumarin in bakery wares and spices via HPLC-DAD was optimised and validated according to ISO 17025 (LOD 1.5 mg kg^{-1}, LOQ 5 mg kg^{-1}, RSDr 3-5% (N=6), RSD_R 3-6% (N=12), Recovery 98-103%, $u_{expanded}$- 0,47-7,15 mg kg^{-1}). Liquid extraction of coumarin was achieved by methanol. Fine bakery wares (n=24) containing mahaleb as unique source of coumarin of 3 categories: commercial, home-made and from craft bakeries, were collected and analysed. Coumarin content in commercial products ranged from 2 to 20 mg kg^{-1}, while in hand-made samples from 7 to 109 mg kg^{-1}. Moreover, 4 authentic ground samples of mahaleb, 4 authentic samples in form of seeds and 7 commercial samples in both forms were analysed. It was found that seeds contain 216 mg kg^{-1} of coumarin in average, while ground mahaleb 426 mg kg^{-1}. This difference could possibly occur due to enzymatic reactions promoted from grinding process. Incubation with hydrochloric acid of mahaleb and bakery ware after extraction of coumarin was carried out to calculate the total coumarin, liberating it from its precursors and glucosides. It was found that 90% of coumarin was bound in mahaleb, whereas in bakery samples tested the free and total coumarin content didn't differ. Thus, coumarin formation is induced upon processing. Two parameters that could induce its release were studied: a) Yeast addition that show no effect and b) Temperature. It was found that coumarin release was induces above 160°C. The kinetics of its formation was studied at 160°C and 180°C. At 180°C, a maximum 40-fold raise of the content of coumarin in mahaleb, after 1h30 baking comparing it to its initial content was observed. After completing this research, it might be advisable to reconsider the coumarin limits for bakery products with mahaleb. The limit of 50 mg kg^{-1} (set by Regulation (EC) 1334/2008) for traditional bakery ware with cinnamon in their ingredient list could possibly be widened to also include traditional bakery ware containing mahaleb.

Keywords: *mahaleb, free and bound coumarin, processing parameters, HPLC-DAD*

Acknowledgement: *I need to thank HRIC laboratory and YIOTIS S.A. for providing me the necessary for carrying out this research during my Internship.*

E5

DEVELOPMENT OF A MULTIPLE-HEADSPACE-SPME AND GC-MS METHOD FOR THE DETERMINATION OF WHITE STURGEON (ACIPENSER TRANSMONTANUS) EGGS AND CAVIAR VOLATILE PROFILE AT DIFFERENT STAGES OF RIPENING

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In a scenario where the global market becomes more and more competitive and challenging, the sensory evaluation and the identification of key compounds responsible for the aroma of caviar are relevant issues for its organoleptic quality. In this study, a method for the determination of caviar volatile organic compounds (VOCs) using MHS-SPME coupled to GC–MS was developed, optimized and validated. The method showed the ability to identify and quantify VOCs in caviar in a relatively short time, without any sample preparation avoiding the formation of artefacts before the analysis. This method allowed a reliable estimation of the VOCs quantity solving the issue of the non-exhaustive extraction due to the SPME working principle. The protocol of MHE involves an exhaustive extraction of targeted analytes by a series of consecutive extractions performed on standard calibration mixtures. This step allowed the determination of the exponential decay of the chromatographic area for each analyte under consecutive extractions, which enabled the estimation of the cumulative chromatographic area for each analyte. Consequently, the chromatographic area of each compound obtained after a single extraction on real samples was used to estimate the total area, which was interpolated in a calibration curve obtained by a linear regression model built by injection of external standard solution. White sturgeon (*A. transmontanus*) eggs and caviar samples were provided by an Italian caviar company (Agroittica Lombarda SpA). Each set of samples was collected at different stages of production: raw eggs (t0, n=4), 60 days (t1, n=4) and 120 days (t2, n=4) of ripening, for a total of 12 samples. The caviar analysed was salted with 3.6-3.8% of NaCl and canned before the storage. Twenty-two key volatile compounds were detected in eggs and caviar samples, mainly represented by 6-, 8- and 9-carbon atoms aldehydes and alcohols (hexanal, octanal, nonanal, 1-octen-3-ol, 2-ethyl, 1-hexanol as most represented) and nonanoic acid, with some significant differences among t0, t1 and t2 samples. The total amount of detected aldehydes decreased from t0 (34.4ng/g) and t2 (27.9ng/g). On the contrary, we found a lower amount of alcohols in t0 (5.6ng/g) than in t2 (13.2ng/g) samples. Caviar collected in our work was constantly stored at -1°C in specific cans practically under vacuum, so it might be suggested that the restricted number of compounds detected by the analytical procedure was due to a partial inhibition of lipid oxidation processes that usually lead to the formation of VOCs. The identification and the quantitative analysis of compounds responsible for caviar flavour described in this research represents an innovation in the field, adding knowledge and providing novelty in deficiency of data available in literature to date, giving a substantial contribution in the characterization of such a precious product.

Keywords: *caviar, flavours, sturgeon, MH-SPME*

Acknowledgement: *The authors would like to thank Dr. Enea Beretta of Agroittica Lombarda SpA for his assistance with the collection and purchasing of caviar samples.*

E6

PROCESSING AND STORAGE EFFECTS ON INDUSTRIALLY PRODUCED ORANGE JUICE AROMA: GAS CHROMATOGRAPHY-OLFACTOMETRY STUDIES

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Since widely consumer favoured fresh hand-squeezed orange juice is an unstable matrix, thermal processing is required to reduce/eliminate enzyme and microbial activities. However, heat treatment can considerably affect the aroma composition of juice, and moreover, aroma-active volatiles can be further altered by subsequent time-temperature storage conditions.
As most of orange juice consumed worldwide is industrially processed, the aim of this study is to present results on orange juice aroma differences - at first in feedstock (raw defrosted juice originated from Costa Rica), afterwards in final juice products from two following production years (2018, 2019). Finally, to point out subsequent aroma changes due to the storage. All these aspects were dealt with special emphasis on output from a combined technique GC/FID-O studies. Analysed juice samples were processed in protective nitrogen atmosphere, and subsequently stored at set conditions during their four month-long shelf-life. The study demonstrated use of headspace-solid phase microextraction (HS-SPME) with GC-MS method, and in parallel, with combined GC-FID/O technique for complex characterisation of the overall aroma of orange juices. It is obvious from the results, that pasteurisation can significantly reduce levels of some reactive or/and thermolabile aroma impact compounds (majority of terpene alcohols, esters, aldehydes, ketones, sesquiterpenes) and to create off-flavours or their precursors via a complex of chemical reactions, particularly Maillard reactions, Strecker degradation of aminoacids, and acid catalysed hydration reactions. Off-flavours such as furaneol, 4-vinylguaiacol, methional, methionol are products of chemical reactions, while another off-flavour, for example guaiacol is an indicator of microbial contamination. In spite of the inert gas application during the whole juice processing, our results suggest that orange juice aroma compositions are affected to a certain extent by storage time with reactions within the juice matrices; the inherent acidic matrix of the orange juice produces acid-catalysed reactions which lead to a reduction of some aldehydes, ketones, terpene alcohols; while levels of furaneol (2018, 2019) and 4-vinylguaiacol (2018) were increased. However, GC-FID/O analyses proved that these changes were not significant sensorially to such a degree, which would result in a markedly worsening of organoleptic properties of analysed juices during the storage. However, a certain loss of citrus freshness, as well as typical orange sweetness were noticed in juice samples on the end of storage period (4th month).
Finally, from an overall aroma point of view, comparing results of analysed orange juice samples of two before mentioned production years indicates, that e.g. diverse climatic conditions in orange fruit production, as well as different contents of fruit pulp in juice products, can also affect significantly their qualitative organoleptic parameters.

Keywords: *orange juice volatiles, aroma quality, odour intensity, storage, gas chromatography-olfactometry*

Acknowledgement: *This contribution is the result of the project APVV-15-0023 „Quality and authenticity of fruit juices – study of relationships between the origin of feedstock, processing technology and quality of fruit juices" and of the project ITMS 26220220175 „Improvement of nutritional and sensorial parameters of fruity and vegetable drinks via inert gases application" implementation, supported by the Research & Development Operational Programme funded by the ERDF.*

E7

DYNAMIC HEADSPACE SAMPLING COUPLING WITH MULTIDIMENSIONAL GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC×GC-MS): APPLICATION TO BEER AROMA

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The most commonly used aroma analyses involve techniques based on headspace enrichment, which consists in the pre-concentration on a sorbent material of the vapor phase released from solid or liquid samples. These non-destructive methods are considered green sample preparation techniques, as they avoid the use of solvents, allow the isolation of VOCs in their natural form and are characterized by minimum sample preparation.
In the present contribution, a method using the most effective sampling (*i.e.* dynamic sampling), separation (*i.e.* multidimensional gas chromatography) and detection (*i.e.* mass spectrometry) techniques is proposed, showing its potential in unraveling aroma profiling in beverages (*e.g.* beer, herbal infusion).
In addition, a neat workflow for data analysis is discussed and used for the successful characterization and identification of different beer flavors, if the steps in the analytical process are properly controlled. From the technological viewpoint, this is the first time that a purge-and-trap (P&T), comprehensive 2D gas chromatography (GC×GC), and mass spectrometry (MS) are exploited in combination. A newly-thought flow modulation approach allowed for multidimensional 2D gas chromatography, with the entire eluate transfer onto the second dimension and the MS detector with no need to divert the flow, making the overall method highly sensitive and selective [2-3].

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Keywords: *mass spectrometry, comprehensive two-dimensional gas chromatography (GC×GC), dynamic headspace sampling, volatile organic compounds*

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E8

SHORT-TERM EFFECTS OF HIGH TEMPERATURE STORAGE ON VOLATILE PROFILES OF FRAGRANT RICE

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Aroma property greatly affects palatability and consumer acceptability of rice. Desirable volatile compounds such as 2-acetyl-1-pyrroline (2-AP) could be lost during long-term or unfavorable storage conditions. To understand changes in aroma property of fragrant rice under high temperature conditions, brown rice of 'Cheonjihyang-1-se', a recently developed fragrant rice variety, was stored at 45˚C for 7 days and daily changes in volatile compounds were determined by headspace solid-phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS). Identification of volatiles were conducted based upon retention index (n-alkane, C8 to C20) and mass spectral library (NIST 14) followed by deconvolution with Automated Mass Spectral Deconvolution and Identification System (AMDIS). During 7-days of storage, total 66 volatile compounds could be identified. According to pattern of quantitative changes, volatiles could be classified into 5 groups as followings: (1) newly produced (e.g., hexadecane), (2) continuously increasing (e.g., decanal which increased by 87% after 7-days of storage), (3) not significantly changing (e.g., p-xylene), (4) continuously decreasing (e.g., D-limonene which decreased by 72% after 7-days of storage), and (5) disappearing (e.g., camphene) after exposure to 45˚C. The amount of 2-acetyl-1-pyrroline, the most important fragrance-determining volatile in rice, decreased by 30%, 47% and 55% after 2, 4, and 6 days of storage under 45˚C, respectively. Partial Least Squares-Discriminant Analysis (PLS-DA) showed that fragrant rice of different storage durations at high temperature could be differentiated by using profiles of 66 volatiles identified in this report. All these results showed changes in volatiles of rice being affected by high temperature conditions, which may result in alterations in flavoring property and overall quality of rice.

Keywords: *flavor, rice, high temperature, volatile, 2-acetyl-1-pyrroline*

Acknowledgement: *This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ013280), Rural Development Administration, Republic of Korea.*

E9

GAINING INSIGHTS INTO THE COMPLEX CHEMISTRY OF CANNABIS AROMA

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In recent years, there has been rapid, worldwide growth of the cannabis industry due to changes in legislation for both medicinal and recreational use. This growth is driving demand for robust testing of the ever-expanding range of cannabis products – from the raw plant material to oils and edibles. The classification of terpenes and terpenoids is an important aspect of cannabis analysis, due to the distinctive aroma and flavour that they impart, as well as their contributions to physiological effects and psychoactivity. Specific terpene profiles can even be engineered by plant breeders in order to give the desired therapeutic effects.
Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS), enables the widest possible range of terpenes to be confidently identified across a range of sample matrices.
However, odour thresholds can differ by many orders of magnitude, so high sensitivity is also essential to capture trace-level, yet pungent, compounds. In this study, we will demonstrate the use of a new multi-faceted sampling platform incorporating trap-based secondary focusing to extend the performance of traditional sampling techniques – namely, headspace (HS) & headspace trap, solid phase microextraction (SPME) & SPME-trap, thermal desorption (TD) and high-capacity sorptive extraction.
The combination of these four techniques allows different sample preparations e.g. flower (typical form), resins, oils, brownies, cakes, coffees, gum, tinctures, gummies, etc, to be analysed for volatiles of interest on a single platform.
We will demonstrate the coupling of this multi-functional sampling platform with GCxGC-TOF MS for comprehensive characterisation of cannabis products.

Keywords: *cannabis, aroma, terpenes, sampling, GCxGC*

E10

COMPREHENSIVE AROMA PROFILING OF FOOD AND BEVERAGES BY GC×GC-TOF MS/FID/SCD

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Aroma profiles of food and beverages are composed of a broad range of chemical classes, including terpenes, phenolics, fatty acids, esters, lactones, aldehydes, as well as nitrogen- and sulfur-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.
In this study, we will demonstrate the use of trap-based secondary focusing to extend the performance of conventional sampling techniques (e.g. headspace and SPME) while retaining fully automated methods. Using real world examples, we will demonstrate unique, high-performance workflows including SPME-trap with enrichment and high-capacity sorptive extraction which allow significant improvements in profiling applications
Nevertheless, the aroma profiles are often highly complex, with important compounds, such as trace-level off-odours, frequently masked by higher-loading components. The enhanced separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) is now frequently used to tackle this challenge.
Here, we apply multi-hyphenated analytical system to obtain comprehensive aroma profiles. The use of parallel detection by three different techniques ensures that three complementary datasets are obtained from a single run:
- Robust quantitation of high-loading species by flame ionisation detection (FID)
- Highly-sensitive, confident identification of aroma-active species by time-of-flight MS (TOF MS)
- Highly specific detection of sulfur odour taints by sulfur chemiluminescence detection (SCD)
We will show that the result of using this multi-functional setup is confident aroma profiling and off-odour detection, with fully automated workflows and simple data processing.

Keywords: *aroma, TOF MS, GCxGC, beer, wine*

E11

AROMA PROFILING OF COFFEE WITH GC, GC×GC, AND TOF MS

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The coffee industry is an important part of the global economy and its final product is one of the most consumed beverages in the world. As expected with commodities, there is a large amount of taste and flavor variation in coffee that is related to differences in the variety and geographical origin of the beans, storage and processing conditions, roasting conditions, and brewing conditions, among other factors. In addition to the expected variation, the aroma profile for coffee is quite complex and comprised of a large number of individual analytes. An understanding of these analytes can be helpful for quality control, process optimization, and also for providing information on flavors that direct consumers to their preferred styles. Non-targeted chemical analysis techniques, like gas chromatography with mass spectrometry (GC-MS) and headspace solid phase micro-extraction (HS-SPME), are well-suited for this type of work. Volatile and semi-volatile analytes were collected from coffee samples, separated, and detected, resulting in identification and relative quantification information for hundreds of analytes. Analytes of interest do not need to be determined prior to acquisition, so the data were generally characterized to investigate the samples and their differences. Comprehensive two-dimensional gas chromatography (GCxGC) increases peak capacity with an additional complementary separation dimension and was also explored here. With GCxGC, more analytes were separated and this additional analytical capability led to an improved understanding of these complex samples.

Keywords: *beverage, GC-MS, GC-TOF MS, coffee aroma, profile*

E12

COMPARATIVE STUDY OF VOLATILE PROFILES OF BRAZILIAN ARBEQUINA OLIVE OIL USING HS-SPME-GC×GC-MS

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Volatile organic compounds (VOC) responsible by the aroma of olive oil result mostly from biochemical reactions during olive processing stage, mainly on malaxation. Multiple factors influence these VOC: olive variety, ripening and edaphoclimatic conditions on its cultivation. C6 aldehydes, alcohols and esters produced by the lipoxygenase cascade (LOX cascade), are the main sources of of green notes in olive aroma; their detection by conventional Gas Chromatography (GC) is challenging, and comprehensive two-dimensional gas chromatography (GC×GC) may be an attractive alternative. We evaluate here the VOC profile of oils from Arbequina olives produced in Brazil using GC×GC-MS. Six samples of extra virgin olive oil from different Brazilian regions were used: Divinolândia/MG (FI), São Sebastião da Grama/SP (FI6) and Maria da Fé/MG (EPA) from 2017-2018 crop, and São Sebastião da Grama/SP (FI19), Formigueiro/RS (OF), Consolação/MG (CM) from 2018-2019 crop. An optimized HS-SPME method was used: 2 g of sample were added to a 20 mL vial kept at 60 ºC and agitated at 200 rpm; the vial was incubated for 10 min prior to 50 min of headspace extraction with a SPME fiber. The extracts were analyzed on a lab-made GC×GC-QMS prototype based on a TQ8040 GC-MS/MS (Shimadzu, Kioto, Japão) fitted with a DB-WAX (D^1) + HP-17 (D^2) column set. The modulation period was 4 s and injector operated in splitless mode at 250 ºC. Hydrogen was used as carrier gas, column oven was programmed from 40 ºC (hold 2 min) to 150 °C, at 3 °C min^{-1}, and then to 250 °C, at 20 °C min^{-1}. The data was processed with GCImage software (Zoex, Houston, TX, USA). Principal component analysis (PCA) of the chromatographic data was carried out on Matlab software after retention time alignment. Results show that 3 principal components (PC) could 51.27% of the variance; PC2 showed a significant separation of the FI samples, which were produced and processed in the same region and mill compared to other samples, which were responsible for 16.18% of total variance. Graphical inspection of PC2 loadings helped to find the compounds that differentiated the samples. Between 75 and 160 compounds were detected depending on sample, and 117 compounds were identified with library match greater than 80%. Some 22 compounds were found only in FI samples, notably (E)-2-Hexen-1-ol and (Z)-4-Hexen-1-ol acetate, responsible for perception of green notes. Other 54 were associated to other samples, and may be related to the different producing regions or extraction procedures – since parameters such as temperature, time and oxygen exposition of olive paste's malaxation may vary. This study observed a regional factor in VOC that was more important than ripening or harvesting season.

Keywords: *comprehensive two-dimensional gas chromatography, extra virgin olive oil, monovarietal, volatile organic compound*

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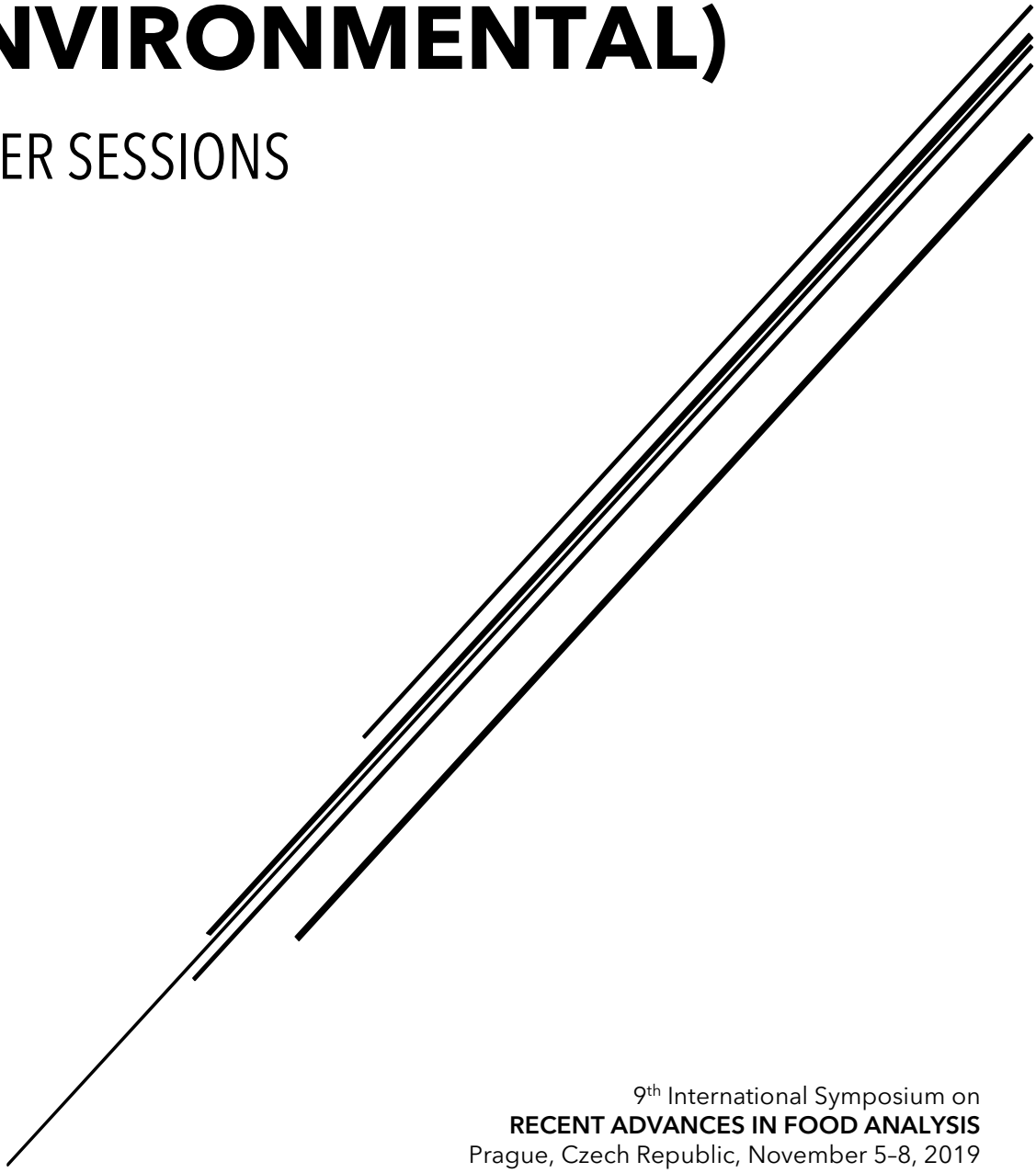
FOOD CONTAMINANTS (ENVIRONMENTAL)

POSTER SESSIONS



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F1

DEVELOPMENT OF EXTRACTION (SAPONIFICATION), CLEAN UP AND DETERMINATION OF MINERAL OIL SATURATED HYDROCARBONS (MOSH) AND MINERAL OIL AROMATIC HYDROCARBONS (MOAH) WITH ON-LINE HPLC-GC-FID ANALYSIS IN FOODSTUFF AND FEEDS WITH HIGH FAT CONTENT + DETERMINATION OF MOSH/MOAH MIGRATION FROM PACKAGING MATER

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The method developed in this research is used for the analysis of mineral oil saturated hydrocarbons (MOSH) and/or mineral oil aromatic hydrocarbons (MOAH) in food/feed with high fat/oil or fatty acid content. MOSH and MOAH fractions are isolated and separated by an on-line HPLC-GC-FID system. They are separated on a silica gel column using a n-hexane/dichloromethane gradient. A method for the determination of migration of MOSH and MOAH from packaging material to the foodsimulator matrix Tenax was also developed and validated

F2

ORGANIC POLLUTANTS AS QUALITY INDICATORS IN AGRICULTURAL APPLICATION OF BIOGAS PRODUCTION WASTES

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As an important prerequisite of sustainable future circular bioeconomy strategies, recycling of both non-renewable and renewable nutrients from organic residues will be important. In this context biogas production plays an ever-increasing role in providing sufficient energy for electricity, heating and transportation. Biogas production is based on carbon-rich substrate originating from sewage treatment, agricultural -, household- and aquaculture wastes. As part of the here propagated circular bioeconomy strategies, the application of remaining organic residues as soil amendment and fertilizer are expected to recycle the nutrients contained in the residues but may also imply a dispersal and accumulation of contaminants on agricultural soils. From soil, contaminants may be transferred into plants, ultimately resulting in animal and human exposure. The development of suitable production pathways for renewable energy production in recent years still do not take potential associated pollutant transfer sufficiently into account. Various technologies have been promoted and applied with the potential of uncontrolled emission of anthropogenic pollution. For instance, the use of biological (waste) material in anaerobic digestion, both as decentralized farm biogas plants as well as municipal plants for handling of, among others, organic household waste, has increased significantly in Europe and the North Americas. This development leads not only to an increasing amount of bioenergy produced, but also to a considerable amount of production waste to be handled properly (i.e., biogas digestate). The most attractive option to manage these digestates is to apply them as organic fertilizer to agricultural land allowing to recover nutrients, primarily nitrogen and phosphorus, and, in addition, potentially improving soil quality by adding organic matter. Unfortunately, such residues may also contain complex organic compound mixtures, salts, anthropogenic pollutants and/or pathogenic bacteria that can adversely affect terrestrial organisms and may accumulate in plants. Thus, the identification of potential pollutant sources and the development of suitable mitigation strategies are a prerequisite for the success of the currently propagated circular bioeconomy.

Keywords: *digestate, organic pollutants, transfer, soil, bioeconomy*

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F3

ARSENIC SPECIATION ANALYSIS USING AN AUTOMATED, SINGLE PLATFORM SAMPLE INTRODUCTION SYSTEM COMBINED WITH ICP-MS

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The influence of a metal ion or metal complex within an environment or biological system can be essential or toxic, depending on the species present. In the case of arsenic, the inorganic species (e.g. As(III) and As(IV)) have a higher bioavailability compared to the organic species (e.g. AsB, DMA, MMA, and AsC). Therefore, detecting the total amount of arsenic in a given sample is not enough in most cases to understanding the level of toxicity. The need to differentiate which arsenic species is equally as important. Arsenic is a naturally occurring element found in water which also accumulates in plants and animals. Many consumer products are thus at risk for elevated arsenic levels, including rice and rice products, apple juice, grape juice, wine, shell fish, seafood, etc. The following presentation will examine what is required for arsenic testing in apple and grape juice, seafood. These analyses were carried out using liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS). In addition, state-of-the-art of a novel inovatitive instrumentation that combines inline dilutions with chromatography will be presented as the LC portion of this instrumentation

Keywords: *arsenic, speciation, food safety*

F4

QUALITY AND RISK ASSESSMENTS OF KILISHI (DRY MEAT) SOLD IN SOKOTO METROPOLITAN

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Due to the process of drying, the dry meat is contaminated with heavy metals mainly through pollution either from the smoke released by vehicles that are passing by the road, burning of firewood, dust and mostly gutters which are also found by the road side. Different samples of wet and dry meat were obtained from different location in the metropolitan for this research, heavy metal analysis was conducted using Atomic absorption spectroscopy and the results obtained for wet samples in chromium 1.72, 0.35, 0.04 and 0.35 mg/100g, the concentration cadmium was 0.02, 0.04, 0.02 and 0.30 mg/100g, for copper the concentration were 3.20, 1.40, 0.54 and 0.24 mg/100g, Zinc content in the fresh meat are 0.40, 1.72, 2.65 and 0.34 mg/100g for sample A_1, B_1, C_1 and D_1 respectively. Nickel was detected in sample A_1 and D_1 with the values of 0.45 and 0.98 mg/100g, below detection limit was observed in the lead. The concentration of heavy metals in the dry meat were 2.25, 0.48, 0.05 and 0.64 mg/100g for chromium, 0.05, 0.06, 0.056 and 0.065 mg100g for cadmium, 5.72, 4.14, 1.72 and 1.24 mg/100g for copper, 1.77, 3.87, 8.99 and 2.81 mg/100g for zinc, in the samples of dry meat for A_2, B_2, C_2 and D_2, for nickel 1.95 and 3.20 were detected only in the sample A_2 and D_2 and lead content was below detection limit in all the dry meat samples. The results of the research confirmed the presence of heavy metals namely Zn, Cd, Cr and Cu in the analyzed samples of fresh and dry meats obtained in Sokoto. Cu and Zn exceeded the levels recommended by international organization. However, the concentration of Cd, and Cr were below the tolerance limits. The consumption of meat in which metal concentrations beyond the tolerated limits detected might be harmful to the health of consumers, those below the tolerance limit though not harmful but might pose health hazards when consumed in large quantities due to bioaccumulation.

Keywords: *kilishi, dry meat, fresh meat*

F5

EXPOSURE ASSESSMENT OF THE POPULATION IN SAUDI ARABIA TO THE TOXIC EFFECTS OF ARSENIC SPECIES, CADMIUM, LED AND MERCURY IN RICE.(ARSENIC SPECIATION METHOD)

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Food quality is a primary concern that has led to the introduction of rigorous legislation setting maximum levels of contaminants in foodstuffs. Heavy metals are strictly regulated because their consumption in food is correlated with some serious health conditions. The objective of the survey is Exposure assessment of the population in Saudi Arabia to the toxic effects of Arsenic species, Cadmium, Led and Mercury in Rice. Inorganic As, Total As, Hg, Pb and Cd has detected by two different methods. The samples are treated by Nitric acid and hydrogen peroxide solution to extracted inorganic arsenic (iAs).Anion exchange will selectivity separated inorganic arsenic from the other arsenic compounds .HPLC coupled to ICPMS to determination of mass fraction of iAs . Also the samples digested by Nitric acid to detect Total As, Cd,Hg and Pb by ICPMS . Total arsenic and its forms (inorganic, organic) examined in 31 samples of rice products that are available on the Saudi market. Average content of total arsenic in investigated rice samples was 0.115mg/kg. The content of inorganic arsenic in rice samples was lower - mean: 0.03 mg/kg, than for organic As - mean: 0.075 mg/kg. From the total arsenic 66% was organic As which be considered as safe to consume compare to iAs. Inorganic arsenic concentration in rice samples depending on origin was in the range 0.017mg/kg - 0.103 mg/kg at India. While rice imported from Pakistan were between 0.09 mg/kg – 0.175 mg/kg. The average value of rice samples from Egypt, Thailand and Italy were (0.025, 0.041 and 0.031 mg/kg respectively. Most prevalent heavy metal detected in the rice samples was lead, Cadmium and Mercury. Pb levels ranged from below detection limits up to 0.02 mg/kg .Mercury was also found in all of the samples tested ranging from 0.0007 to 0.04 mg/kg. Cadmium concentrations was below MRL with average 0.018 mg/kg.Reported in the present study results for inorganic arsenic Total As, Cd, Hg and Pb in rice samples were significantly lower than the permitted maximum level set by Commission Regulation (EU) 2015/1006 as well as in FAO/WHO standards.

Keywords: *rice, inorganic As, heavy metals*

Acknowledgement: *would also like to acknowledge with appreciation to Dr Jens Jørgen Sloth Head of Research Group, Senior Researcher at Technical University of Denmark whose contribution in developing the arsenic speciation method*

F6

AUTOMATED CLEAN-UP OF POLYCYCLIC AROMATIC HYDROCARBONS IN SUNFLOWER OIL FOR GC-MS

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Food samples need to be analyzed for unwanted compounds like polycyclic aromatic hydrocarbons to ensure consumer safety. Therefore, the analytes need to be extracted from the sample matrix and cleaned-up in a way that enables the injection to a suiting instrument like a gas chromatography mass spectrometer (GC-MS). The Solid phase extraction method EZ-Pop NP has been successfully shown to clean-up polycyclic aromatic hydrocarbons in oil, prior to injection to mass spectrometry instruments. However, clean-up by EZ-Pop NP solid phase extraction is labor intensive and uses 15ml acetonitrile for the elution-step of each column. Here we show a new developed micro-SPE method, also called mini-tube, to automate the clean-up of polycyclic aromatic hydrocarbons in sunflower oil using a GC-EI-Q-Orbitrap system with a TriPlus RSH autosampler. As the method is automated, the labor is reduced. In addition, the mini-tube needs only 4µl of sample and 360µl acetonitrile for elution, and therefore significantly less organic solvent than the original EZ-Pop NP method. Our results add up to already developed automated mini-tube methods, whereas all prior published methods analyzed QuEChERS-extracts in acetonitrile.[1–4] Our developed method is therefore the first automated mini-tube method analyzing a pure sample. Furthermore, we show how a complex SPE method can be transferred to the mini-tube concept. Our LOQ values are exceeding the regulations, but we discuss how sensitivity can be increased for future applications of screening for PAH levels.

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[4] Y. Sapozhnikova, J. Chromatogr. A 2018, 1572, 203–211.

Keywords: *PAH, edible oil, µSPE CLean-up, QuEChERS, PAHs*

F7

PERFLUOROALKYL SUBSTANCES IN BOAR LIVER – INFLUENCE OF NEW EFSA TOLERABLE WEEKLY INTAKES FOR PFOA AND PFOS ON HUMAN CONSUMPTION

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In 2018 EFSA published new health-based guidance values for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in food. For PFOA a tolerably *weekly* intake (TWI) of 6 ng/kg body weight (bw) and for PFOS a TWI of 13 ng/kg bw was established [1]. These values are much more stringent than the former tolerable *daily* intakes (TDI) of 1500 ng/kg bw for PFOA and 150 ng/kg bw for PFOS, respectively [2].
In 2014 and 2018 the occurrence of 27 perfluoroalkyl substances (PFASs) was analysed in a total of 44 samples of boar liver and 16 samples of boar meat from the south-western part of Germany. In all liver samples a broad spectrum of perfluoroalkyl carboxylic and sulfonic acids were detected with concentrations of PFOA and PFOS ranging between 2 and 820 µg/kg. In 88% of the meat samples, however, no PFASs were detected. As national or European maximum levels for PFASs in foodstuff are missing an assessment of PFOA and PFOS concentrations was performed based on the EFSA guidance values.
The results show that by applying the former TDI for PFOA consumption of at least 47 kg per day of the lowest contaminated liver sample (2 µg PFOA/kg) exceeds the TDI (applies for an adult with 70 kg bw). In contrast to that consumption of only 190 g per week of the lowest contaminated liver sample leads to an exceedance of the current TWI for PFOA (applies for an adult with 70 kg bw). Furthermore, consumption of only 1 g per week of the highest contaminated liver sample (820 µg PFOS/kg) leads already to an exceedance of the current TWI for PFOS (applies for an adult with 70 kg bw).
Due to the high concentrations of PFASs in boar liver and the new EFSA TWIs consumption of boar liver is not recommended.

Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food, Scientific Opinion of the Panel on Contaminants in the Food chain, EFSA Journal 2018;16 (12):5194
European Food Safety Authority; Perfluoroalkylated substances in food: occurrence and dietary exposure, EFSA Journal 2012; 10(6):2743

Keywords: *environmental contaminants, PFAS, human consumption*

F8

UTILIZING 624 GC COLUMN SELECTIVITY AND LOW BLEED STATIONARY PHASE FOR THE ANALYSIS OF PURGEABLE VOLATILE ORGANIC COMPOUNDS BY GC-MS

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Volatile organic compounds (VOCs), both synthetic and naturally-occurring are a global environmental concern and can be found in a variety of samples including waste water, drinking water, and ground water. The traditional approach to VOC analysis is GC-MS (Gas Chromatography coupled to a Mass Spectrometer) using purge and trap extraction or an alternative extraction technique based on regulatory adherence. These compounds can potentially cause harm to human health as well as the surrounding environment. As a result, regulatory agencies have set standards for monitoring VOCs in all samples regardless of water source. Due to the varying compositions and range of volatilities of VOCs, a highly selective GC column is required for analysis to provide optimal separation of critical VOCs in a short run time. Additionally, the use of a Mass Spec certified GC column is necessary to reach top performance criteria. An added challenge is method capability to handle various sample matrices as dirtier samples such as wastewater and sludge can greatly decrease column lifetime. The present work provides improvement in separation and baseline to EPA Method 8260 for Volatile Organic Compounds. The highly selective ZB-624PLUS stationary phase allows for identification and quantification of 74 VOCs, while maintaining a rapid analysis time of 15 minutes. The high temperature resistance of 300/320 ^{0}C and low bleed provides from ZB-624PLUS GC column provides excellent performance and peak shapes of critical VOCs even after 113 injections of various matrices including groundwater, wastewater, and sludge.

Keywords: *VOC, 624, EPA 8260, drinking water*

F9

PER AND POLYFLUORINATED ALKYLSUBSTANCES (PFAS) ANALYSIS IN DRINKING WATER, SEDIMENTS, AND FOOD SAMPLES BY QUECHERS, SPE, AND LC-MS/MS

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Per and Polyfluorinated Alkylsubstances (PFAS) are a class of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications including surface treatment for textiles, packaging materials, non-stick cookware, and fire-fighting foams. PFAS are characterized by a hydrophobic fluorinated alkyl chain and a hydrophilic functional group. They are persistent in the environment due to the exceptional stability of the C-F bond. These have been detected throughout the global environment, food products, even human plasma. PFAS are associated with various adverse health effects, they are bioaccumulative, ubiquitous, and their analysis level requirements are very low to account for an expected lifetime of exposure. There are several methods available for the extraction and analysis of PFAS in aqueous samples. However, very few procedures are available for extracting these compounds in solid matrices such as sediments and food samples. Presented are three methods making use of various sample preparation techniques for the analysis of PFAS. The methods include, direct inject technique for drinking water, QuEChERS for sediment samples, and QuEChERS followed by SPE for food samples (milk, eggs, and fish tissue). All are validated LC-MS/MS procedures.

Keywords: *PFAS, fish, LC-MS/MS, QuEChERS, dairy*

Acknowledgement: *Syljohn Estil at Sanitation Districts of Los Angeles County and Agustin Pierri at Weck Laboratories*

F10

DETERMINATION OF TOTAL ARSENIC AND WATER-SOLUBLE ARSENIC SPECIES IN THE BFR MEAL STUDY

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Arsenic is ubiquitous in the environment and occurs in both inorganic and organic form. Due to the toxic potential of inorganic arsenic (iAs), the European Food Safety Authority (EFSA) recommends minimizing the intake and called for more data on arsenic species in food to improve nutrition-based exposure assessment. Total arsenic, iAs, dimethylarsinic acid (DMA) and arsenobetaine (AsB) were determined in foods as part of the BfR MEAL Study (meals for exposure assessment and analytics in foods), which is the first Total Diet Study in Germany.
Determination of total arsenic was performed for 870 samples by inductively coupled plasma mass spectrometry (ICP–MS) after microwave digestion. During sampling, regionality, seasonality and type of production for more than 350 foods were taken into account. Water-soluble arsenic species were first extracted with trifluoroacetic acid and hydrogen peroxide at 95 °C and under repeated sonication and subsequently measured by a coupled system of anion exchange high performance liquid chromatography with ICP-MS / MS (HPLC-ICP-MS / MS) using aqueous malonic acid as mobile phase. To assess extraction efficiency, the content of total arsenic in the extracts was determined. Since AsB is not fully resolved by the anion exchange method, samples that showed signals at low retention times were subjected to cation exchange HPLC-ICP-MS / MS using aqueous pyridine as mobile phase. According to the Commission Regulation (EU) No 2015/1006 arsenic species were determined in varieties of rice and rice-based products. Fish, fish products and seafood (mussels, shrimp and prawn, squid and octopus) were determined with particular focus on AsB as its most prevalent organic water-soluble arsenic compound which is known to be virtually non-toxic for humans. In addition, samples with an arsenic amount more than 0.01 mg/kg or 0.05 mg/kg for wet or dry foods, respectively, were also subjected to speciation analysis.
In 360 samples (41 % of all samples), the amount of total arsenic was below the limit of quantification (LOQ: 0.002 mg/kg for wet foods and 0.01 mg/kg for dry foods). The highest arsenic amounts were determined in fish, fish products and seafood from 0.01 mg/kg up to 6.4 mg/kg. As expected, AsB could be identified as predominating water-soluble arsenic species within these samples. In rice and rice-based products as well as other selected foods, iAs and DMA could be determined. No rice sample violated legal limits of iAs (non-parboiled milled rice: 0.20 mg/kg; parboild rice: 0.25 mg/kg; rice wafers: 0.30 mg/kg; rice destined for the production of food for infants and young children: 0.10 mg/kg).

Keywords: *total arsenic, total diet study, inorganic arsenic*

F11

DETERMINATION OF DIOXIN CONCENTRATIONS IN FISH BY GAS CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Fishery products have been identified as the main source of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs), which are collectively referred to as dioxins, in the Japanese diet. Gas chromatography–high-resolution mass spectrometry (GC-HRMS) is the most widely used method for determining dioxin-concentrations. This method is highly sensitive and selective; however, it requires expensive instruments and highly trained analysts. Recently, gas chromatography–tandem mass spectrometry (GC-MS/MS), one of the less expensive analytical methods, has been used to determine dioxins in foods. However, analytical performance data obtained using GC-MS/MS with fish are very limited. We therefore evaluated the performance of a GC-MS/MS method when analyzing dioxins in fish samples.
We used an Agilent 7890A/7000B gas chromatography tandem mass spectrometer. The instrument limits of detection of the spectrometer ranged from 0.08 to 0.5 pg for injected PCDD/Fs and 0.07 to 0.1 pg for injected DL-PCBs. These values were 3-32 times higher than those with GC-HRMS. We initially analyzed a certified
Referencefish sample (WMF-01) using the present method. The concentrations of the certified
Referencedioxin congeners were within the uncertainty limits of the certified
Referenceconcentrations. The present method was also evaluated by analyzing 14 fish samples, and the dioxin concentrations were compared with those determined using GC-HRMS. The concentration ratios of the two methods for almost all quantified congeners were 0.8-1.2, indicating that the concentrations of the dioxin congeners were very similar with both methods. The toxic equivalent (TEQ) concentrations for the fish samples ranged from 0.78 to 4.6 pg TEQ/g with GC-MS/MS and 0.79 to 5.2 pg TEQ/g with GC-HRMS. The TEQ concentrations determined by both methods correlated very well (r = 0.99).
Overall, our results indicate that GC-MS/MS is a useful method for determining dioxin concentrations in fish, although GC-MS/MS was somewhat less sensitive than GC-HRMS.

Keywords: *Dioxins, fish, GC-MS/MS, GC-HRMS*

Acknowledgement: *This work was supported by a Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan.*

F12

ANALYSIS OF PERFLUOROALKYLATED SUBSTANCES IN WATER, PART B: OCCURRENCE IN TAP WATER IN THE CZECH REPUBLIC

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Per- and polyfluoroalkylated substances (PFASs) as a group of potentially harmful environmental pollutants are currently in the center of interest of health and safety authorities. In 2018, the European Food and Safety Authority (EFSA) issued new values of a tolerable weekly intake (TWI) for perfluorooctane sulfonate (PFOS) – 13 ng/kg bw per week and perfluorooctanoic acid (PFOA) – 6 ng/kg bw per week (1). Based on the EFSA opinion, together with fish and seafood products a drinking water was classified as one of the most important contributor to the mean chronic exposure to some PFASs. The main aim of our study was to investigate of a large set of tap water samples originated from various localities in the Czech Republic. Based on obtained data, the daily intake of PFAS via water was estimated and evaluated with respect to the recent TWIs. For the analysis a total of 24 PFASs, the extraction method based on SPE followed by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was validated and then applied for the investigation of 57 samples of samples collected during 2018 and 2019. The most abundant contaminants were perfluorocarboxylated acids (PFCAs) represented by PFOA (in 96% of samples, median 0.42 ng/L) followed by perfluorononanoic acid (PFNA, median 0.27 ng/L) and perfluorodecanoic acid (PFDA, median 0.39 ng/L) in more than 86% of samples. PFOS isomers were found in only 50% of samples in concentration ranging from < 0.02-16.3 ng/L. Nevertheless, the similar occurrence and concentrations were determined also for other target analytes – perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS). The amount of PFASs in tap water samples collected in various places (e.g. households) but originated from the same water supply was almost identical. Regarding the comparison of results in water from various supply area (e.g. town, countryside, mountains, wells) the occurrence of PFASs differs significantly. Based on the calculations of daily intake for the average consumer (70 kg body weight, 2 liters) it was estimated that TWI was double exceeded for PFOA by consuming of water with the highest concentration (51 ng/L). Regarding to PFOS, the TWI is filled by 70% by drinking of water from the area with the highest PFOS content (2.8 ng/L). Nevertheless, using the average occurrence of PFOS and PFOA in other samples (without the highest amounts), the TWI is filled by 2%. In conclusion, until now there has not been any data available on the PFASs occurrence in drinking water in the Czech Republic. This study shows that the drinking of commonly available tap water can significantly contribute to overall exposure to PFASs.

1. EFSA PANEL ON CONTAMINANTS IN THE FOOD CHAIN (CONTAM), et al. Risk to human health related to the presence of PFOS and PFOA in food. EFSA Journal, 2018, 16.12: e05194.

Keywords: *PFOA, PFOS, tap water, UHPLC-MS/MS*

Acknowledgement: *Financial support from specific university research (MSMT No 21-SVV/2019).*

F13

LONG-TIME MONITORING OF QUATERNARY AMMONIUM COMPOUND CONTAMINATION IN AUSTRIA: ARE THERE STILL REASONS FOR CONCERN?

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Quaternary ammonium compounds (QACs) are widely used as disinfectants in the food industry due to their antimicrobial activity. Main areas of application are the dairy and meat industry, small-scale ice-cream producers and other food businesses dealing with milk-based foodstuffs. Since 2014, a maximum residue limit of 0.1 mg/kg for the two main groups of these substances (benzyalkyldimethylammonium chlorides and dialkyldimethylammonium chlorides) is effective in the European Union. Over many years, the Austrian Agency for Health and Food Safety has been monitoring these contaminants in different groups of animal-based food (milk, ice cream, sausages, etc.). This poster will give an overview on the trends that are visible in the long-time monitoring and thus whether and how official controls have improved the situation of QAC contamination over several years. Despite the fact that the topic of QAC contamination has been in the limelight for some time, cases of extremely high-contaminated samples still occur. A case of a milkshake from a fast food restaurant, which was harmful to health, will be presented.

Keywords: *quaternary ammonium compounds, contaminants, animal-based food, monitoring, data*

Acknowledgement: *Eveline Dorn, Barbara Steffl, Anton Turkowitsch*

F14

CHEMCOCK: THE MODIFIED REFEREN CEPOINT INDEX (MRPI) - AN APPROACH TO ASSESS RISKS OF FOOD CONTAMINANT MIXTURES

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People are exposed to a variety of potentially harmful food contaminants via their daily diet. Risk assessment of such chemical cocktails still represents a challenging task. Especially regarding food contaminants, for which comprehensive toxicological data are often not available, existing methods for mixture risk assessment are not perfectly suited. The project ChemCock aimed to deal with these difficulties and to present first calculations of associated risks for the Austrian population.
One main aim of the project ChemCock was to determine a suitable and applicative approach for the risk assessment of food contaminant mixtures as standard method for risk assessors of food safety authorities or industry.
Several existing methods for cumulate risk assessment were evaluated regarding their applicability to food contaminant cocktails.
We evolved a new approach, the modified reference point index (mRPI), which combines the advantages of two standard methods, the Hazard Index and the Reference Point Index. Furthermore, we established a decision tree for the determination of specific uncertainty factors, by which the mRPI becomes an easily applied method for cumulative risk assessment even in a data poor field like food contaminants.

Keywords: *risk assessment, contaminants, chemical mixtures, combined exposure, modified*

Reference point index

Acknowledgement: *The research was funded by the Austrian Federal Ministry of Labour, Social Affairs, Health and Consumer Protection.*

F15

CHEMCOCK: SOME EXAMPLES FOR CUMULATIVE RISK ASSESSMENT OF CHEMICAL MIXTURES OF CONTAMINANTS

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Within the national project "Risk Assessment of Chemical Cocktails in Food (ChemCock)", the Austrian occurrence data on contaminants in food and consumption data were analyzed in order to assess the cumulative risk for the Austrian population groups of children and adults.
After the method of the modified
Reference point index (mRPI) has been established, the approach was used to estimate the cumulative risk to contaminant mixtures in food.
Estimation of average and high concentrations of the individual contaminants or contaminant groups in food was based on the lower bound - upper bound approach. For adults and children, two scenarios each for average and high consumption were calculated to assess the dietary exposure assuming that all contaminants tested in the respective food categories co-occur with each other. The mRPI was applied to estimate the cumulative risk for the cumulative assessment groups (CAG) of nephrotoxicity and neurotoxicity.
For adults and children, the mRPIs exceeded the comparative value of 1 in the CAG for nephrotoxicity in each exposure scenario; this CAG represented the highest risk, with cadmium contributing the most to the cumulative risk of nephrotoxicity.
In the CAG for neurotoxicity, the mRPIs at average intake were below or in the range of 1 indicating a low risk for children and adults. At high consumption, the mRPIs exceeded the comparative value in both population groups; indicating some risk with acrylamide being the main contributor to the cumulative risk.
For all those scenarios for which a risk could not be excluded in this first cumulative risk assessment, further refinement of the risk assessment should be carried out.

Keywords: *risk assessment, contaminants, chemical mixtures, combined exposure, modified reference point index*

Acknowledgement: *The research was funded by the Austrian Federal Ministry of Labour, Social Affairs, Health and Consumer Protection.*

F16

TEMPORAL TRENDS IN LEVELS OF DDT AND ITS METABOLITES IN EDIBLE MARINE SPECIES FROM THE BLACK SEA COAST, BULGARIA

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Organochlorine compounds (like DDT and its metabolites) are persistent and can accumulate in fatty tissue and this fact defines fish as an appropriate species for environmental pollution monitoring. Concentrations of DDT and its metabolites DDE and DDD were measured in marine fish, collected from Bulgarian Black Sea coast in 2007, 2009, 2011 and 2015 in order to investigate the temporal trends of these pollutants. The indicator fish species: goby *(Neogobius melanostomus),* sprat *(Sprattus sprattus sulinus),* horse mackerel *(Trachurus Mediterraneus ponticus)*, grey mullet *(Mugil cephalus)* and bluefish *(Pomatomus saltatrix)* were chosen in accordance Qualitative descriptor 8 (Marine Strategy Framework Directive). The organochlorine pesticides were determined in tissue of fish species by gas chromatography system with mass spectrometry detection (GC/MS Ion Trap).
The total DDTs (as sum of DDE, DDD and DDT) ranged from 3.48 (goby 2015) to 224.28 ng/g wet weight (bluefish 2009). The ratio of most persistent metabolite p,p-DDE to total DDTs was found over 0.6 in all investigated species and suggested a lack of recent inputs of DDT pollution. Levels of DDTs in goby, sprat and grey mullet significantly decreased in 2011 and 2015. The dietary intake of DDTs through fish consumption was estimated in order to assess human health risk.
The climate change is expected to increase toxic emissions from warm zones and atmospheric transport will intensify exposure in northern food chains. Although the temporal trends showed decreasing of DDTs levels, monitoring of these compounds in the future is necessary.

Keywords: *DDTs, fish, Black Sea, Bulgaria*

F17

CHARACTERIZATION OF C10-C17 CHLORINATED PARAFFINS IN OVEN-BAKED PASTRY PRODUCTS AND UNPROCESSED PASTRY DOUGH BY HPLC-ESI-Q-TOF-MS

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Chlorinated paraffins (CPs) are structurally complex mixtures of polychlorinated n-alkanes with varying chain lengths and degrees of chlorination. CPs are classified according to their carbon chain lengths: short-chained CPs (C_{10}–C_{13}, SCCPs), medium-chained CPs (C_{14}–C_{17}, MCCPs), and long-chained CPs (C_{18+}, LCCPs). CPs have a broad range of industrial uses, predominantly in the metalworking field and polyvinyl chloride processing, as well as plasticizers and flame retardants. Despite classification of SCCPs as POPs, the annual worldwide production of CPs remains high (~ one million tons per year). CPs are chemically stable and highly lipophilic compounds and over the past decades CPs have been reported with alarming frequency in almost all environmental matrices. CPs can biomagnify through food webs and display endocrine-disrupting characteristics.
Exposure to CPs poses a potentially major threat to human health while several studies have assessed the presence of CPs in domestic items (e.g., household baking oven doors and kitchen hoods), showing that significant emission sources of CPs are present in the kitchen environment. These observations suggest that migration of CPs might occur during food preparation and contribute to an additional dietary exposure to CPs.
Despite the instrumental advances, analysis of CPs remains a challenging task due to thousands of possible congeners. Gas chromatography (GC) in combination with electron capture negative ion (ECNI) low-resolution mass spectrometry (LRMS) has been the commonly applied analytical technique for over two decades. However, an obvious drawback for applications of LRMS is its lack of selectivity because other organochlorine contaminants and CPs themselves can cause interfering MS peaks. In order to eliminate this issue, several studies have relied on high-resolution MS (HRMS). As of now, the majority of recent CP detection methods relay on GC coupled to time-of-flight MS (TOF-MS) or Orbitrap-MS techniques to separate and characterize CPs. Moreover, some studies reported that HPLC techniques can be applied to chromatographic separation of CPs, thus making the analysis of CPs even more accessible for the scientific community.
In our study, we present an easily applicable HPLC-Q-TOF method to analyse CPs without any additional use of ionization enhancers (e.g., DCM). The aim of this study was to assess the levels of SCCP contamination in thermally processed foods, in this case oven-baked pastry products. The acquired SCCP and MCCP homologue profiles were compared to unprocessed dough. Data of this type can lead to further insights regarding CP transformations occurring during the food preparation and provide complementary information about possible contamination sources.

Keywords: *SCCP, MCCP, HPLC-ESI-Q-TOF-MS, oven-baked pastry products, unprocessed pastry dough*

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F18

MICROPLASTIC PRESENCE AND CHARACTERIZATION IN MUSSELS AND FRESHWATER FISH: STUDY ON THEIR POTENTIAL ROLE AS VEHICLES OF CHEMICAL CONTAMINANTS

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Microplastics (MPs) are increasingly documented in aquatic environments and their trophic transfer along food webs is raising concern for food safety. Scientific findings confirmed a wide diffusion of MPs in biota, highlighting potentially adverse effects on nutritional value, inflammatory reactions and effects at molecular and cellular levels, up to modulation of physiological functions. MPs can also act as vectors of hydrophobic pollutants adsorbed from water and released after MPs ingestion by aquatic organisms.
The main aim of this study was to characterize MPs occurrence in freshwater fish and marine mussels of commercial interest. Levels of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and trace metals were also measured, to investigate the role of MPs as potential vehicles of these contaminants.
Fish species (*Perca fluviatilis, Rutilus rutilus, Abramis brama* and *Carassus auratus*) were collected from three Italian Lakes (Piediluco, Corbara and Trasimeno), while *Mytilus galloprovincialis* were obtained from six sites (including both aquacultures and field locations) identified along to the central Adriatic coast. For MPs extraction and characterization, gastrointestinal tracts of fish and the whole tissues for mussels were processed applying a validated procedure. Extracted particles were microscopically observed, photographed, measured through a stereomicroscope, and categorized according to both size classes and shapes. Polymer typology of extracted particles was assessed using a μFT-IR microscope [1].
6-NDL-PCBs and 15PBDEs were analyzed by GC-MS/MS in isotopic dilution [2,3]. Pb, Cd, As, Ni, Mn Cr, Co, Zn and Cu were determinate with ICP-MS after microwave digestion, while Hg was analysed by Cold Vapor Atomic Absorption spectroscopy.
Main results suggested that ingestion of MPs is a widespread phenomenon also in the freshwater environment, with the 50% of fish and 25% of mussels positive to their ingestion. In particular, freshwater fish showed a higher frequency of ingestion respect to the marine ones (50% vs 30%). Moreover, in these species extracted MPs are mainly lines shaped and polyester (PEST) made and mostly related to domestic discharge, in contrast to the marine ones where ingest MPs are mainly fragments made of polytene (PE), originated by mechanical and physical fragmentation of macroplastic items.
PBDEs and PCB were below the method LOQs in all the samples of *C. auratus analysed*, while detectable levels were measure in mussels, always below maximum permitted levels [4]. Eventual correlation with the presence of MPs should still be studied.

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[2] Tavoloni T. et al, RAFA 2013
[3] Piersanti A. et al, Food Anal Methods. 2018;11:355–366
[4] Commission Regulation (EC) No 1881/2006

Keywords: *microplastics, organic contaminants, trace metals, freshwater fish, mussel*

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F19

EU COMPLIANT ROUTINE QUANTITATIVE DIOXIN, DIOXIN-LIKE COMPOUNDS BY GC-MS/MS WITH ADVANCED ELECTRON IONISATION SOURCE

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Regulatory changes in Europe in 2014 allowed GC-MS/MS to be used for confirmatory analysis and control of maximum levels (MLs) and action levels (ALs) of dioxins and dioxin-like PCBs in food and feed samples. In this study, the performance of a GC-MS/MS system equipped with an Advanced Electron Ionization (AEI) source was evaluated for the routine analysis of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in solvent standards and food/feedstuff samples. Sample extracts were analysed on identical Thermo ScientificTM TSQTM 9000 GC-MS/MS systems. Chromatographic separation was performed using a TG-Dioxin capillary GC column. Acquisition, processing, and reporting of the data were performed using Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System (CDS) software. Excellent agreement between the measured TEQ values and the supplied
Referencevalues from the EURL was obtained. A custom LOQ standard was also analysed at regular intervals throughout all PCDD/F sequences, in order to demonstrate the sensitivity required to maintain LOQs compliant with 1/5th maximum levels. Ion ratio tolerances were maintained at ±15% and RF deviation of less than 30% from the calibration average. System stability was tested by analysing dioxin samples for approximately two weeks.

Keywords: *dioxins, dioxin-like PCBs, food, feed, advanced electron ionization*

F20

ANALYSIS OF CHLORATE AND PERCHLORATE IN DIETARY FOOD SAMPLES USING LC-MS/MS

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Chlorate and perchlorate (ClOx) in food are of concern due to the harmful effect on human and animal health. Determination of ClOx in food is a bit challenge due to the trace level and their high polar properity. Ion chromatography coupled with tandem mass spectrometry (IC-MS/MS) or LC-MS/MS with ion-exchanging based column were commonly used, however, suffering the high-cost, short column life and relatively complicated operation. A new LC-MS/MS method was developed in this study for analysis of ClOx in food.

The food samples surrogated with 18O labeled internal standard were extracted by water and methanol. The extracts were then purified by an Envi™ Carb SPE cartridge or QuEChERS procedure. With the CSH PFP column and acetonitrile-0.1% formic acid used as eluent, chlorate and perchlorate were analyzed by a Shimazu LCMS-8060 triple quadrupole mass spectrometer under negative electronspray ionization mode and multiple reaction monitoring (MRM).

Good linearities were achieved for chlorate and perchlorate over the range of 0.5-1000 μg/L and 0.05-100 μg/L, respectively, with correlation coefficients R2>0.998. The quantification limits of the method were 0.2 μg/kg-1.0 μg/kg for perchlorate and 1.0 μg/kg-5 μg/kg for chlorate, respectively. Mean recoveries ranged from 80.3% to 123.8%, with precisions no more than 20%, which complied with the regulations for determination of trace contaminants residues in food matrix. The 144 composite dietary samples collected from 12 provinces in Chinese Total Diet Study were analyzed and perchlorate was detected 91.7 percent of dietary samples, with a concentration range of 0.14 μg/L-70.91 μg/kg. Relatively high level of perchlorate occurred in vegetables, fruits and grains, corresponding to the concentration range of 3.11-46.44 μg/kg, 4.03-12.28 μg/kg and 3.14-70.91 μg/kg, respectively. The estimated dietary intake of perchlorate for a standard male in China averaged 0.65 μg/kg bw/day, which closed to the

Referencedose recommended by National Accademy of Science, US, however, was higher than the value resulted from American Diet Study in 2005-2006 and the relative study conducted in Canada.

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F21

HELIUM SAVINGS AND FLOW OPTIMIZATION FOR MAXIMIZED PRODUCTIVITY AND COST SAVINGS FOR PBDE, DIOXIN AND PCB ANALYSIS WITH MAGNETIC SECTOR GC-HRMS WITH DUALDATA OPTION

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Helium shortage is a global concern, affecting laboratories and operational costs. Analytical solutions need to have options to avoid challenges due to helium shortage. 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 Dioxins with PBDEs. Due to the typically acquisition time of those applications the PBDEs can be acquired while the Dioxins are already injected but still in the waiting time frame. In such a sequence for each Dioxin sample, an additional PBDE run can be acquired in the same time compared to a standard sequence only for Dioxins on a single GCMS System. 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. To address helium shortage, the DualData XL Option can be combined with helium saver module. Using the helium saving module, helium consumption can be reduced drastically.

F22

TOTAL DIET STUDY IN SUB-SAHARAN AFRICA HIGHLIGHTS THE OCCURRENCE OF POPS AND OTHER CONTAMINANTS AND RESIDUES IN SMOKED FISH

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Introduction. We carried out a multi-center Total Diet Study in Benin, Cameroon, Mali and Nigeria (2014-2018) for assessing the dietary exposure to a wide spectrum of chemical contaminants and residues in a representative sampling of the populations' diet.
Materials and Methods. We derived food consumption patterns from household budget surveys and allowed for the identification of a food list that covered the average diet in excess of 90% in eight study centers. We collected and prepared 4020 samples of the food list according to local customs and pooled by 12 subsamples to form composites. We tested those food composite samples for various chemicals, including PAHs, PCDD/Fs, PCBs, BFRs, PFAS and various classes of pesticides.
Results. Among the various core foods that we sampled and tested for food chemicals, smoked fish stood out for various reasons. The PAHs levels exceeded the EU maximum tolerated limit in 100% of smoked fish, collected Benin, Cameroon and Mali. We did not collect any smoked fish from Nigeria, where it is not as significant in the diet as it is in the other countries. In Mali, smoked fish contained six different pesticides, including 5-18 mg/kg of chlorpyrifos, in Sikasso and Bamako, respectively. The higher PFOS concentration in smoked fish from Mali compared to other countries may be explained by the concomitant presence of pesticides in this commodity, in Bamako and Sikasso. The difference in PCDD/F and PCB profiles in smoked fishes compared to non-smoked fishes suggests that the origin of the contamination can be the combustion material used in the smoking process. Moreover, we also identified the presence of secondary metabolites, such as aflatoxin B1 and cereulide in some smoked fish composites.
Conclusions. The characteristics of this Total Diet Study, including the multi-center approach, the spectrum of analytes as well as the analytical results, are unprecedented. Of all the foods collected, smoked fish represented the highest concentration of a number of food chemicals, and particularly of POPs. Appropriate management actions based on the measurement of the chemical contamination profiles of smoked food products will allow better control of the smoking process as well as the storage and preservation of the foods concerned.

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Ingenbleek L, Hu R, Lopes Pereira L, et al. (2019) *Food Chemistry X*. 2019; in press.

Keywords: *food contamination data, PCDD/F, polychlorinated biphenyls, flame retardants, PFAS*

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F23

ROBUSTNESS OF SPME ARROW IMMERSION SAMPLING: POLYCYCLIC AROMATIC HYDROCARBONS IN DRINKING WATER

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Analyzing polycyclic aromatic hydrocarbons (PAHs) in drinking water becomes challenging especially for hard water (high mineral content water) and/or when sodium thiosulfate being added for treating chlorine content in drinking water during analysis. In this poster, repeatability for determination of PAHs in drinking water using SPME arrow (PDMS) immersion technique with gas chromatography mass spectrometer (GC/MS) was carried out. Single SPME arrow had been used in this experiment to determine its durability. The repeatability (n=100) which characterized as %RSD was less than 11% for all analytes, ranged between 1.08 % - 10.90 %. The relative peak area of 100th injection against 1st injection peak area for all analytes ranged between 77.54% to 119.44%; indicating SPME arrow fiber is dutiful for at least 100 injections with immersion technique. In conclusion, fully automated SPME arrow immersion technique with GC/MS analysis is an excellent option to analyze PAH compounds in drinking water; with higher throughput and robustness.

Keywords: *polycyclic aromatic hydrocarbons (PAHs), solid phase micro-extraction (SPME), SPME arrow, immersion, PDMS*

Acknowledgement: *Emanuel Coelho, Célia Ferreira and Cristina M. M. Almeida. J. Braz. Chem. Soc., Vol. 19, No. 6, 1084-1097, 2008. Andreas Kremser, Maik A. Jochmann and Torsten C. Schmidt. Anal Bioanal Chem, 408:943–952, 2016.*

F24

ESTROGENIC AND ANDROGENIC ACTIVITY OF HYDROXYLATED / METHOXYLATED METABOLITES OF BDES / CBS AND RELATED LIPOPHILIC ORGANIC POLLUTANTS

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Persistent organic pollutants (POPs) are known to show endocrine disrupting (ED) activity, including interactions with hormone receptors. In this work yeast based bioasay was addopted for evaluation of ED potency of highly lipophilic metabolites of POPs. Estrogenic / androgenic activity of some native brominated biphenyl ethers (BDEs) / chlorinated biphenyls (CBs), and their hydroxylated / methoxylated metabolites was assessed. No significant POP metabolism by the yeast cells was observed under applied conditions. The developed method was sensitive with EC50 values $6.5*10^{-11}$ M and $4.5*10^{-9}$ M calculated for E2 and DHT, respectively. Both CBs and BDEs show weak estrogenic activity negatively correlated with the degree of their halogenation, but their metabolites are significantly more potent xenohormones. 4-OH-2,2′,4′,6′-TeCB was the most potent estrogen receptor (ER) agonist among all tested compounds; its activity was only 1,000 times lower than that of native E2.

Keywords: *POPs, estrogen, androgen, hydroxylated, yeast*

F25

ENDOCRINE DISRUPTING POTENCY OF ORGANIC POLLUTANTS PRESENT IN COD LIVER OIL

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In this work an activity of xenobiotic mixtures containing persistent organic pollutants isolated from commercial fish oil samples against sex hormone receptors, including estrogen and androgen was assessed. The applied bioassay was based on transgenic yeast strains. Mixtures of chemicals isolated from fish oil using the semi-permeable membrane dialysis technique may interact with human steroid sex hormone receptors in various ways: the tested samples showed both estrogenic and anti-androgenic activity. Calculated 17β-estradiol equivalents for the tested samples ranged between 0.003 and 0.073 pg g^{-1} (fat). Anti-androgenic activity expressed as the flutamide equivalent concentration was in the 18.58–216.21 ng g^{-1} (fat) range. Polychlorinated biphenyls and various DDT metabolites were the main fish oil pollutants influencing the receptors. Additivity and/or synergy between chemicals was observed in the ER/AR mediated response.

Keywords: *EDC, DDE, DDT, POPs, fish oil*

F26

SIMPLE AND FAST AUTOMATED SPE CLEAN-UP FOR DETERMINATION OF PERFLUOROALKYL SUBSTANCES IN FOOD MATRICES

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Perfluoroalkyl substances (PFASs) are widely used in consumer products and industrial applications and can be found ubiquitously e.g. in the environment, in food and in human tissues. Due to their ubiquitous presence also in laboratory equipment (i.e. plastic tubes, fittings, etc.) analysis of these compounds is quite challenging and good control of procedural blank contamination is essential. Quantitative analysis of PFASs requires an efficient sample preparation method employing sample extraction and clean-up usually by using solid-phase extraction (SPE). Manual solid-phase extraction is, however, time consuming and thus unfavorable in routine analysis where a high number of samples has to be analyzed.
Hence, the aim of the present work was to develop a trace-analytical method employing an automated SPE clean-up for the qualitative and quantitative determination of 27 PFAS in food matrices by LC-MS/MS. Analytes of interest for this method are perfluoroalkylcarboxylic acids (C4 – C14), perfluoroalkylsulfonic acids (C4 - C12) as well as perfluoroalkyl sulfonamides and selected telomers.
Analytes were extracted with acetonitrile and ultrasonication. SPE clean-up was based on a weak anion exchange sorbent. As automated system, LCTech FREESTYLE SPE technique was used. It's a robotic system free of fluorinated plastics e.g. PTFE. In order to achieve optimal results, different washing and elution solutions were tested. Concentration steps and solvent changes were performed to enable optimized and more sensitive detection. Quantification was performed by an isotope dilution method using ^{13}C-labelled compounds to account for matrix effects and to compensate differences in recovery rates.
An overview of the main test results (in beef matrix) and a comparison of the various conditions will be shown.

Keywords: *PFOS, PFOA, LC-MS/MS, environmental contaminants*

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F27

ANALYSIS OF CONTAMINANTS IN BEVERAGES USING ICP-MASS SPECTROMETRY

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Analysis of Contaminants in Beverages Using ICP-Mass Spectrometry
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Beer is the most popular alcoholic beverage in Europe and enjoys a particularly high status due to the German Beer Purity Law of 1516 (the "Reinheitsgebot"), which uniquely defines the ingredients of beer to be hop, malt, yeast and water and makes the German Beer Purity Law the oldest food law in the world. Statistically, per capita beer consumption in some European countries was more than 100 liters in 2017 [1]. For these levels of consumption, the question arises: just how healthy is beer and what does beer contain? Beer contains hops, malt, yeast and water, as well as all major B vitamins, bitter substances, minerals and trace elements (e.g. Ca, Na, Mg and Zn) that are important for human nutrition. However, undesirable substances such as pesticides and heavy metals are also found. Maximum levels for certain contaminants in food are set in Commission Regulation (EC) No 1881/2006 for the following contaminants: nitrate, mycotoxins, dioxins, polycyclic aromatic hydrocarbons (PAH), metals and more [2]. For simultaneous multi-element analysis at ultra-low levels such as As, Se and Sb the inductively coupled plasma mass spectrometer ICPMS-2030 has been used according to the quality standards described in the European brewery convention (EBC). In this study commercially available beverages such as beer and wine have been measured. Thanks to the ICPMS-2030 system, analysis of beer could be performed without any time consuming sample preparation. All analyzed samples are only degassed with ultra sonication. After this treatment they are acidified with nitric acid and diluted with deionized water – then directly aspirated to the nebulization system of the ICPMS-2030. 7 different elements are simultaneously quantified: Analytical results for a variety of beers are presented.

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[2] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006

F28

LEVELS OF PCDD/FS AND PCBS IN FOOD, SAMPLED IN BADEN-WÜRTTEMBERG - COMPARISON WITH THE NEW TWI PUBLISHED BY EFSA

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The State Institute for Chemical and Veterinary Analysis (CVUA) in Freiburg performs analysis of PCDD/Fs and PCBs in the framework of official food and feed control for the German state of Baden-Württemberg (BW). About 600 different food samples are analyzed per year. With this data it's possible to obtain an overview of the background contamination of food sampled in BW.
In addition to maximum and action levels, established in Regulation (EC) no 1881/2006, to control and reduce levels of PCDD/Fs and PCBs, also the risk assessment should be considered. In 2001 the Scientific Committee on Food, a committee of the European Commission, published a tolerable weekly intake (TWI) of 14 pg WHO-TEQ/kg bodyweight (bw). According to the final report from the Scientific Co-operation on Questions Relating to Food (SCOOP), based on data from 1982-1999, the average dietary intake of PCDD/Fs and PCBs for an adult person has been approximately 1.2 - 3.2 pg WHO-TEQ/kg bw/day [1]. This means that a considerable part of the European population exhausts or exceeds this TWI. As per the German Federal Institute for Risk Assessment (BfR) in 2010 the German population exhausts the TWI to 90 – 121 % on average [2].
A new TWI of 2 pg WHO-TEQ/kg BW was published by EFSA in November 2018. In the light of this considerable reduction a comparison of the current mean background contamination with the new TWI is of interest. Therefore the mean PCDD/F and PCB levels of different food matrices, sampled in BW in 2018 were used and the results of the second national consumption study of the Max Rubner-Institute (MRI) [3] as well as the results of the EFSA Comprehensive European Food Consumption Database have been considered [4].
Summarizing the mean contamination levels of seven different matrices (eggs, fish, poultry, milk, dairy products, beef- and pork meat; n = 447), involving consumption data [3] and bodyweight (70 kg), this results to an intake of 1.3 pg WHO-TEQ/kg bw/week. This implies an exhaustion of 65 % of the current TWI. Using the results of the EFSA Consumption Database the exhaustion is a little bit lower.
Even if this is only a simplified consideration, it seems, based on the used analytical results that the human's exposure due to the background contamination in BW is below the current TWI.
All food samples were analyzed under accreditation according to ISO/IEC 17025 using validated GC-HRMS method.

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Keywords: *PCDD/F, PCB, TWI, background contamination*

F29

AN LC-Q-ORBITRAP METHOD FOR THE DETERMINATION OF THIRTY-THREE PERFLUOROALKYLATED COMPOUNDS IN LIVER

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Perfluoroalkylated substances (PFASs) have been produced since the 1950s and they represent a wide group of highly stable synthetic compounds used in various industrial applications. They are found, for example, in food packaging, non-stick coatings, fireproof foams, paper coatings, fabrics and personal care products. Over the past decade, PFASs have proven to be ubiquitous in water, air, food, wildlife and humans thanks to their high resistance to typical environmental degradation processes. The aim of this work was the development and validation of an analytical method for the determination of a wide group of PFASs in animal liver at low picograms per gram level. Thirty-three analytes were included in the method scope together with twenty-one labelled compounds used as internal standards. Two grams of homogenized liver were extracted and purified following the protocol suggested by Kärrman et al. [1] with slight modifications. The quantification was performed by liquid-chromatography coupled to Q-Orbitrap analyser (LC-Q Exactive, Thermo Scientific) using ESI negative ionization mode and full scan/SIM acquisitions. With regard to the optimization of chromatographic conditions, the high number of analytes (33) with very different polarities and molecular weights (from 214 u to 913 u) prevented the achievement of acceptable peak shapes for all the compounds. In order to dilute the high percentage of "strong" phase (80% MeOH) contained in the dissolution mixture of the final extracts, a peek tube was installed between the injector and the analytical column, improving peak symmetry and reducing broadening. This arrangement allowed to increase the injection volume (from 5 to 20 µL), too. Severe measures and internal quality control procedures were implemented in order to minimize PFAS contamination from labware and equipments. The validation study was performed spiking liver samples of different animal species (bovine, pig and poultry) at eight concentrations: 2, 5, 10, 25, 50, 100, 500 and 1000 ng/kg on wet weight basis. The results were satisfactory with intra-laboratory reproducibility CVs lower than 20% and trueness from 80 to 110%. Detection and quantification limits were from 2 to 50 ng/kg. Finally, a certified reference material (IRMM-427 – fish tissue) was analysed obtaining results within the reported ranges (Certified value ± Uncertainty).

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Keywords: *perfluoroalkylated substances, LC-Q-Orbitrap, animal liver, method validation*

Acknowledgement: *This work was supported by the Italian Health Ministry (Ricerca Corrente IZSSA01/16 "Development and validation of non/multi-target methods for identification and quantification of per-and poly-fluorinated chemicals (PFASs) in the food chain, to support risk assessment").*

F30

BFRS (PBDES AND HBCDS) IN FRESHWATER FISHES AND CRUSTACEANS FROM TRASIMENO LAKE – CENTRAL ITALY

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Polybrominated diphenyl ethers (PBDEs) and Hexabromocyclododecanes (HBCDs) are Brominated flame retardants (BFRs) used in a wide variety of commercial and industrial products to retard or prevent the possible ignition of fire. BFRs are additives not chemically bound to the polymers, hence they can easily leach out. These lipophilic and poorly degradable organic pollutants are, therefore, easily released in the environment [1,2] and they accumulate in wildlife and human tissue [3,4]. The main routes of BFRs human exposure are diet, inhalation and dust ingestion [4,5]. Among food, fish and fishery products have the highest BFRs content.
This study involved the analysis of 15 PBDE congeners (28, 47, 49, 66, 77, 85, 99, 100, 138, 153, 154, 183, 197, 206, 209) and 3 HBCD isomers (a, b, g) in fish and crustaceans collected in Trasimeno Lake (Umbria Region; Central Italy). Five species were investigated: crucian carps (n=14), European perches (n=18), tenches (n=14), eels (n=10) and red swamp crayfishes (n=2 pools).
The samples were analyzed either by GC-MS/MS (PBDEs) or LC-MS/MS (HBCDs) in isotopic dilution (LOQ 10 pg/g; 100 pg/g only for BDE-206 and -209), performing a common sample preparation: QuEChERS-like extraction followed by a two steps clean-up [6].
PBDEs and HBCDs were all below the LOQs in crucian carps, European perches and tenches; only traces of the BDE-47 and a-HBCD were detected. Different were the eels, in which BDE-47, -49, -100, -99, -154 concentrations were above LOQs and S15PBDEs (*lower bound* approach) ranged between 265 and 921 pg/g. In eels also HBCDs were measured: a-HBCD was present in all the 10 samples considered, and, in some specimens, traces of b- and g-HBCD were detected; S3HBCDs ranged from 158 up to 1142 pg/g.
Contamination in red swamp crayfish was peculiar. 20 specimens were collected and divided into 2 pools (female and male), of 10 animals each. In both pools, all the 15 BDE congeners were below LOQs while the three a-, b-, g- HBCD isomers were quantified with not negligible levels. The male pool seemed to be significantly more contaminated than the female (S3HBCDs: 3369 pg/g and 138 pg/g, respectively).
All species showed the same PBDEs contamination pattern as expected: 47 > 100 > 154 > 49 > 99; a-HBCD was always the most abundant congener. Only in crayfish the g- isomer was dominant above all the others. Consequently, more red swamp crayfishes should be analyzed to confirm the present findings.

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Keywords: *brominated flame retardants, PBDE, HBCD, freshwater fish*

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F31

SOURCES OF NICOTINE IN DRIED MUSHROOMS

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A discussion about the source of Nicotine (NIC) in boletus (Boletus edulis) was started in the EU in 2009. No clear conclusion about the nicotine source in wild dried mushrooms was reached [1,2]. In 2010 the specific MRL of 2.3 mg/kg for dried wild mushrooms was established.
There was one single case of high nicotine content in cultivated mushrooms due to a nicotine-containing substrate of chicken feathers which had been deloused with nicotine. Various theses have been developed and disproved. As ever, the highest nicotine levels can still be detected in Chinese mushrooms, especially from the province Yunnan. Parallel to our study of nicotine findings in Indian tea[3], we postulated the ambient agro-climatic situation in China and more precisely in Yunnan could be the cause.
Besides being the most common location for the picking of boletus, Yunnan is the province in China, famous for its tobacco growing and production. NIC is highly volatile. It can be transported per air and condensed on the earth.
Altogether we measured more than 20 different dried mushrooms for nicotine and for the main nicotine metabolite, cotinine. We also differentiated between the stump and the hut of the mushrooms and also analyzed sponge (boletus) as well as lamella (champignon) mushrooms. The NIC findings were higher in the huts of sponge mushrooms, followed by their stumps in Chinese products. We didn't see any differences between NIC findings in different tissues of the lamella mushrooms, but we could identify the two following trends:
1. Where the Cotinine/NIC proportion was 1-5 % we would ascribe the contamination to tobacco producing. This is always the picture in Chinese boletus (Yunnan) with high NIC amounts (1.0-5.0 mg/kg) and there is no difference between whole mushrooms and caps. That proved the thesis of M. Anastassiades and A. Fernandes-Alba[4]. The contamination is from soil (and air).
2. In European dried mushrooms, however, the cotinine and NIC levels were about the same with a maximum of 0.5 mg/kg. Their source is very probably the environmental tobacco smoke (ETS) or aging (oxidation) nicotine in the air. The amounts of NIC are significantly lower than in Chinese boletus. NIC Amounts in huts is higher than in the whole thallus. The source of the contamination is clear air only.

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Keywords: *nicotine, mushrooms, contaminants*

F32

RAPID DETERMINATION OF POLYCHLORINATED BIPHENYLS ULTRA-TRACES IN WATER BASED ON MICROEXTRACTION AND HIGH RESOLUTION MASS SPECTROMETRY

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Environmental pollution by polychlorinated biphenyls (PCBs), classified as "persistent organic pollutants", is today a major public health issue because of their abilities to bioaccumulate and biomagnificate through the food chain. EPA and ANSES define maximum levels for these substances in water and food products, often of the ppt order. The aim of the present study was to develop a sensitive analytical approach able to rapidly detect PCB contamination ultra-traces in water while being reproducible and easy to settle for a routine evaluation. Starting from water samples spiked with indicators PCBs, microextraction techniques usually dedicated to the extraction of very low molecular weight compounds have been coupled with gas chromatography (GC) and Orbitrap® high resolution mass spectrometry (HRMS) in order to reach the targeted levels of sensitivity. Headspace solid phase microextraction (HS-SPME), dynamic headspace (DHS) and stir-bar sorptive extraction (SBSE) coupled with GC-HRMS were thus compared, with a test of different trapping polymers to improve analytical performances and insure efficiency and reliability for PCBs detection. The lowest detected contamination levels were reached in HS-SPME with a polydimethylsiloxan/divinylbenzene (PDMS/DVB) coated fiber. Limits of detection for indicators PCBs were between 0.3 and 0.6 ppt. Based on an analysis by HS-SPME with PDMS/DVB fiber coating, PCBs have been sought in real water samples originated from clouds, which were collected at the puy de Dôme station (France) during campaigns under the influence of different air masses (urban-anthropogenic, marine, continental, etc.). Further investigations have to be undertaken to assess if HS-SPME remains the best microextraction technique for more complex food matrices, in which competition between PCBs and other matrix compounds could occur.

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F33

DETERMINATION OF ORGANIC CONTAMINANTS IN INSECTS REARED AS PROTEIN SOURCE FOR ANIMAL FEEDING

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In a context of scarcity of natural resources, decline of the agricultural land areas, and high dependence regarding proteins for animal feed, the development of edible insect farming is an alternative, efficient and sustainable solution to address food and nutrition security. In particular, insect rearing can provide a good protein source with significantly shorter life cycles than traditional livestock (Caparros Megido et al., 2016). In front of the emergence of the insect-based food industry and the opening of the European regulations for the use of insects as protein source in animal feed since July 2017, it is now essential to ensure the health safety of insects and/or insect-based products in livestock and humans. Whereas researches about biological risks have been already undertaken, research is needed on the chemical risk assessment, from the earliest stages of insect farming to the production of insect-derived products and their digestion and metabolisation by livestock / humans. In taking the grain beetle *Tenebrio molitor* as model of insect farming, the present study aimed to develop an analytical method to assess the safety of insects regarding the main chemical contaminants susceptible to contaminate finally the insect-derived products. We focused our attention on environmental pollutants (PCBs, HAPs) and pesticides (used in wheat production or POPs). Based on an efficient extraction protocol coupling ASE (automated solvent extraction), GPC (gel permeation chromatography) and vacuum concentration, targeted contaminants have been determined by GC-ToF/MS. Performance of the developed method have been characterized. Thanks to this MS-based analytical method, further investigations can be now undertaken, especially to study the potential phenomena of bioaccumulation and biomagnification of organic contaminants related to the rearing of insects and their consumption, or to compare the health quality of the different production chains used in insect rearing.

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F34

SEPARATION OF THE MINERAL OIL AROMATIC HYDROCARBONS OF THREE AND MORE AROMATIC RINGS FROM THOSE OF ONE OR TWO AROMATIC RINGS

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Petroleum products are complex mixtures of hydrocarbons made from crude oil. The mineral oil hydrocarbons (MOH: mineral oil hydrocarbons) can be subdivided into saturated (MOSH) and aromatic (MOAH) hydrocarbons. Unlike the commonly known polycyclic aromatic hydrocarbons (PAH), the majority of MOAH are highly alkylated and possibly partially hydrogenated [1]. Exposure to MOSH and MOAH can occur to humans orally either through food or through cosmetic and pharmaceutical products such as lip balms and laxatives, which can consist almost entirely of MOH [2].
According to the 2012 opinion of the European Food Safety Authority (EFSA), "MOAH with three or more, non- or simple-alkylated, aromatic rings may be mutagenic and carcinogenic, and therefore of potential concern" [3]. For this reason, a distinction must be made between the mono- / diaromatic fraction (MDAF) and the tri- / polyaromatic fraction (TPAF) within the MOAH, since the latter has a higher toxicological relevance [4].
In this work, we present a successful analytical method for the separation and quantification of MOAH in fractions according to the number of aromatic rings (MDAF and TPAF). This separation was achieved by donor-acceptor complex chromatography (DACC) on a π-acceptor phase. The qualitative composition of both fractions and the concentrations of the respective compounds were determined by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometer detection (GCxGC-TOF-MS) and by coupling of liquid chromatography to gas chromatography with flame ionization detection (LC-GC-FID). The analytical performance of the method was demonstrated on cosmetic raw materials petrolatum and paraffinum liquidum.

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Keywords: *mineral oil hydrocarbons, donor-acceptor complex chromatography, two dimensional gas chromatography, mono and diaromatics, tri- and polyaromatics*

F35

DEVELOPMENT AND VALIDATION OF PERCHLORATE AND CHLORATE ON AGILENT'S NOVEL HILIC-Z,P-COLUMN USING LC-MSMS

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Perchlorate and chlorate are difficult analysable compounds due to low mass and chemical characteristics. A robust and sensitive method is developed using Agilent's novel HILIC-Z,P-column. This method is validated for all Food and Feed including Food additives, Alongside manure, chemical fertilizers, soil, oils & fats including fatty acids and other salts can also be analyzed.
LC-MS-MS system
•UPLC Agilent 1290 Infinity II
•Sciex Triple Quad 6500+ in negative ESI mode
•Liquid chromatography Triple-Quadrupole mass-spectrometry (LC-MS/MS)
•Agilent, InfinityLab Poroshell 120 HILIC-Z,P
150 mm x 2.1 mm, 2.7 µm
•Hydrophilic interaction chromatography zwitterion PEEK-lined (PEEK; polymer polyetheretherketon)

Conclusion: A robust and sensitive method is developed for the analysis of Perchlorate and Chlorate on HILIC-Z,P-column using LC-MSMS for Food, Feed and related matrices.

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OCURRENCE OF CADMIUM AND LEAD IN COCOA BEANS FROM BRAZIL

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Cocoa beans (*Theobroma cacao L.)* world production is of 4.6 Thousand tons, which are mainly produced in West Africa, Central America, South America and Southeastern Asia. High levels of cadmium in cocoa have been reported In Latin America. Many countries import beans from these different regions and process them for production of derived products (liquor, cocoa powder, cocoa butter, chocolate). In order to ensure safety of derived cocoa products, regarding the presence of inorganic contaminants, maximum levels for cadmium and lead in chocolates, cocoa liquor and cocoa products have been established in European Union, Mercosul and Brazil. The objective of the present study was to establish an ICP-OES method for determination of cadmium and lead in cocoa beans and evaluate the contamination of 73 samples from the main producing regions in Brazil: Bahia (n=32), Pará (n=29), Rondônia (n=8) and Espírito Santo (n=4). Method validation presented precision with coefficients of variation under 6% and accuracy with recovery values between 90 and 104% for both contaminants. Limits od detection and quantification for cadmium and lead were 0.5 and 1.5 μg kg^{-1} e 7.0 e 22 μg kg^{-1}, respectively. Mean results (range) detected for cocoa beans samples from Brazil (mg kg^{-1}) were: cadmium = 0.091 (<0.0015-0.818) and lead = 0.792 (<0.022-2.52). Four percent of the samples showed cadmium levels over maximum permitted by Brazilian regulation (0.3 mg kg^{-1}) and 60% surpassed maximum lead levels (0,5 mg kg^{-1}). Highest levels were detected in samples from Pará and Bahia regions. Despite reports from literatures, results from the present study showed that besides cadmium, lead may also be a significant contaminant in cocoa and possibly likewise in cocoa products.

Keywords: *cadmium, lead, cocoa, ICP-OES*

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F37

DETERMINATION OF PHTHALATES IN BABY FOOD COMBINING ACETONITRILE-BASED EXTRACTION WITH LOW-TEMPERATURE CLEANUP AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS

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A simple, easy and cost-effective sample preparation method has been introduced for the determination of seven phthalates in baby food by gas chromatography–mass spectrometry (GC-MS). The developed method includes acetonitrile-based extraction followed by addition of salts sodium chloride and magnesium sulfate in order to induce phase separation, and then low-temperature cleanup at –18°C. As a great result, reduction of more of 50% in matrix co-extractives content was verified in the final extract by gravimetric measurements, when compared to crude baby food extract. Adequate performance characteristics were achieved including analytical selectivity, high sensibility with LOQs between 1 and 10 μg kg^{-1}, linearity in solvent and matrix-matched calibration curves, good recoveries (73–110%) and precision (RSD ≤ 16%), under repeatability and within-laboratory reproducibility conditions. The validated method was applied to twenty commercial fruit-based baby foods and the compounds BBP, DBP, DEHP, DEP and DnOP were detected in some samples with levels varying from 1.4 to 90.3 μg kg^{-1}, confirming the suitability of the proposed method for routine analysis.

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THE PAH INTERNAL STANDARDS TOOLBOX: THE EUROSTARS "13C CRM" PROJECT

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Polycyclic aromatic hydrocarbons (PAHs) occur in oil, coal, and tar deposits, are formed by incomplete combustion of organic matter, and are found as pollution in air, water and soil. Amongst the PAHs are some of the most toxic compounds known. Some of the PAHs are known to be carcinogenic, mutagenic, and teratogenic. Because of their wide distribution, it is therefore important to monitor these compounds.
PAH compounds of particular toxicological and environmental concern are monitored using internationally recognized methods. In the United States, the EPA (Environmental Protection Agency) has listed 16 priority PAHs based primarily on occurrence, while in the EU 15+1 priority PAHs are listed based on risk assessment.
Internal standards (ISs) are needed for quantitative analysis of PAHs. The benefit of using internal standards with physico-chemical properties similar to those of analytes is that both systematic and random errors will be minimized.
Chiron have developed several serious of PAH internal standards, deuterated, monofluorinated versions as well as unlabelled native compounds eluting different from the native once.
Deuterated internal standards of PAHs have been widely used. Chiron has also developed mono-fluorinated PAHs (F-PAHs) as internal standards, due to the closely similarity of F-PAHs to the parent PAHs.[1,2] Deuterated PAHs and F-PAHs are applied as internal standards for GC-MS, GC-ECD, and GC-FID analysis have been investigated.
Now we present a complete set of ^{13}C labelled the16 EPA PAHs prepared through the EUROSTARS project "^{13}C CRM". While the ^{13}C labelled compounds elute identical to the natives, both on GC and LC, the deuterated and monofluorinated compounds elute different and can be used for other applications.
Synthesis and availability of the various IS-alternatives will be presented.
Thus, a complete tool-box of various choices of internal standards for PAH analysis is now available, deuterated, mono-fluorinated, as well as ^{13}C labelled. The choice of which internal standard to be selected depends on the method and in particular the choice of instrument and detector. Pro and cons for the various IS alternatives will be discussed, and a recommendation for the choice of internal standard to select is presented.

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Keywords: *PAH, Internal Standards toolbox, 13C labelling, Monofluoro labelling*

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THE EUROSTARS CHLOFFIN PROJECT, NEW STANDARDS FOR THE QUANTIFICATION OF POLYCHLORINATED PARAFFINES

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The CHLOFFIN project is a collaboration between Chiron AS, the EU commission (the Joint Research Center JRC, Geel), and the Free University of Amsterdam, and the ultimate aim is to develop a set of clearly defined reference materials for CPs for quantification. The CHLOFFIN project started October 2019 and will last for three years.
Polychlorinated paraffines (CPs) is a class of industrial chemicals used as high-temperature lubricants in metal-working machinery and as flame retardant plasticizers in vinyl plastics. Less common applications include the use as flame retardants in rubber, paints, adhesives and as sealants. The total global production remains unknown but is believed to exceed several of millions of metric tonnes pr year. CPs are highly toxic to mammalians, via bioaccumulation and potential carcinogenic to humans, thus short-chain CPs have been prohibited by the POP Regulation in the EU since 2012. CPs represent a category of POPs that need to be continuously monitored – and no suitable and generally accepted reference standards are commercially available yet.
Industrially, the CPs are synthesized by direct chlorination of *n*-alkane feedstock with molecular chlorine at elevated temperatures and pressures, and sometimes in the presence of UV-light. CPs fall into three categories, C10-C13 (short, "SCCP"), C14-C17 (medium, "MCCP"), and C18-20-C30 (long, "LCCP"). They are further sub-categorized into their weight content of chlorine, 40-50%, 50-60% and 60-70% CPs are analyzed by GC using ECD detector, or more sophisticated by high resolution gas.chromatography/electron capture negative ion-mass spectrometry (HRGC/ECNI-MS).
Chiron offers a broad range of polychlorinated CPs and the ultimate goal for the CHLOFFIN project is to develop a standard with defined composition and response factors which are similar to the industrial mixture. The Chiron standards are single molecule compounds. They are useful in the quantification and as standards for CP determination, for dividing them into the various classes according to carbon length and chlorine content.

Keywords: *CPs, Quantification, CRMs, Reference standards*

Acknowledgement: *EUROSTARS and The Norwegian Research Council*

F40

ANALYSIS OF PERFLUOROALKYLATED SUBSTANCES (PFASS) IN WATER, PART A: SPE COLUMNS AS A SOURCE OF BACKGROUND CONTAMINATION IN PFASS TRACE ANALYSIS

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A solid-phase extraction (SPE) is a key technique utilized for per- and polyfluoroalkylated substances (PFASs) determination in water samples, as it enables to analyze these (ultra)trace levels analytes.
In this troubleshooting study, we map our experience in application of the SPE technique for isolation of 24 PFASs (including 11 perfluoroalkyl carboxylic acids and 9 perfluoroalkyl sulfonic acids) from water prior to the final analysis using ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) in terms of background contamination originating from the used SPE columns (Oasis WAX, Waters). The existing method was successfully modified by following steps: (a) a sample amount reduction from 500 mL to 100 mL, (b) enlargement of a sample volume injection from 5 to 50 µL and (c) a change of the final extract solution. As a result, we obtained 5 – 10 times lower limits of quantification (LOQs), which have emphasized the problems with background contamination. Routinely in our laboratory, we have to examine water samples from both 'hot spots' where leaks of PFASs happened (concentrations in the range of 100 – 10 000 ng/L) and drinking water samples in which ultra-trace levels of targeted substances are typically determined (more information are presented in the poster *Analysis of perfluoroalkylated substances (PFASs) in water, part B: Occurrence of PFASs in tap water in the Czech Republic*). Therefore, it is important to properly wash all used extraction devices (manifold, tubing,…) to avoid potential cross-contamination. Also, other materials and solvents are carefully checked for the presence of targeted compounds. Despite that, high concentrations mainly of perfluorooctanoic acid (PFOA) were measured in procedure blanks several times and SPE columns (Waters) were identified as the source of blank contamination, other material used was previously tested and evaluated as a PFASs-free. For PFASs-positive SPE columns, a decontamination procedure provided by the producer and its modification with a high amount of solvents were applied and SPE columns obtained from another producer (Strata-X-AW, Phenomenex) were also tested.
PFOA was present at concentrations above the LOQs in all 4 tested batches of columns (Waters). In one batch, PFOA was determined at level corresponding concentration 40 ng/L of water (LOQ = 0.025 ng/L). The decontamination protocol from Waters was insufficient and even two-cycles of decontamination using a larger volume of solvents was not sufficient. Finally, Strata-X-AW columns were found as suitable for our method.
In a trace level environmental contaminants analysis, the use of a target-analytes-free material is essential. Decontamination can be solvents and time-consuming approach with insufficient results. With regard to the frequent use of the SPE columns in this type of analysis, it is important to point out the possible background contamination to prevent inaccurate data.

Keywords: *PFOS, PFOA, SPE water, UHPLC-MS/MS*

Acknowledgement: *Financial support from specific university research (MSMT No 21-SVV/2019).*

F41

STUDY ON THE OCCURRENCE AND DISTRIBUTION OF DIOXINS (PCDDS/PCDFS), DIOXIN-LIKE PCBS AND INDICATOR PCBS IN HAIRY CRAB

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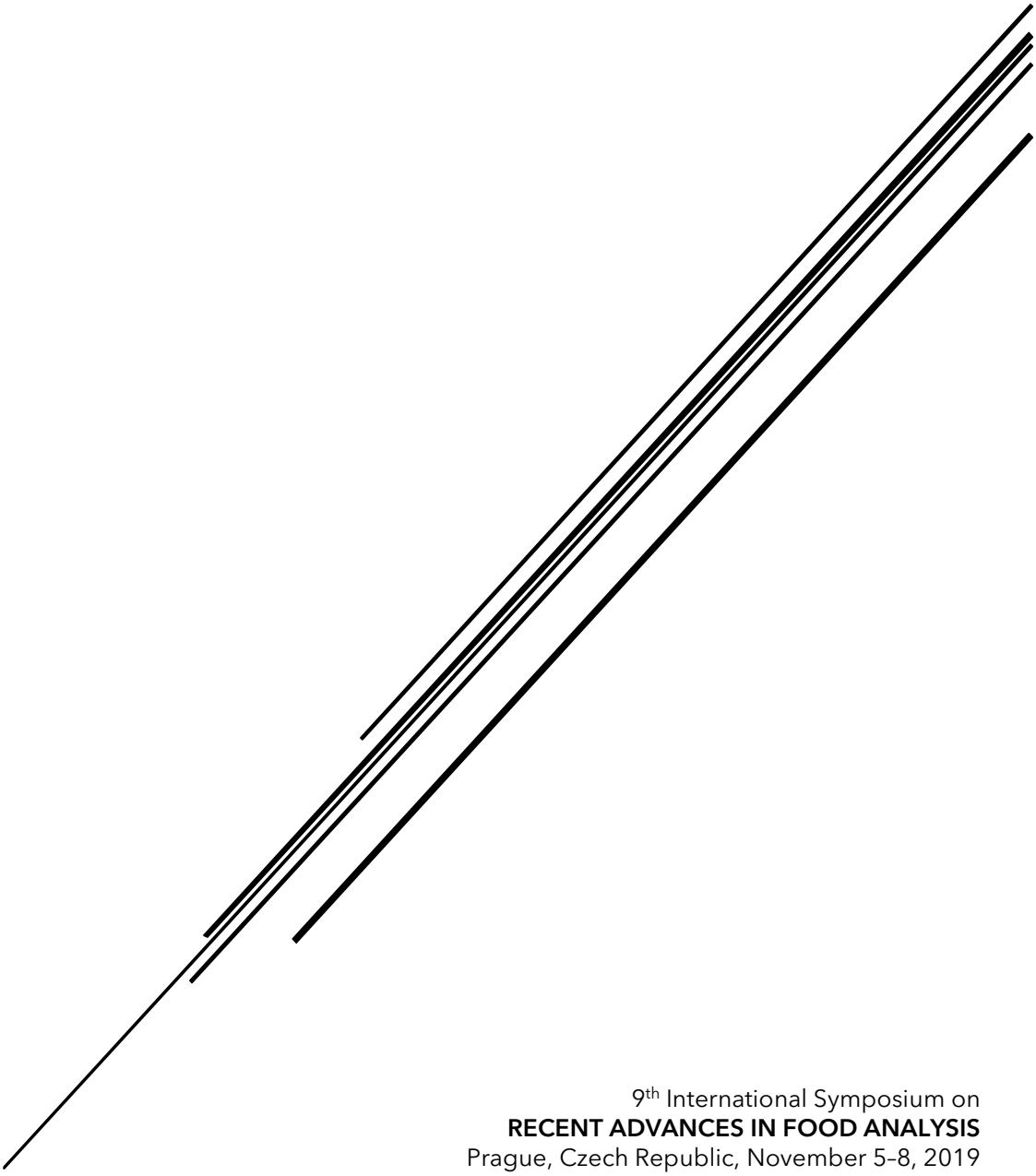
The hairy crab, also known as Chinese mitten crab or *Eriocheir sinensis,* is native to rivers, lakes, estuaries and other coastal habitats of eastern Asia. It is one of the most commercially valuable crab in Asia where its ripen roe is a delicacy in Chinese cuisine. The edible portion of the crab can be divided into muscle meat and brown meat. The muscle meat is present in the claws, legs and body, while the brown meat is found in the shell cavity at the top of the crab, which has the highest fat content. This study aims to determine the occurrence and distribution of PCDD/Fs, dioxin-like PCBs and indicator PCBs in muscle meat and brown meat of hairy crab. Brown meat was shown to exhibit an elevated level of contamination with mean concentration of 9.6 pg TEQ g^{-1}, 19.0 pg TEQ g^{-1} and 140.0 ng g^{-1} fresh weight for PCDD/Fs (sum), PCDD/Fs and dioxin-like PCBs (sum), and indicator PCBs (sum) respectively. These levels were 57, 76 and 177 times higher than those in the muscle meat. The significantly higher level of the above contaminants in brown meat could be attributed to the bioaccumulation effects of these persistent organic pollutants (POPs). Hairy crab, being a bottom feeder, has the tendency to enrich lipophilic contaminants from aquatic sediments, leading to high level of dioxins and dioxin-like PCBs in the brown meat. Given the fact that Chinese dietary habit of consuming the entire edible portion of the crab including the brown meat, exposure to dioxins and dioxin-like PCBs increase significantly. While consumption of hairy crabs containing dioxins and dioxin-like contaminants may not pose acute health risk, the potential long-term health concerns should be addressed through regulatory measures and consumer advisory to moderate the consumption of this seasonal delicacy.

Keywords: *dioxins, dioxin-like PCBs, indicator PCBs, hairy crab, bioaccumulation*

FOOD FORENSICS

POSTER SESSIONS



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G1

ELEMENTAL ANALYSIS (TOTAL AND SPECIATION) OF WINES FROM VARIOUS REGIONS OF THE WORLD

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Wine is a popular beverage of choice throughout the world and can range in quality, cost, taste, and flavor. The perception of wine is generally characterized by the overall score received from a wine critic or the popularity among consumers. However, analytical testing is an important aspect for determining the quality of the wine from the perspective of food safety. The testing can be broken into a few categories: molecular or organic content and elemental or inorganic content. The elemental content can affect various properties of wine; some inorganics (e.g. S, P, Fe, Mg, or Ca) can affect the flavor, color, aroma, and rate of precipitation, whereas elements such as As, Cd, Hg, and Pb are considered toxic and pose great risk for the consumer.
The most common method for elemental analysis is inductively coupled plasma-optical emission spectroscopy or -mass spectrometry (ICP-OES or ICP-MS). ICP-MS is normally the preferred choice of instruments since it offers detection limits that are generally in the ng/L range. For certain elements (e.g. As or Cr) detecting the total amount in the wine does not provide all relevant information when wanting to assess levels of toxic species present. For example, arsenic has various forms that exist as both organic or inorganic species. Inorganic arsenic (As III or As V) is more bioavailable, thus is more dangerous as compared to organic arsenic species (AsB, AsC, DMA, or MMA). In the case of chromium, Cr III is consider an essential element that may play a crucial role in the human body, whereas Cr VI is toxic.
This work will present an automated method for measuring total elemental content and elemental speciation in wine using a single sample introduction platform in combination with an ICP-MS. The total elemental measurements will include both essential and toxic elements in the panel.

Keywords: *asenic, chromium, speciation, food*

G2

A NOVEL ANALYTICAL APPROACH FOR THE DETECTION OF ILLEGAL USE OF CMC FOR INFLATION OF THE WEIGHT OF PRAWNS AND RELATED PRODUCTS BY FTIR TECHNIQUE

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There have been articles and video clips circulating on Internet, claiming that there exist illegal activities in some countries whereby prawns were injected with a gel-like substance to make the prawns looked firm, fresh and big in size with inflated weight. The gel-like substance was suspected to contain hydrated carboxymethyl cellulose (CMC). The use of CMC to inflate product weight for illegal economic gain constitutes a serious food fraud. Nevertheless, to our knowledge, there has been no simple and effective analytical method for verifying whether those accusations were true or false.

Visual inspection was proposed by some research workers. This involved slicing the head/belly of prawns and squeezing out the inner contents to observe the color and states of the substance oozing out of the prawn body. Unfortunately, it is hard to make firm conclusion whether the substance is due to the injected CMC or the normal natural tissues of prawns since CMC hydrocolloids formed are nearly transparent. There were also recent attempts to use sophisticated equipment such as low-field nuclear magnetic resonance (LF-NMR) spectroscopy and magnetic resonance imaging (MRI) for the related authenticity checks. However, these expensive instruments are neither commonly available nor field-deployable, which limit their actual usage when routine sample checks for CMC are required.

Herein, we developed a rapid and low cost analytical approach, based on Fourier-transform Infrared Spectroscopic technique (FTIR), for the combat against food fraud pertaining to the above-described illegal use of CMC for prawn and related products. Several regions of FTIR spectrum, corresponding to stretch/bending bands of the -C-O-, C-O-C- and -O-H- bonds of CMC molecules, were exploited for the confirmation of the presence or absence of CMC in suspected prawn samples. Through the examination of FTIR spectra of leaked substances resulting from the application of a heating process to prawn samples, CMC at an adulterated level as low as 0.5% of the total prawn weight can be detected with confidence. This novel technique was successfully adopted for our recent market surveillance testing of imported prawn products.

Acknowledgement: *We thank our vendor of Agilent for the FTIR testing that greatly assisted the research*

G3

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 sourc e ingredients.

Keywords: *isotope fringerprint, IRMS, stable isotopes, origine, authenticity*

G4

ELEMENTAL ANALYSIS IN FOOD FOR RISK ASSESSMENT AND PROVENANCE STUDIES

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To ascertain the quality of food and food products, the analysis of toxic, essential and nutritional elements has become a routine task for food quality monitoring. Elements such as arsenic, cadmium, mercury or lead can enter the food chain via a series of pathways including, but not limited to, industrial pollution or environmental contamination. Recent public alerts on arsenic or lead contaminations in our daily food or water supply have contributed to increased attention on this particular issue, but there is also a high demand for clear information on nutrients and contaminants from health-conscious consumers.

Elements such as the rare earth's (e.g. Nd, Gd) can cause significant false positive signals on critical analytes such as arsenic or selenium, as a result of the formation of doubly charged ions in the plasma. Elimination of these interferences is crucial to obtain correct results. In addition, the appearance and distribution of these elements may give additional insight into the provenance of foodstuffs.

To keep up with the demands of the market, analytical laboratories need to be capable of analysing a high number of samples, containing both major and trace levels of a variety of elements, in the shortest possible time. This can usually be accomplished by using single quadrupole ICP-MS instruments, with a single measurement mode applied for analysis of all the target elements in a suite. This single mode approach dramatically reduces the measurement time required per sample and reduces analysis cost.

However, some interferences, such as the doubly charged ions of rare earth elements mentioned above, require triple quadrupole ICP-MS instruments to consistently remove them.

At the same time as quantifying the target set of analytes, screening a sample set for other analytes that don't require full quantification can be accomplished using a full mass scan, allowing unexpected elements in the sample to be identified, even months after the original analysis.

This paper reviews various strategies, including the use of collision/reaction cell (CRC) technology with both single and triple quadrupole ICP-MS instrumentation, for the accurate analysis of trace elements in different food samples.

Keywords: *food, metals, trace elements, ICP*

G5

ANALYSIS OF PHYTOCANNABINOIDS IN PLANT OILS: BENEFITS PROVIDED BY SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC) COUPLED TO HIGH RESOLUTION MASS SPECTROMETRY (HRMS)

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A fast supercritical fluid chromatography – high resolution mass spectrometry based method (SFC-HRMS/MS) for the determination of 17 phytocannabinoids in plant oils has been developed and validated. Analysis of phytocannabinoids in oils is a challenging task not only due to a difficult matrix, but also because of a structural similarity and high concentration variability of the target analytes. Prior the instrumental analysis, samples were only dissolved in hexane and then separated on a normal phase Acquity UPC2 Torus DIOL column using 5 mM ammonium formate and 0,1% formic acid in methanol:water (95:5, *v/v*) as mobile phases modifier; mass spectrometer with Q-TOF mass analyzer was used as the detector. The repeatabilities of measurements were in the range 4 – 14 % and the limits of quantitation ranged from 0.25 to 1.0 mg/kg for all analytes/oil matrix combinations. These results were comparable to those obtained by the commonly used reverse phase UHPLC-HRMS/MS based methods, nevertheless, thanks to a low lipids retention under SFC conditions used, the sample analysis time was significantly reduced. Great attention was also paid to the evaluation of matrix effects as significant differences were found among plant oils (rapeseed, olive, hemp and linseed oils were analyzed). To compensate the matrix effects, deuterated internal standards of cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC) and cannabinol (CBN) were used.

Keywords: *cannabis, SFC-HRMS/MS, phytocannabinoids, hemp oil*

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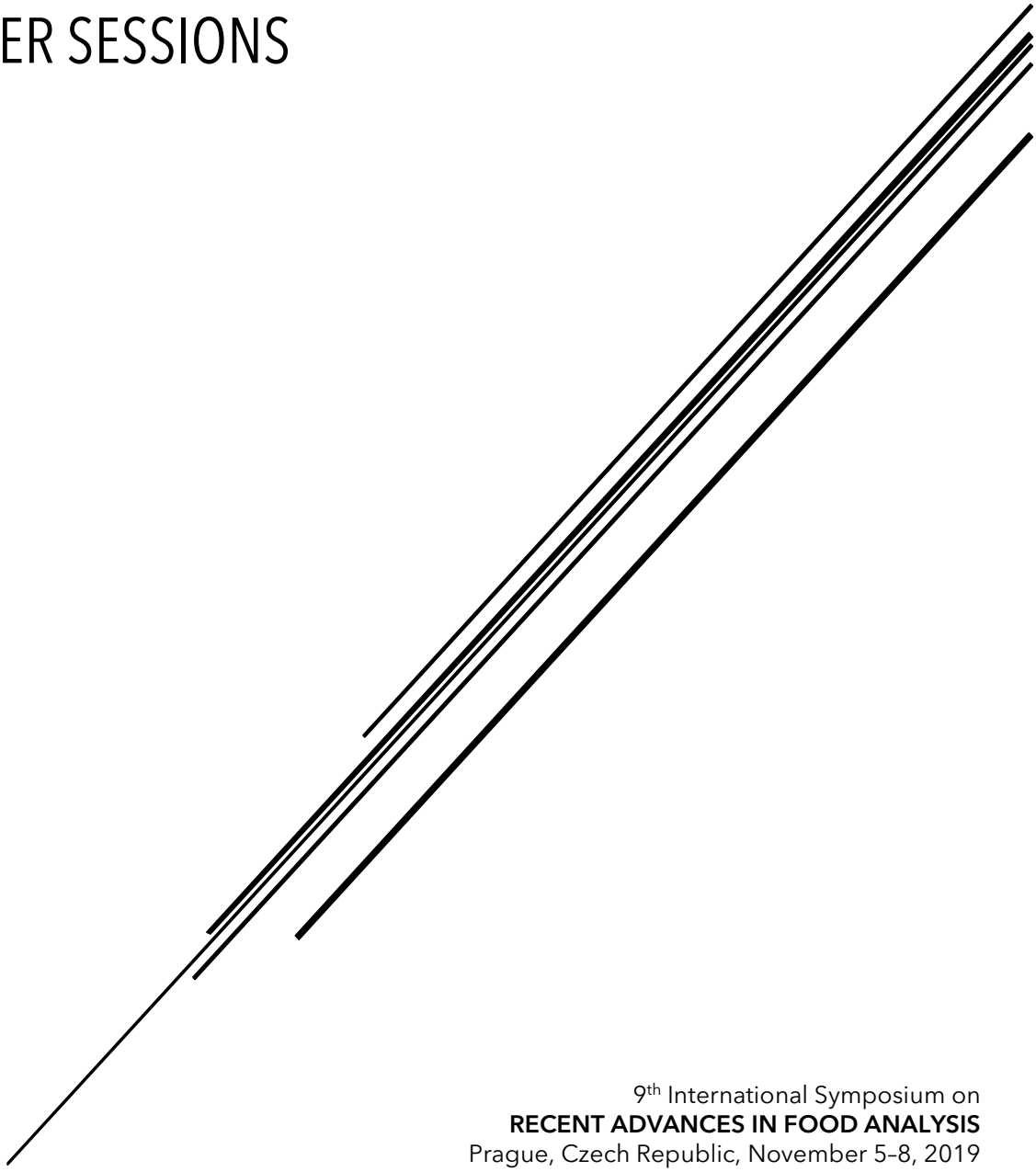
GENERAL FOOD ANALYSIS

POSTER SESSIONS



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H1

ASSESSMENT OF CITRUS PEELS CHEMICAL COMPOSITION BEFORE AND AFTER ESSENTIAL OIL EXTRACTION

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Citrus fruits are among the most popular fruits in the world, due to their pleasant taste but also due to their content in bioactive compounds that can have a positive effect on human's health. The increase consumption of citrus fruits-based products (e.g. juices) led to an increase in the overall quantity of citrus peel waste. Representing almost half of the fruit mass, citrus peel can be regarded as a valuable source of natural antioxidants and pigments, essential oils, fibers, etc. When used for the extraction of essential oil the remaining citrus peel residue is discarded. In order to exploit this by-product in a more sustainable way, the aim of our study was to evaluate the chemical composition of several citrus peels before and after the extraction of essential oil in order to assess the possibility to further recover the bioactive compounds from the peels depleted of essential oil.
Five citrus peels samples were taken into study, namely orange, grapefruit, lemon, lime and tangerine. The extraction of essential oils was performed by microwaved-assisted extraction using an Ethos X system from Milestone, Italy. Before and after the essential oil extraction the citrus peels proximate composition (protein, lipids, carbohydrates, ash, dry matter) as well as their total phenolic and flavonoid contents, antioxidant activity and fatty acids profiles were determined.
The obtained results underline the rich composition in bioactive compounds of citrus peels samples before the essential oil extraction. Even though some losses in bioactives concentrations and some shifts in their profiles were observed, the citrus peels resulted after the essential oil extraction still contained appreciable amounts of these compounds that maintained their functional activities and can be recovered and exploited in value-added products.

Keywords: *citrus peel, bioactive compounds, food waste, sustainable exploitation*

Acknowledgement: *This work was supported by a grant of the Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-TE-2016-0973 and by a mobility grant of the Romanian Ministry of Research and Innovation, CNCS - UEFISCDI, project number PN-III-P1-1.1-MC-2019-0549, within PNCDI III.*

H2

IODINE LEVELS AND DIETARY GOITROGENS IN SOME LOCALLY GROWN GRAINS CONSUMED IN SOKOTO STATE, NIGERIA

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Environmental ingredients such as soil, water, food and salt are influential in human and animal iodine nutritional status. Research to date, on iodine levels, has been predominantly conducted in neighbouring states of Sokoto. Few studies, if any, are available on iodine levels and some of its goitrogenic factors in the study area. This cross sectional study designed to assess iodine levels and some dietary goitrogens in selected grains from three geo-political zones of Sokoto State. Grains iodine concentrations were determined by cerium IV reduction method. Cyanogenic glucosides content was determined by hydrocyanic acid method. Glucosinolates was estimated enzymatically. Thiocyanide was measured by benzidine hydrochloride method. Totalpolyphenols were determined by oxidation of phosphotungustomolybdic method. There was a significant difference ($p<0.05$) in grains iodine levels between SWZ and SEZ. For dietary goitrogen, total polyphenols were significantly ($p<0.05$) different between SCZ and SEZ. The grains grown in the study area are generally iodine deficient and these do not produce sufficient iodine to reach the adults recommended dietary allowance of 150 µg day. Therefore, grains grown in this state, are not considered good sources of dietary iodine.

Keywords: *Iodine, levels diatery goitrogens grains, geo-political zones, Sokoto State*

Acknowledgement: *Our sincere appreciation goes to Sokoto State Government especially the State Ministry of Environment for granting us permission to conduct this study. We also want to appreciate Tetfund for making this work real*

H3

A NEW FLUORIMETRIC ASSAY AIMING TO MEASURE CARBONYL CONTENT IN BOTH SOLUBLE AND INSOLUBLE PROTEIN FRACTIONS OF MUSCLE FOODS

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Muscle foods, particularly fish products are highly exposed to oxidative stress during processing and storage, resulting in oxidative modification of proteins. Protein oxidation reactions are known to cause decrease in sensory and nutritional quality, as well as unwanted changes in textural characteristics of muscle foods.
Despite rapid advances in high-throughput protein analyses, determination of protein carbonyls in insoluble protein fractions such as membrane and scaffold proteins, has been a major challenge in biological and food science, and so far remains a bottleneck in quantitative analysis. Accumulation of carbonyls is a dynamic process occurring under oxidative stress and thus spatial cellular and tissue carbonyl distribution in both soluble and insoluble protein fractions might change over time. However, total carbonyl content has only been studied in soluble protein fractions by a number of analytical techniques based on a specific chemical derivatization of carbonyl groups with hydrazines, hydrazides and hydroxylamines. The derivatized carbonyl groups can be detected spectrophotometrically by using ELISA kit or Western blot method, as well as chromatography, mass spectrometry, and fluorescence spectroscopy. Therefore, it was extremely important to develop new methods to analyse carbonyl spatial distribution and content in muscle foods to attribute carbonyl levels to certain soluble and insoluble protein species.
We developed a simpler, faster and cheaper method to assess carbonylated protein level in muscle foods, including both soluble and insoluble protein fractions, which is based on a direct reaction of protein carbonyls with 7-(diethylamino)coumarin-3-carbohydrazide (CHH). For the assay development, a direct fluorescent labelling of protein extracts by coumarin-hydrazide was used. The proposed fluorescence microscopy assay represents a rapid, non-invasive and cost-efficient method aiming to not only quantify carbonyls in both soluble and insoluble proteins, but also provide information on their spatial localization and distribution in the tissue by fluorescence imaging. The protocol was verified for proteins extracted from different fish species and compared with DNPH-based immunocytochemistry.
Thus, a novel fluorescence non-invasive detection method proposed in the study can help estimate the carbonylation extent in both soluble and insoluble proteins, considerably reducing sample amounts in comparison with existing methods such as ELISA or Western blotting.

H4

EVALUATION OF BEEF CONSUMPTION PATTERNS OF ROMANIAN CONSUMERS

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The beef intake in Romania is very low compared to other states from European Union according to FAO data: Romania 4,7 kg/head/year - France 23.8 kg/head/year or Germany 13.1 kg/head/year (FAO Food consumption, 2013). This might be due firstly to the scarcity of traditional gastronomic preparations with beef, and secondly, due to lack of beef cooking knowledge of population. Beef cuts options available on Romanian market are *Black Angus* tenderloin, t-bone steak, ribeye steak, sirloin steak and brisket. *Black Angus* is considered an ideal choice for a successful sensory experience. For the last 15 years, Romanian *Black Angus* farms were created to cover the need of HORECA channel.

The objective of this study was to evaluate the consumer's preferences and habits patterns about beef consumption. To achieve this goal, a survey with 16 questions was distributed online to consumers (n=115, from which 9% were removed from data interpretation because they were non-consumers of beef). The participants responded about the following topics: (1) frequency of beef consumption, (2) motivation of beef consumption, (3) purchasing channels, (4) the most preferred beef cut, (5) selection criteria of beef cut, (6) preferred degree of doneness, (7) most frequently consumed gastronomic preparations with beef, (8) inspirational channels for gastronomic dishes with beef and (9) demographic data. The data were analyzed on Microsoft Office Excel.

The results indicated that the consumers prefer beef meat for its taste (33%) and due to perception of beef as being a healthy product (44%). Most of consumers (57%) prefer to buy meat from a shop dedicated to meat distribution. This result might explain the high score (3.94) obtained by the manufacturer in the selection criteria of beef, compared to maturation degree (3.20), packaging (3.15) and Angus species (3.06). Only 8.6% of respondents have beef intake a few times per week, and more than 90% have beef less than once in a month. This low rate of beef intake might be due firstly to high price of beef meat on Romanian market compared to other types of meat available on the market. Secondly, in Romanian gastronomy fresh beef meat is traditionally used to prepare the only four dishes with beef: beef soup, steak, schnitzel, "mici" (a Romanian traditional sausage grilled without membrane prepared from mixed meat – beef and pork).

In conclusion, more consumer education is needed, with focus especially on the beef consumption quality, recipes with beef and quality criteria of beef. Labeling of the products with information about the beef cuts and appropriate cooking methods for the specific cut, might be helpful to increase the beef intake.

Keywords: *beef consumers, matured meat, Black Angus, consumption patterns*

H5

NURTIONAL PROFILE AND ANTIOXIDANT POTENTIAL OF SARCOCEPHALUS RUSSEGGERI FRUIT

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The proximate, minerals, fatty acids, anti-nutritional factors, total polyphenols, flavonoids, antioxidative vitamins and radical scavenging activity of *Sarcocephalus russegeri* fruits were evaluated using standard methods. The *S. russegeri* fruits have 73.67% moisture, 4.28% ash, 8.78% crude protein, 12.46% crude fibre, 14.28% crude lipid, 60.20% available carbohydrate and 404.44 kcal/g of energy value. The fruit mineral content (in mg/100 g Dry weight) are: Na (406.66), K (5933.33), Ca (43.53), Mg (58.14), P (43.99), Fe (4.64), Zn (3.00) and Mn (1.02).The anti-nutritional agents present include phytate (7.60 ± 0.20 mg/100g), oxalate (5.55 ± 0.15 mg/100g), tannins (19.48±0.03 mg/100g), HCN (6.90±0.02 mg/100 g) and nitrate (0.01±0.003 mg/100 g). To predict the effect of phytate and oxalate on Ca, Fe and Zn bioavailability, phytate/oxalate to nutrients ratios were calculated. The calculated [Oxalate]/[Ca] (0.06), [Oxalate]/[(Ca+Mg)] (0.02) and [Phytate]/[Ca] (0.1) molar ratios are below the critical level of 2.5, 2.5 and 0.5 respectively. However, [Ca][Phytate][Zn], [Phytate]/[Zn] and [Phytate]/[Fe] are above 0.5, 1.5, and 0.4 respectively. This is an indication that Ca, Fe and Zn may be hindered by the phytate content of *S. russegeri* fruit. The composition of fatty acids in the fruit was saturated fatty acids (51.39%), monounsaturated fatty acids (26.05 %) and polyunsaturated fatty acids (13.37%). The bioactive compounds found in the fruit were flavonoids (12.97±0.05 mg/100ml), phenolics (9.52±0.02GAE/g), vitamin A (0.01±0.004 mg/100g) vitamin E (4.86±0.10 mg/100g) and vitamin C (2.80±0.12 mg/100g). The radical scavenging activity was determined and the IC50 value was 0.32.

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H6

OCCURRENCE OF HISTAMINE IN FRESH AND MARINATED FISH COMMERCIALLY AVAILABLE IN POLAND IN 2014-2018

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Histamine is biogenic amine naturally occurring in the human. This amine is also present in certain foods like chess, fermented meat products, wine or fish and fish products, containing free histidine and is formed by bacteria during spoilage in fish and products. Formation of histamine in fish is related to free histidine content in fish muscle and the presence of bacterial histidine decarboxylases produced by microorganisms such as *Morganella morganii, Morganella psychrotolerans, Photobacterium damselae, Photobacterium phosphoreum, Raoultella planticola, Hafnia alvei, Klebsiella pneumoniae, Escherichia coli, Staphylococcus, Vibrio* and certain environmental conditions. The criteria for histamine are laid down (in Commission Regulation No. 2073/2005 on microbiological criteria for foodstuffs) only for fish, fishery products and fish sauce produced by fermentation of fishery products. The histamine concentration in fishery products from fish species associated with a high amount of histidine, except fish sauce produced by fermentation of fishery products, is considered satisfactory if among tested nine samples, the average concentration is ≤ 100 mg/kg, a maximum level of histamine of two samples has a concentration between 100 and 200 mg/kg and none of the sample exceeds the limit of 200 mg/kg. Batch of fish/fishery products is unsatisfactory, if the mean value observed exceeds 100 mg/kg or more than two values are between 100 and 200 mg/kg or one or more of the values observed are > 200 mg/kg. Histamine occurs in many species of fish with dark meat with higher level of histidine, particularly fish species of the families: *Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae* and *Scombresosidae*. High contamination of histamine, in fish and fish products may cause food poisoning in humans. Scombrotoxic fish poisoning (SFP) is associated with the consumption of contaminated fish from *Scombridae* family. SFP is a chemical intoxication and causes many symptoms in humans.
The aim of this study was to determine the level of histamine contamination in all species of fresh fish and marinated fishery products available on Polish market. Histamine was determined by high performance liquid chromatography with diode array detection (HPLC-DAD). In our research a total of 347 samples of fish and 43 samples of marinated/salted fish were investigated. In 39 (11.2%) samples of fresh fish the concentration of histamine ranged 3.64 – 42.40 mg/kg, except only one sample of salmon which contained 153.4 mg/kg of histamine. In 40 samples of marinated fish (herring) histamine concentration was between 3.88 to 121.94 mg/kg in 33 samples. In three samples of salted herrings the concentration of histamine was in range of 11.99 to 219.98 mg/kg (two samples contain more than 200 mg/kg. This study showed that fresh fish available on Polish markets were safe for consumers. However, marinated herring consumed in excess can cause consumer SFP poisoning

Keywords: *histamine, fresh fish, marinated fish, SFP, HPLC-DAD*

H7

ALIZARIN RED S DETECTION AND EVALUATION OF THE BIOACCUMULATION POTENTIAL USING THE EUROPEAN EEL AS AN EXAMPLE

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Alizarin red S (ARS) is an often used chemical marker substance for fish. It is an important tool for fish stock monitoring and large-scale management programs. For the endangered European eel (Anguilla anguilla) ARS turned out to be the most promising option for standard mass marking. However, due to lack of data regarding the accumulation potential of ARS in edible muscle tissues, not only for eels but for fish in general, a statement was concluded by the German Federal Institute for Risk Assessment aspired to define whether marking of eels with ARS could pose a risk to the health of consumers. In the present study we developed a liquid chromatography–mass spectrometry (LC-MS/MS) protocol for ARS detection and estimated the bioaccumulation potential of ARS in muscle tissue using the example of the European eel. By developing and optimizing the detection method for ARS in fish muscle tissue, a detection limit of 8.9 µg kg^{-1} could be achieved. In total, 250 marked eels which differ in size (6.74 – 57 cm) and time after marking (0 days up to 3 years) have been analyzed for ARS content. The highest ARS concentration of 6056.75 µg kg^{-1} fish muscle was detected immediately after marking. Already one year later the ARS concentration was below detection limit. A new method for ARS detection in fish muscle tissue, followed by an application on recaptured marked eels was able to show that ARS accumulation in the fish muscle is highly unlikely.

Keywords: *anguilla anguilla, Alitarin Red S, bioaccumulation potential, LC-MSMS, fish marking*

H8

EVALUATION OF NUTRITIONAL POTENTIAL OF FICUS CARICA FRUITS

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The proximate, minerals, amino acids and anti- nutrients compositions of *Ficus carica* fruit were evaluated. The proximate analysis revealed the following: moisture 81.67%, ash 4.33%, crude lipid 11.67%, crude protein 4.42%, carbohydrates 75.75%, crude fiber 3.38% and energy value of 425.10kcal/100g on dry weight basis. Magnesium (182.55mg/100g), calcium (57.44mg/100g), and potassium (268.33mg/100g) were the predominant macro elements present in the fruit. Iron (17.50mg/100g), manganese (1.04mg/100g) and copper (1.98mg/100g) were the micro elements detected in appreciable amount. Essential amino acids were above the recommended level by Food and Agricultural Organization/World Health Organization (FAO/WHO) for adults. The results of anti-nutrients to nutrients molar ratios are below the critical levels known to inhibit the availability of some minerals element. The present investigation showed that *Ficus carica* fruits are rich source of many important nutrients that appear to have a very positive effect on human health.

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H9

FEASABILITY OF ALTERNATIVE ATMOSPHERIC PRESSURE IONIZATION SOURCES TO REDUCE MATRIX EFFECT IN FOOD ANALYSIS

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Nowadays, chromatography coupled to mass spectrometry plays an important role in food analysis. The determination of contaminants at low concentration levels or the identification of possible adulterants, as well as the use of mass spectrometry profiles to facilitate the food characterization, are some of the important and frequent applications in this field. Among the mass spectrometry related techniques, liquid chromatography coupled to mass spectrometry (LC-MS) is the techniques often chosen to determine a wide range of compounds (from relatively no-polar compounds to polar and ionic compounds). The sensitivity, selectivity and the unequivocal confirmation are some of the main characteristics appreciated in these techniques. The ionization in LC-MS is commonly performed by electrospray (ESI), even the well-known important influence of the sample matrix and other coeluting compounds in the ionization of target analytes, which results in ion signal suppression or ion signal enhancement thus hindering the quantitative analysis.
In this work, the feasibility of alternative atmospheric pressure ionization sources to the electrospray is evaluated. Electrospray (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) capabilities to overcome matrix effects in food analysis are compared. In this way, both the ionization performance and their behavior in the quantitative analysis using different calibration methods (external calibration, matrix-matched calibration, etc.) are compared. As examples in this work, the ionization and matrix effect of pigments and banned food dyes when they are determined in both oil and spice samples using API sources are evaluated. The results of these studies show that although the performance of APCI and APPI in terms of precision, selectivity and linearity was similar to ESI, the matrix effect was always below 20%, which allow the quantification of target compounds by external standard calibration and the improvement of the throughput in control laboratories.

Keywords: *matrix effects, food analysis, atmospheric pressure chemical ionization, atmospheric pressure photoionization, electrospray*

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H10

SALT CONTENT IN BREAD: STUDY OF THE INFLUENCE OF THE ANALYTICAL STRATEGY AND PREPARATIVE STAGE ON THE COMPARABILITY OF RESULTS BETWEEN LABORATORIES. ANALYSIS BY ION CHROMATOGRAPHY AND ATOMIC ABSORPTION

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Noncommunicable diseases (NCDs) are the main contributor to mortality and morbidity globally. Elevated sodium intake has been associated with a number of NCDs (including hypertension, cardiovascular disease and stroke), and decreasing sodium intake may reduce blood pressure and the risk of associated NCDs. Populations around the world are consuming much more sodium than is physiologically necessary following World Health Organization (WHO) recommendations: 2 g sodium/day (equivalent to 5 g salt/day).
WHO members have agreed to reduce 30% the salt intake before 2025. To reach this aim, the starting point of each country around the world is very different. A global review has identified 59 programs to reduce salt intake, 38 countries with programmed aims and 9 countries with laws for some foods. 17 countries have informed to have already reached reductions, mainly for bread. In Spain the average salt intake is 9.8 g/day, 19% of ingested sodium coming from bread.
The amount of salt in food can be obtained from chloride-based analytical methods and sodium-based analytical methods. The different ways of treating the samples have great influence in the final results. Authors do not agree if different techniques are equivalent.
In 2005 a first project was launched in Spain to reduce the salt content of bread. Afterwards some other local projects have been carried out. As each study has followed a different process of sample treatment and sometimes, a different analytical technique, this gave way to study if these differences have a significant influence in the results. So, sample drying temperature and time, the way of extraction of chloride, and performing the analysis of chloride by ion chromatography (IC) or the analysis of sodium by atomic absorption (AA) are to be studied.

Keywords: *salt, bread, extraction, ion chromatography, atomic absorption*

H11

NITROGEN/PROTEIN AND CHNS DETERMINATION OF INSECT-BASED FOOD BY FLASHSMART ELEMENTAL ANALYZER FOR FOOD QUALITY AND LABELING

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Regulations require that labels of food products report on their energy values, to ensure transparency for consumers and enabling them to perform quality/price comparison. Protein content of food products is important from many points of view: legal, nutritional, health, safety and economical. Over the last few years, insects-base food has been legalized in various countries and its demand has increased, although averagely it is more expensive than other alternatives. Insect-base food contains high amount of proteins and has low value in fats. For this reason, the accurate determination of the protein amount, through the determination of nitrogen, is fundamental to report the nutritional value of finished products. The Dumas method (combustion method) has shown to have greatly improved the determination of nitrogen, making analyses 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). The Thermo Scientific FlashSmart Elemental Analyzer, based on the dynamic flash combustion of the sample, meets a wide array of important requirements of laboratories such as accuracy, day by day reproducibility and high sample throughput. As in the last years the cost for helium, which is used as carrier gas for the Flash*Smart* EA, has increased. For this reason, the Flash*Smart* EA can use argon as alternative gas, ensuring the same analytical performance.
In this presentation data on Nitrogen/Protein and CHNS determination of insect-based food products, obtained with an analytical system based on dynamic flash combustion of the sample are reported. Also, the performance of the system and the reproducibility of the results obtained is shown, illustrating also the performance by using argon as an alternative carrier gas.

Keywords: *labelling, QA/QC, CHNS, combustion, food quality*

H12

OPTIMIZATION POLYPHENOLS' EXTRACTION FROM FOODS OF PLANT ORIGIN

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Antioxidants present in large amount of food products are characterized by high beneficial effects on our health and well-being. They play significant role in prevention of diseases of affluence. Spinach is an example of food of plant origin rich in antioxidants, especially polyphenols. This green, leafy vegetable has low energy content and is being increasingly consumed, but frequently after processing.
The aim of this work was to optimize the extraction of antioxidant compounds from foods of plant origin, based on the example of spinach. In addition, the effect of processing on the antioxidative capacity of the prepared spinach samples, fresh and frozen, dried by various methods, was investigated and compared. For this purpose, the samples of both types of spinach were prepared by drying at a temperature of 40°C and freeze-drying. In addition, fresh spinach leaves were dried at room temperature. Then, samples were homogenized and the prepared material was used in further analyzes.
To find the efficient extraction method several parameters were checked. Firstly, the best solvent with optimal proportions was found, among acetone, methanol, isopropyl alcohol, ethanol and acetonitrile. Afterwards, three extraction techniques were used - assisted by shaking, ultrasonic and microwave. Temperature and time of analytical approaches were also optimized. The results were assessed using Folin-Ciocalteu method, which is preferable method to evaluate polyphenols content.
Based on the obtained results, it was concluded that the highest absorbance values corresponded to extracts prepared during a 25-minute extraction using microwaves at 110°C. The best solvent was acetone mixed with water in the ratio 1:1. Using those parameters and Folin-Ciocalteu method, validation process was performed and the obtained results were highly satisfying (recovery: 101-102%, RSD: 1.11-1.83%).
Spinach, which leaves were subjected to the lyophilization process, characterizes with the highest antioxidant potential. It has also been shown that eating fresh spinach leaves, instead of processed ones, is much more beneficial for the consumer in terms of the antioxidant compounds content in the product consumed.

Keywords: *antioxidant potential, extraction, polyphenols, spinach*

H13

ENANTIOMERIC DETERMINATION OF CYSTEINE BY CAPILLARY ELECTROPHORESIS USING Γ-CYCLODEXTRIN AS CHIRAL SELECTOR OR ITS COMBINATION WITH IONIC LIQUIDS

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In the last years, ionic liquids (ILs) have attracted great attention in separation techniques. In particular, ILs with a chiral anion and/or cation have been used in capillary electrophoresis (CE) as chiral selectors mainly in combination with other selectors such as cyclodextrins (CDs) and antibiotics [1]. This combination in dual systems has been mainly employed for the separation of model drugs and to a lesser extent for amino acids.
The aim of this work was to develop new analytical methodologies by CE to achieve the enantiomeric separation of the protein amino acid Cysteine (Cys), the most abundant biothiol in plasma and serum, which is supplied from food and as a product of homocysteine synthesis [2]. With this purpose, γ-CD and a novel chiral ionic liquid (CIL) were used as sole chiral selectors and their combination in a dual system was also evaluated. To carry out this study, Cys was derivatized with 9-fluorenylmethoxycarbonyl chloride (FMOC-Cl). The use of γ-CD as the sole chiral selector or of a dual system based on the combination of γ-CD/CIL in phosphate buffer at pH 7.0 enabled to achieve the enantiomeric separation of Cys in a short analysis time (less than 8 min) with high resolution values (2.6 and 5.0 for the single and dual system, respectively). The combination of both selectors originated a reversal in the enantiomeric migration order for Cys, as observed in a previous work for homocysteine [3]. To demonstrate the suitability of the developed methodologies, their analytical characteristics were evaluated and the methods were subsequently applied to the quantification of L-Cys in food supplements.

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[3] M. Greño et al. J. Chromatogr. A, 2018, 1568, 222-228

Keywords: *chiral capillary electrophoresis, chiral ionic liquid, cyclodextrin, cysteine, food supplement*

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H14

ANALYSIS OF UNDERIVATIZED AMINO ACIDS IN WINE BY HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY COUPLED TO SINGLE QUADRUPOLE MASS DETECTOR

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Amino acids in grapes are a very important nitrogen source for wine yeast growth during alcoholic fermentation and also influence the organoleptic properties of wine as precursors of alcohols, aldehydes, and esters. The amino acid composition in wine is influenced by yeast strain, fermentation conditions, the grape variety, and the terroir. Proline is the predominant amino acid in wine, reaching 30% to 85% of total amino acid content.[1] Since proline is poorly consumed by wine yeast during anaerobic alcoholic fermentation[1], it can be used to differentiate wine products according to grape varieties and terroirs, and can also be useful for quality control purposes. Accurate analysis of amino acid content during wine manufacture and aging can be of great value, in terms of the monitoring of levels of nitrogen available to wine yeast and the differentiation of products, as well as the detection of adulteration.
For the analysis of amino acids in food and beverage, liquid chromatography methods with pre-or post-column derivatization have been most widely used. Although the derivatization technique enables retention and separation of polar amino acids on reversed-phase columns with sensitive detection by UV- or fluorescence detectors, this method has some drawbacks such as reagent interference, a required sample handling step and resultant additional labor and exposure to toxic chemicals for the analyst, as well as systematic and random errors during the sample handling step.[2]
Hydrophilic interaction liquid chromatography (HILIC) combined with mass detection is an alternative for the analysis of amino acids because amino acids can be directly analyzed without derivatization. HILIC is a good option for the retention and separation of the amino acids, which are mostly highly polar compounds. The use of a mass detector allows accurate quantification of analytes in complex samples despite incomplete separation of the analytes.
In this presentation, a HILIC-single quadrupole mass detection method for analysis of 22 underivatized amino acids will be reported, including the optimization of buffer salt concentration and the ion source parameters in the mass detector, as well as method validation. The addition of ammonium formate buffer of 20 mM to the mobile phase resulted in the enhancement of peak shape and separation. Application of the developed method to a wine sample will be discussed, especially with a focus on quantification of 17 amino acids that were reported previously[1], using external calibration. Finally, proline quantification using an isotopically labeled internal standard will be also discussed, with the comparison of internal and external results.

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H15

ELECTROACTIVE NANOCARBON FOR FOOD ANALYSIS

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The use of an electroactive nanocarbon material as the electrochemical label in the polymerase chain reaction (PCR) for DNA detection is being investigated due to the various advantages it presents over the more commonly employed fluorescent labels such as simpler protocol, lower cost of materials and potential enhancement in the specificity and efficiency of the PCR process.
The effectiveness of graphene oxide nanocolloids (GONC) as electrochemical label for PCR was demonstrated by monitoring the intrinsic reduction signal after conjugating GONC material with single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). GONC labelled DNA primers were then employed for PCR amplification of the 35S promoter sequence, which is commonly found in genetically modified food supply.
We found that upon conjugation of GONC material to both ssDNA and dsDNA, a significant reduction peak was still observed from the intrinsic electroactivity of GONC, with a linear relationship between the current intensity and GONC-DNA conjugate concentration. Finally, the suitability of GONC for electrochemical PCR was confirmed by the use of GONC labelled primers for PCR amplification of the 35S promoter sequence in genetically modified maize powder, where an amplified band for the PCR product was observed after gel-electrophoresis.

Keywords: *electroactive nanocarbon, graphene oxide, food analysis, PCR, electrochemistry*

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H16

RELIABLE CHARACTERIZATION OF FUROCOUMARINS IN BEVERAGES AND FOODS THROUGH LIQUID CHROMATOGRAPHY COUPLED TO TRIPLE QUADRUPOLE MASS SPECTROMETRY IN COMBINATION WITH THE LRI SYSTEM

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In the last decade, most of the scientific articles has focused on the evaluation of adverse effects connected with coumarins (C) and furocoumarins (FC) dietary intake. Many effects are due to the oral administration of FC; however, only the content of coumarin in bakery products is currently restricted by the official Regulation of the European Parliament [1]. In the meantime, the European Food Safety Authority suggested to maintain the tolerable daily intake of 0.1 mg coumarin/kg bw allocated in the 2004 Opinion [2]. FC are naturally occurred compounds in citrus species and together with C and polymetoxyflavones (PMF) are the main components of the non-volatile fraction in citrus essential oil. PMF are well known for their antioxidant properties, whereas C and FC are currently under investigation due to new evidences concerning their harmful effects on human health, especially after ingestion [3].
The main goal of this research was to validate a new sensitive HPLC-MS/MS method to analyse oxygen heterocyclic compounds (OHC) at trace level in finished products. The qualitative and quantitative profiles of these components, are characteristic of each specie, therefore are commonly used to characterize essential oils. In this research, the main purpose was the development of a new method to quantify OHC at trace level, in citrus beverages and foods.
Taking into account the usefulness of a new analytical approach thought for the quality control of FC in food, the method was based also on the application of the Linear Retention Index system (LRI). The retention data provided by the LRI were used in combination with Full Scan and MS/MS (in Multiple reaction Monitoring acquisition mode) spectra, to achieve a more reliable identification, especially in case of fully automated data processing, when the library alone could fail, for instance in case of isomer compounds. Calibration curves of 35 targets were created in MRM mode and limits of quantification resulted in very low values. Several *Citrus* beverages, among alcoholic and non-alcoholic drinks (home-made and commercial) were analysed. For some sample the amount of FC was significant. The content of OHC could be related to the production procedures, or to the addiction of specific ingredients such as citrus oils, distilled or cold pressed, or citrus extracts or juices.
Based on the results obtained, the regulatory bodies should carefully evaluate the risk related to OHC dietary intake for the formulation of potential new Opinions in this field.

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[2] Coumarin in flavourings and other food ingredients with flavouring properties. The EFSA Journal (2008) 793, 1-15.
[3] Girennavar, B., et al. Bioorg. Med. Chem., 2006, 14:2606-2612.

Keywords: *furocoumarins, HPLC-QqQ, linear retention index, citrus beverages, quality control*

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H17

DETERMINATION OF PFAS IN DIFFERENT COMPLEX BIOTA MATRICES

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Per- and polyfluorinated alkyl substances (PFAS) are a large group of man-made organic compounds where at least one carbon is fully fluorinated. These compounds are widely used since the 1950s in multiple applications and are a byproduct of manufacturing non-stick materials. PFAS are a large, diverse and ever expanding group of more than 4700 individual substance, including perfluorinated carbonic acids as well as more complex substances. The health effects of PFOS, PFOA, PFHxS, and PFNA have been studied relatively well. Some observed effects were: interference with the body's natural hormones, affecting growth, learning, and behavior of infants and older children and others. They may also increase cholesterol levels, affect the immune system and increase the risk of cancer. Since 2009, PFOS and PFOA are listed in Annex B to the Stockholm Convention (decision SC-4/17) and there are numerous health based regulatory values and evaluation criteria in regional to international guidelines. Here, we provide a method for the determination of PFAS in soil, plants, meat and food products. In Organic samples like plants, animal tissue or food extraction is even more critical, since different PFAS may accumulate in different structures and the tissue needs to be degraded to release all enclosed compounds. Furthermore some organic compounds can impede the subsequent LC-MS/MS analysis and have to be carefully removed from the sample. A two stage SPE clean up can be applied to address complex matrices. Since every method shows suits specific groups of PFAS, it is the easiest way to restrict analysis to PFOA and PFOS. However, depending on the source of PFAS contamination these may not be the predominant analytes. Especially if the source of a contamination is not known, it is necessary to broaden the scope of analysis. It is now possible to measure roughly 30 to 40 specific PFAS, for which standards are available with increasing trend.

H18

IMPROVING SAMPLE TREATMENT METHODOLOGIES USED TO MONITOR CHANGES RELATED TO THE OXIDATIVE DEGRADATION OF PEANUTS

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Peanuts are one of the most widely used legumes as a snack food due to its taste and nutritional value. Among its basic components, fatty acids are the major component of peanuts; they are present with higher of 50 % w/w.
On the one hand, this fact prevents cardiovascular disease. Although, high content fatty acids can cut their shelf-life down making their post-harvest handling and storing complex. The main problem is when of polyunsaturated fatty acids (PUFAs) are exposed to light and air, they undergo oxidation reactions and produce undesirable flavours.
This work is part of a research project which investigates different ways of reducing the spoilage of peanuts. Consequently, in this work we were focusing on developing methodologies to monitor changes in their quality over time. Traditionally, changes in the fat fraction composition are evaluated after extraction of the oil of the samples. This step is both time and reagents consuming, hence efforts devoted to minimize the sample treatment are worth. To this end different alternative sample treatment methodologies were tested in order to improve (i.e. reduce or symplify) the complexity of the sample treatment procedure.
Values of different physicochemical parameters were determinde in peanuts, as welll in peanuts oil, stored under different conditions (i.e. several temperatures and oxygen levels). Fatty acids content, tocopherol content, FTIR analysis and volatiles profile were monitored during the study. Different procedures of oil extraction were employed; a commercial fat extractor and an electronic press oil machine. The use of the direct milled peanuts, that is without oil extraction, was also tested. Results showed that no differences were found when the analysis was carrie out using the milled nut when compared to the results registered after oil extraction for all parameters. Hence a reduction in the analysis time and a reduction in the analysis costs were noted.

Keywords: *lipids, oils, peanuts, oxidation*

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H19

THE DEVELOPMENT OF A MULTICLASS METHOD FOR ANALYSIS OF PERSISTENT ORGANIC POLLUTANTS USING GC ORBITRAP TECHNOLOGY

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The conventional method of quantitative analysis of multiclass persistent organic pollutants (POPs) in food and environmental samples is usually time and cost-consuming. It requires multiple sample preparation steps and distinct chromatographic and mass spectrometric methods for each compound class. The development of a multiclass method for the simultaneous analysis of a wide range of POPs becomes very important for routine sample testing in food and environmental laboratories. In the current study, a new multiclass method for the analysis of polyaromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and chlorinated paraffins (CPs) utilizing a Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer is developed. It offers shorter analysis time, superior selectivity through chromatographic and mass resolution and low limits of quantification for over 100 compounds belonging to six compound classes. In addition, the method has the flexibility to provide additional data for targeted or non-targeted screening. It will be applied to routine process and a customized mass spectral library will be constructed for further optimization of the process. Moreover, the development of a multiclass sample preparation method as well as an automation of the overall sample preparation and analysis process are in progress.

H20

ELEMENTAL ANALYSIS – A QUICK AND EASY WAY TO MEASURE THE "RISK POTENTIAL" OF PALM OIL

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Palm oil is widely used in the food industry. Due to its easy accessibility, good functional properties, and low prices, palm oil enjoys tremendous popularity as an ingredient in many products, such as margarine, bakery products, confectionary, and sausages, or pure as oil. But there are also drawbacks. Aside from the contentious debate about its impact on the environment, there is another, less frequently illuminated aspect – harm from the carcinogenic effects of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters. It is formed during the production process, when naturally occurring chlorine-containing compounds in palm oil are exposed to temperatures above 150 °C. In order to minimize the 3-MCPD content, not only final products but also starting and intermediate materials must be monitored so that it is possible to intervene early with regulatory measures. Determination Identification of the chlorine content has established itself as a quick method to assess the risk potential. Elemental analysis is the most suitable technology here, given the widely varying matrix properties of the samples to be analyzed, the desire to differentiate between organically and inorganically bound CI compounds, and the broad concentration range.

Keywords: *palm oil, 3-MCPD, elemental analysis*

H21

ANALYSIS OF TRACE IMPURITIES IN SUNFLOWER OIL BY HR ICP-OES

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As a major constituent of food, sunflower oil is subject to many regulations concerning food safety, for example, decree 1881/2006 of the European Union, which defines limits for toxic heavy metals (e.g., Pb, Cd, Hg) in foodstuffs. On this basis, trace impurities originating from cultivation farming (fertilizers, air pollution), oil extraction, and refining processes (catalysts) need to be analyzed for purposes of food safety. At the same time, nutritional properties need to be ensured by monitoring the contents of macro minerals like phosphorus, calcium, or magnesium. For the analysis of edible oils, unspecific spectral emission lines originating from the organic matrix often hamper the analysis of trace elements like Phosphorous due to spectral interferences. The here discussed methodology involves a simple sample preparation via dilution in kerosene and the subsequent direct measurement with a high-resolution ICP-OES in order to avoid troublesome interferences and to achieve improvements in the quality of the baseline fit, precision and method robustness and limits of detection.

Keywords: *edible oil, sunflower oil, ICP-OES*

H22

QUANTIFICATION OF LYSOZYME IN CHEESE BY LC-MS/MS

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Lysozyme, an enzyme present in egg white, is used as food additive under the number E 1105. Lysozyme acts as preservative by inhibiting bacterial growth. Eggs are listed as food allergens, and thus E1105 has to be declared as allergen. Lysozyme is often used during the manufacturing of certain cheeses. It is also reported that Lysozyme is used in kimchi pickles, sushi, Chinese noodles and wine production. Currently, lysozyme is quantified by ELISA assays in various matrices. Specifically in cheese, HPLC methods with UV detection are also used. To avoid potential matrix effects, enhance specificity by comparison to UV detection and to enable multiplex detection with other allergens, an LC-MS/MS method was developed to detect specific lysozymes peptides in cheese.

Cheese samples were grated and suspended in a NaCl buffer for Lysozyme extraction and centrifuged. Proteins from the supernatant are reduced with dithioerythritol and free cysteines are alkylated with iodoacetamide prior to tryptic digestion. After desalting on C_{18} solid-phase extraction cartridges, the digests are injected into a liquid chromatograph hyphenated with an Orbitrap Q-Exactive tandem mass spectrometer (Thermo Scientific, Palo Alto, CA). For control and method development, the same method was used on lysozyme. Digested lysozyme peptides were assigned to their respective peptide sequences using their precursor and fragment ion masses. For quantitative analysis, internal standard peptides labelled with ^{13}C and ^{15}N to achieve a mass difference of 9 units were used (Thermo Scientific, Palo Alto, CA).

Lysozyme digests were used to identify proteotyptic peptides for quantification. The presence of these peptides was subsequently confirmed in Grana Padano cheese with declared additive E 1105. Lysozyme peptide 33-44 (FESNFNTQATNR) and 116-124 (GTDVQAWIR) were selected based on their specificity, the absence of methionine and cysteine and their fragment ion abundance. The two peptides were not observed in digests of Parmigiano Reggiano DOP cheese, where addition of E 1105 is not allowed. Next, heavy-isotope labelled winged forms of the two selected peptides (*i.e.* the same peptides with two amino acids on the N-terminal side and three amino acids on the C-terminal side) were used as internal standard for lysozyme quantification. Such winged peptides have the advantage of at least partially normalizing the tryptic digestion efficiency of cheese samples. Preliminary results show that accurate quantification of Lysozyme can be achieved over a large concentration range. Also, application of the same method for different food matrixes as well as processed food seems possible.

Keywords: *protein, allergen, mass spectrometry, cheese, authenticity*

H23

RELATIVE RESPONSE FACTOR-BASED SIMULTANEOUS DETERMINATION OF TOCOLS, SQUALENE, AND PHYTOSTEROLS IN RICE BY USING GC-FID AND GC-MS

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Traditionally rice has been an important food crop for carbohydrate intake. Recent consumer preferences, however, are changing into higher palatability and health beneficial effects of rice. Studies on nutritional value of rice include quantitation of phytochemicals, during which cautious handling and management of authentic standards are required, especially when external standard-based calibrations are adopted. To develop a more stable and convenient analysis method to quantify lipophilic phytonutrients in rice, rac-tocol (CAS 119-98-2) was selected as an internal standard (IS) and relative response factors (RRF) of α-, β-, γ-, δ-tocopherols (T) and tocotrienols (T3), squalene (SQ), campesterol (CA), β-sitosterol (SI), and stigmasterol (ST) relative to IS (*m/z* 388) were determined by injecting authentic standards of 5 different concentrations into GC-FID and GC-MS. All phytochemicals showed acceptable linearity (R^2>0.98) within tested concentrations rage and intra- and inter-day repeatability (RSD<5% in GC-FID and <10% in GC-MS). The RRF values of each phytonutrient obtained from GC-FID and GC-MS (*m/z*) were as followings; α-T: 0.96, 0.55 (430); β-T: 0.93, 0.92 (416); γ-T: 1.11, 1.10 (416); δ-T: 1.04, 1.18 (402); α-T3: 0.98, 0.88 (424); β-T3: 0.71, 1.24 (410); γ-T3: 0.99, 1.42 (410); δ-T3: 1.10, 3.15 (396); SQ: 0.73, 0.14 (81); CA: 1.06, 3.51 (400); ST: 1.05, 3.21 (412), and SI: 1.09, 3.23 (414). When developed RRF was applied into a study determining phytonutrient contents in brown rice of 184 rice germplasm accessions, results estimated by using RRF showed highly positive correlation (R^2>0.94) with values determined by conventional external standard-based calibration method in all tested phytonutrients. All these results showed practicality of using RRF developed in this report as an expedient method for quantitation of tocols, squalene, and phytosterols in rice.

Keywords: *response factor, rice, vitamin E, quantification, GC-FID*

Acknowledgement: *This work was supported by a grant from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center No. PJ013280), Rural Development Administration, Republic of Korea.*

H24

CHALLENGE: DETERMINATION OF POLYMERIZED TRIACYL GLYCEROLS IN FRYING OILS BY HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY WITH EVAPORATIVE LIGHT SCATTERING DETECTOR (HPSEC-ELSD)

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Frying is one of the most popular food preparation procedures; it is used all over the world. In the frying bath, at temperatures typically ranging between 150 - 190 °C, not only desirable, flavour significant compounds are formed, but also undesirable products are originated from thermally unstable triacylglycerols through chemical reactions such as hydrolysis, oxidation and polymerization.
The major products of these reactions are non-volatile polar compounds and triacylglycerol polymers (TGP). TGP are large molecules with linkages of -C-C-, -C-O-C- and -C-O-O-C- between individual triacylglycerols, the extent of their formation depends on the oil type, frying temperature and time / number of frying cycles. With regards to adverse effects of TGP on a fat/oil nutritive value, TGPs should be monitored as frying oil/ fat quality markers, their content should not exceed 12% (w/w).
For a routine determination of TGP in frying fats/oils, size exclusion chromatography with a refractive index detector (HPSEC-RID) is commonly used. As a detection alternative, evaporative light scattering detector (ELSD) can be employed. The major advantage of ELSD is the possibility of gradient elution and its higher sensitivity, nevertheless, the linear range of its response is rather limited; moreover, it is dependent on a chemical structure of analytes. Under these conditions, the quantification of TGP by internal normalization is a challenging process because there are no standards of TGP to calculate the response factor.
In our study, we developed and optimized HPSEC-ELSD method for determination of TGP in frying oils. For analysis, we used narrow bore columns, which effectively save consumption of mobile phase solvent. The results of frying oils measurements by the new method were compared with those obtained by ´classic´ method employing RID for TGP detection; then conversion factor was calculated. Using HPSEC-ELSD, 56 samples of used frying oils from restaurants and fast foods were measured to assess the quality of fried food in Prague. Only two of them exceeded the limit of 12 % TGP, which is the ´quality limit´ established by some European countries.

Keywords: *polymerized triacylglycerols, HPSEC-ELSD, frying oils*

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H25

NEAR INFRARED HYPERSPECTRAL IMAGING FOR NONDESTRUCTIVE QUANTIFICATION OF FOREIGN MATERIALS IN CEREAL GRAINS

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Cereal grains and seeds are staple foods for humans and represent a very important part of the diet. High quality standards in product specifications compels producers not only to supply the grains without damage (healthy grain) by insects or fungi, but also without foreign materials. Foreign bodies in cereals include damaged kernels, ergot, animal excreta and other cereals (such as barley, rapeseed, maize, oats and rye). Generally, improper cleaning of storage sites and transportation vehicles, inadequate weed control during production, and the inability of the cleaning equipment to remove similar-sized grains are the main reasons for cereals being mixed with other grain types. Traditional methods used to determine the amount of contaminants in cereal are manual and time consuming. To make quick and accurate decisions about the grain quality and grade, robust automated technologies are needed. Near infrared (NIR) hyperspectral imaging (HSI) is a recent technology which has proven to be effective in grain quality inspection.
The objectives of the present study are to evaluate the use of NIR hyperspectral imaging for nondestructive quantification of foreign materials in cereal grains and seeds. In particular, wheat, barley and rapeseed grains were supplied by an important producer industry in Navarra (Spain). The sampling process included two stages: sample collection from different farmers during the harvesting period and hyperspectral analysis. In total, 55 samples were analysed. 10 samples per product were used for classification models construction and 5 for validation. Additionally, 10 samples included mixed cereal grains to verify the performance of the model. For the hyperspectral measurement, 25 g sample was placed inside a Petri dish. NIR hyperspectral images were collected using Specim FX-17 camera working at 900-1700 nm range. Image correction was performed to get the reflectance value of each pixel in full wavelength spectral band.
Principal component analysis (PCA) was used for exploring individual pixels of the images as well as for calculating spectral features in the object-wise approach. The spectral data were pre-treated with standard normal variate (SNV) transformation prior to the analysis in order to remove effects of scattering. Selected PCs were used to compute classification models for the observed groups of samples. The quality of classification was evaluated both visually, by using prediction maps, as well as by calculation of sensitivity, specificity and classification accuracy of the models. The main benefit of the system used in the current study is that it is capable of screening both single and bulk kernels. Because the system uses line-scanning technology, it is possible to scan bulk samples and still identify single objects. Ideally, this is what would be required in industry where bulk samples can be analysed and individual kernels identified and sorted.

H26

RELATIONSHIPS BETWEEN THE ORIGIN OF FEEDSTOCK AND QUALITY OF ORANGE JUICES

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Orange juices represent the most popular soft drink due to their flavour and aroma. The variety and fruit maturity, origin of feedstock, way of fruit harvesting, post-harvest-treatment or preserving technology (pasteurization, application of inert gases) can significantly influence qualitative properties of orange juices.
The aim of the presented work was to study the relationships between the origin of feedstock and quality of stored orange juices (7 °C, darkness, 152 days ~ 5 months). For these purposes, orange juices of different origin of feedstock (Costa Rica vs. Spain, season 2018) were tested to their ability to scavenge $ABTS^{\bullet+}$ and TEMPOL radicals by EPR spectroscopy. Concentrations of total polyphenols compounds (TPC) as well as colour changes were assessed by means of UV-VIS spectroscopy. Changes in concentrations of sugars as well as in ascorbic acid and hesperidin were monitored by HPLC-RID and HPLC-DAD, respectively. Besides that, basic characteristics such as relative density, pH, °Brix, titratable acidity and formol number were monitored in order to obtain complex information on juices. The data obtained were statistically processed by ANOVA and by multivariate statistical methods in order to assess the effect of feedstock origin on the monitored qualitative parameters.
The results obtained confirmed that the quality of analysed orange juices is significantly influenced by feedstock origin, as both groups of samples were unambiguously differentiated by statistical methods. Gradual worsening of all monitored characteristics in analysed orange juices was noticed. Generally, Costa Rican juices were characterized by higher antioxidant activity, ascorbic acid and hesperidin content ($P < 0.05$). Regarding colour, Spanish samples were lighter and total colour difference was lower in comparison with Costa Rican juices.

Keywords: *orange juice, origin of feedstock, quality*

Acknowledgement: *This contribution is the result of the project APVV-15-0023 „Quality and authenticity of fruit juices – study of relationships between the origin of feedstock, processing technology and quality of fruit juices" and of the project ITMS 26220220175 „Improvement of nutritional and sensorial parameters of fruity and vegetable drinks via an inert gases application" implementation, supported by the Research & Development Operational Programme funded by the ERDF.*

H27

QUICK AND COST EFFECTIVE METHOD TO DETERMINE WATER CONTENT OF SPORT NUTRITION FOOD AND RAW MATERIALS BY NIR INSTRUMENT

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Our goal was to develop a simple and cost effective method to measure moisture content sport nutrition foods and supplements and its raw material at the Scitec, Ltd. The sport nutrition food production procedure is a powder blending technology, normally the process does not contain heating, freezing or any other steps which can reduce its microbiological contamination. Next to the strong hygienic rules, the measuring control of the water content and keeping it low is necessary regarding to quality assurance reasons. The commonly used measuring method with drying oven takes 3-4 hours, and with the fast-heated gravimetric methods takes 3-20 minutes which is too much time for quality control in the industrial field. That was the reason for this method development.
To get
Reference data, it was measured by fast-heated gravimetric instrument, it was validated to the MSZ 20900-1:1987 method.
NIR spectrometer was used in reflection mode to quantify the water content. Second derivative function transformations gave the most accurate estimation function the used spectrum ranges were 8748.1-8563; 7205.2-7027.8; 5361.5-5037.5 $cm^{-1.}$
The method development is based on the measured spectrums,-
Referencedata PLS regression. In the case of semi-and finished product. We had to count the huge matrix effects therefore half of the samples, were artificially moisturized in a gas humidifier. For validation, 1598 sports nutrition food spectra (proteins, mass gleaners, pre-post workouts, Capsule-Tablets powder) were used, with a water content of 1 to 26m/m%. The performance of the method was determined by cross validation (R^2:92.56; RMSECV: 0.783; RPD:3.67; Bias: 0.0198) and re-measuring samples of known concentration. Test validation was also executed which resulted similar parameters.
To measuring the raw materials was also challenging because of its huge diversity. In this study 273 different raw materials were studied (protein powders, sweaters, amino acids, vitamins, additives etc.).2943 spectra were taken. The water content range was 0.05-20 m/m%. Unfortunately, we were not be able to build a prediction model with a good performance properties. Therefore, this huge range was divided into two ranges and two methods. The first range was 0.1-3 m/m% (1357 spectra) the second was 3-20 m/m% (1264 spectra). The performance of the first method was determined by cross validation (R^2:85.26; RMSECV:0.306; RPD:2.6 Bias:-0.0008125) and re-measuring samples of known concentration. The performance the second method was determined by cross validation (R^2:90.51; RMSECV:0.525; RPD:3.25;Bias:-0.00201) and re-measuring samples of known concentration. Of course both of them were test validated which resulted similar parameters.
According from the obtained results, it can be concluded that the developed methods are able to measure sport nutrition foods, supplements and raw materials, the measuring takes 15 second which resulted short enough for quality control time for expectations.

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H28

FOOD COMPLAINT CASES INVESTIGATION AND SURVEY OF TOTAL VOLATILE NITROGEN CONTENT IN A VARIETY OF MEAT IN HONG KONG

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Food safety is of significant importance to human health and is closely related to our daily lives. Government Laboratory adequately provides various testing support including the examination of food samples for foreign substances, deterioration with respect to pH, titratable acidity (TA), total volatile nitrogen (TVN), etc., and other parameters such as pesticide residues and veterinary drugs, catalase activities, heavy metals, toxins, preservatives, colour matters, nutritional labelling and meat authenticity.

Food complaint cases often interrelated to food incidents. In view of the reports of the Brazilian meat incident in March 2017, a substantial amount frozen and chilled meat and poultry meat samples from Brazil was taken at the import and retail level to Government Laboratory for testing of meat deterioration in terms of TVN as a precautionary measure. The TVN content provides a suitable indicator regarding the freshness of meat with TVN increasing as spoilage starts.

After the Brazilian meat incident, the Hong Kong Government has enhanced surveillance on frozen and chilled meat and poultry meat available in the local market for better safeguard public health and ensure the quality of meats for sale in Hong Kong. Meat samples including beef, pork, mutton, chicken, duck and goose were taken at the import and retail level for testing of meat deterioration. From March 2017 to August 2019, a total of 840 samples were tested for the TVN content by Government Laboratory. Determination of TVN comprises the measurement of various volatile nitrogen-containing compounds including trimethylamine, dimethylamine, ammonia and other volatile amines associated with spoilage. As the TVN value of the food sample increases as spoilage progresses, it provides a suitable scientific indicator for monitoring deterioration of meats, seafood and their products.

In this poster, the analytical results for the TVN study will be presented. Focus will be on the overall quality of various meat types, based on comparison with TVN values in available standards. This on-going study will help develop a TVN database for different meat and their products. Proposal to extend the scope of the study will also be discussed.

Keywords: *deterioration, TVN, meat, food complaints*

H29

DETERMINATION OF THE CONTENT OF HYDROXYMETHYLFURFURAL (HMF) IN FRESH BEE HONEY PRODUCED IN BOSNIA AND HERZEGOVINA (B&H) BY HPLC DAD METHOD

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HMF as a natural compound in honey is the subject of many scientific research, mainly for two reasons, its toxicity, and its presence in honey and foods of plant origin as indicator of age of food, its way of saving or processing. Contents of HMF in honey depends of many factors, geographic area, climate, varieties of honey, quality of honey and especially way of processing and storage. Except of some benefits for the health of people and bee, undoubtedly, its harmfulness. For the bees high content of the HMF could lead to death of the bee colony. For humans it could lead to a different harmful effects, and for that reasons international standards determined its maximum allowed quantity, for both continental and tropical areas of origin of honey. In this paper is presented intra-laboratory validation of HPLC DAD method for determination of content of HMF in the B & H honey, as well as the results of the testing of fresh samples of honey of different variety and geographic origin from the 2018. The precision and accuracy of the validation are made on the concentrations of 6 ng/mL (60 ng/g), 4 µg/mL (40 µg/g) and 10 µg/mL (100 µg/g). Validation results showed that there was no interference, the method is specific, the results of a calculated LOD and experimentally obtained agree with more than 96%. All other parameters are satisfactory, LOQ (RSD < 2.4), precision (RSD < 1.3), accuracy (RSD < 2.7) where recovery was of 73%-120%. The measurement uncertainty is calculated taking in account accuracy, precision, and other sources. Expanded measurement uncertainty is calculated for k = 2, and it had the following values: 5.6% to 60 ng/g, 0.8% for 40 µg/g and 5.1% for a 100 µg/g. All the validation parameters are made with six repetitions by two analyst. In the season of 2018 right after the picking, 21 of the next honey samples were collected: 8 acacia, 4 honey dew, 3 chestnut, 4 meadow, 1 flower and 1 heather of honey. The obtained results range from 0.02 mg/kg (honey locust) to 27,40 mg/kg (medow). The obtained results show this method is very reliable for the determination of HMF in honey, and fresh B & H honey could be very recommended for consuming in terms of low contents of HMF.

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H30

OCCURRENCE AND MOLECULAR CHARACTERISTICS OF CLOSTRIDIAL STRAINS ISOLATED FROM POLISH HONEY SAMPLES

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A high concentration of sugars and the presence of antimicrobial substances in honey are not favourable conditions for survival and growth of vegetative microflora. However, even in such environments, the presence of anaerobic spore-forming bacteria is possible. From the epidemiological point of view, the most important pathogenic species is *C. botulinum*. This pathogen's occurrence was reported in many publications as a potential risk factor for infant botulism. On the basis of epidemiological reports, the World Health Organization has recommended that infants under one year of age should not be given honey.
The aim of this study was isolation and molecular characterization of *C. botulinum* strains isolated from Polish honey.
Material and Methods: The study was carried out on 240 honey samples (1 sample = 1 apiary) from 16 provinces (15 samples per province) in Poland. For examination of samples, direct centrifugation (DC) method was used with enrichment in TPGY (Tryptone Peptone Glucose Yeast Extract) broth and differentiation on Willis – Hobbs and FAA (Fastidious Anaerobe Agar) media. DNA was extracted from liquid culture and agar media and subsequently subjected to real – time PCR screening analysis for *ntnh* gene detection. Total RNA extraction was conducted after screening analysis of suspected isolates and expression of *ntnh* gene was analysed. Genomic DNA isolated from *C. botulinum* strains was subjected to NGS analysis and botulinum gene cluster was thoroughly characterized with using Bioinformatics tools (RAST, MEGA,HMMER).
Results: The eight bacterial strains with phenotypic features characteristic for *C. botulinum* were isolated from the examined samples. The obtained colonies exhibited the characteristic lipolytic properties.
The *ntnh* gene occurrence and expression were detected in only 5 isolates. These strains were isolated from samples collected in Lubusz, Greater Poland, and the Warmia-Masuria provinces.
Characterization of botulinum cluster indicated on two of isolates belongings to toxin type A, subtype A1 and three of them were classified to toxinotype B, subtype B1.
The number of clostridia spores sufficient for infection is undetermined; however, even a single colony forming unit of *C. botulinum* could cause botulism symptoms in an infant. The obtained results have shown that risk assessment of the entire honey harvesting process should be undertaken in order to ensure the microbiological safety of the product, especially for infants and people with weakened immune systems.

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H31

HIGH-THROUGHPUT ANALYSIS OF CAFFEINE IN SOFT DRINKS

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The conventional procedure of analysis for quantitative estimations in chromatography is based on performing subsequent injections of samples to produce individual chromatograms which are saved as individual data files. The interpretation of multiple results is usually performed by integration of peak area providing a high level of precision and accuracy for quantitative determinations. For the high throughput analysis, a simpler and more direct procedure utilizing multiple injections within a single experimental run was described in litterature.[1] This approach allows a direct visualization of high amount of chromatograms within one graph without need for peak integration and graphing offering the benefit of simplicity, speed, and ease of understanding by non-experts.[2] The technique was successfully applied for monitoring of synthetic reactions, catalysis, formulation, purification screening and biomarker analysis.[2] A highly efficient UHPLC system with a fast injection unit and a mass spectrometer dramatically enhance the advantage of the technique supporting both, high throughput and compound-specific detection. This poster presents an application of the technique on an example of high throughput analysis of caffeine in soft drinks.

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Keywords: *caffeine, high-throughput, soft-drinks, mass spectrometry, UHPLC*

H32

BROMINATED OILS IN SOFT DRINKS BY COMBUSTION ION CHROMATOGRAPHY (CIC)

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Brominated vegetable oil (BVO), is a food additive used to emulsify citrus flavoring and provide a stable mixture in carbonated beverages. BVO is a heterogenous fatty acid composed of plant-based oils which are brominated at the previously unsaturated bonds. Analytical methods are needed for BVO determinations to maintain the beverage quality and to meet labeling requirements. However, the determinations are challenging because BVO exists as a non-polar suspension in an ionic sample matrix. Pyrolytic combustion ion chromatography is an ideal approach to eliminate the sample matrix and increase sample homogeneity. In this application, BVO was determined in beverage samples by pyrolyzing a 50-µL aliquot at 1000C under inert atmosphere and combusted with oxygen, water, and peroxide to obtain bromide. Bromide and other ions in the collected aqueous sample are separated by anion-exchange chromatography on a 4 x 150 column using electrolytically generated 23 mM KOH at 1.0 mL/min and detected by suppressed conductivity. Using overlap mode for the 5-min combustion, the total run time is 13 min. BVO was determined in three carbonated beverage and two ginger beer samples. Bromide from total BVO was found at expected levels in one of the carbonated beverages and at trace levels in another carbonated beverage. The method had good accuracy with recoveries within the 80-120% and good reproducibility with <5% RSDs.

Keywords: *combustion ion chromatography, cic, brominated vegetable oil, BVO, food additive*

H33

SELECTIVE AND SENSITIVE DETERMINATION OF BROMATE IN BREAD BY IC-MS

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Potassium bromate is an odorless and tasteless white crystal that is commonly used in the baking industry. It is often added in flour and flour products to improve the product quality. It acts by oxidizing thiol groups of the gluten protein in flour and in the process forming disulfide bonds. The overall effect is to make bread rise in the oven, and increase loaf volume and texture. However, potassium bromate is considered a carcinogenic and nephrotoxic substance. Bromate has been listed as a potential carcinogen at low levels by the International Agency for Research on Cancer (IARC). Due to its hazardous properties, the concentration of bromate must be carefully monitored in bread. Ion-exchange chromatography with suppressed conductivity detection is the most commonly applied technology for the bromate measurements. However, conductivity detection has low selectivity, especially when applied to complex food matrices in which many compounds are ionic and thus can possibly interfere with bromate detection. Here we developed a new method using ion chromatography coupled with single quadrupole mass spectrometry (IC -MS) for a selective and sensitive determination of bromate in flour and flour products. The Thermo Scientific ™ ISQ™ EC Single Quadrupole Mass Spectrometer allows seamless integration of IC with MS, taking advantage of the strengths of both techniques. IC separation with eluent generation and suppressed conductivity detection provides chromatographic selectivity and analytes in the ionic form. Electrospray ionization (ESI) introduces the liquid IC stream (after suppression) as a fine spray into the MS source.
Flour samples were extracted with high-purity water and subjected to a series of simple clean up steps before they were analyzed on the IC-MS system. We used a recently introduced Thermo Scientific™ Dionex™ IonPac™ AS31 column to separate bromate from matrix anions. The IonPac™ AS31 column is a high capacity column which allows large injection volumes, thus allowing the determination of low bromate concentrations. In this work we analyzed various samples including flour, burger buns, white bread, bread (baked using bromated flour), etc. The method showed good precision and accuracy with recovery of 90–110%. The limit of detection (LOD) and the limit of quantitation (LOQ) of bromate in the solution were 0.1 ppb and 0.34 ppb, expressed as $H^{79}BrO_3^-$ (m/z 127), respectively, which corresponded to 5 µg/kg and 17 µg/kg, respectively, in bread.

Keywords: *IC-MS, bromate, ion chromatography, mass spectrometry, bread*

H34

ADULTERANT QUANTIFICATION IN UHT MILK USING CUSTOMIZED MEMS IR SOURCES AND LOW-COST LINE ARRAY ATR SETUP

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The continued urbanization and decentralization of agricultural goods production has led to longer delivery chains and thus increased the requirements for food quality control. Quality analysis as early as possible is, especially in emerging countries, rather challenging. The analysis of goods in a professional laboratory is for various reasons not suitable for everyday use. During the last decades, infrared spectroscopy has been proven applicable in many food analysis applications and is considered an attractive choice especially due to its non-destructive nature and to the little sample preparation required. Nevertheless, the method struggles against its image being expensive, stationary laboratory device and tedious to calibrate. In the recent years, multiple approaches for miniaturized devices have been realized especially in the near-infrared range. Compared to near-infrared approach, one benefit of mid-infrared range is distinct absorption bands, which reduces the amount of samples required for calibration and additionally increases the robustness of the generated models.

The focus of this presentation is to demonstrate the performance of a cost-sensitive line array ATR system with optimized MEMS-based light source in quantification of adulterants in milk. Together with the detector and optics, the light source should be carefully chosen and optimized to provide maximum performance and efficiency. The emissivity of the electrically modulated thermal infrared emitters used is approximately 0.85 in the whole measurement range of 5.5 – 11 µm (1818 – 909 cm^{-1}). The setup consists of a line array of three EMIRS200 IR sources (Axetris AG, Kägiswil, Switzerland) with an optimized reflector built into a Pyreos PY0727 ATR (Pyreos Ltd., Edinburgh, UK) spectroscopy evaluation kit. The infrared array sensor has an integrated linearly variable filter. The IR energy from the light sources passes through a multi-reflection zinc selenide ATR crystal and is received by a Pyreos PY0728 128 pixel linear array. The tested setup has no moving parts, is clearly hand-held size and the material costs of the system are a fraction of a standard FTIR-spectrometer.

The measurements were conducted by separated spiking of UHT milk with powder-form ammonium sulfate and melamine (Grade 99%, Sigma-Aldrich, St. Louis, USA). Both analytes are used in milk to increase the apparent Kjeldahl protein where ammonium sulfate is, additionally, used to mask the density change when water is added (*P.W. Hansen and S.E. Holroyd, doi: 10.1111/1471-0307.12592*). The modelling was conducted with PLS_Toolbox (Eigenvector Research, Inc., Manson, USA) using Partial Least Squares regression. For both analytes, 1st derivative was applied and three Latent Variables were used. The root-mean-square error of prediction was 0.11 g/l in 0 to 3 g/l range for ammonium sulfate and melamine – comparable to commercialized devices and close to the accuracy of the gravimetric Reference used.

Keywords: *ATR, milk analysis, MEMS emitter, line array, infrared spectroscopy*

H35

ANALYSIS OF ACYLGLYCEROLS IN EDIBLE OILS BY GAS CHROMATOGRAPHY USING A UNIQUE STATIONARY PHASE

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Characterization of edible oils is essential to the food industry due to the amount of fraudulent activity that surrounds these products. Some edible oils (e.g. Extra Virgin Olive Oil) carry high value, therefore making it an easy target for fraud. By mixing different vegetable oils (e.g. palm, sunflower, etc.) with high quality olive oils, manufacturers and suppliers increase their oil yields to make larger profits on counterfeit olive oils. For these reasons, it is important to obtain a triacylglycerol (TAG) fingerprint of edible oils to know that they have not been adulterated with other oils. In addition, the freshness of oils can be determined by looking at the ratio of 1,2 to 1,3-diacylglycerols (DAGs). By using a unique gas chromatography (GC) stationary phase without bleed interference and retention time shifting due to phase loss, one is able to resolve TAGs and DAGs, and a full analysis of the edible oil can be conducted for oil adulteration and degradation. The analysis and results for these oils will be presented along with an examination of column bleed at high GC operating temperatures.

Keywords: *triacylglycerol, diacylglycerol, triglyceride, oil, GC*

H36

QUANTIFICATION OF MYO-INOSITOL PHOSPHATES IN CASHEW NUT BEVERAGES

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Consumption of plant-based beverages is steadily increasing. They are considered an alternative for milk or as a health, lactose and gluten free option. Plant-based beverages may also function as a source of nutrients and bioactive compounds, among others minerals. Mineral deficiencies are still considered a public health problem affecting more than 2 billion people world-wide. Besides low mineral intakes, low mineral bioavailabilities are responsible for mineral deficiencies. Some food constituents such as *myo*-inositol phosphates, could chelate minerals among others iron and zinc and reduce their absorption by the human body. Thus, the presence of *myo*-inositol phosphates (IP3, IP4, IP5, IP6) could negatively affect the nutritional quality of foods such as plant-based beverages. However, information about the *myo*-inositol phosphate concentrations of plant-based beverages is scarce. The aim of this study was therefore to quantify *myo*-inositol phosphates in cashew beverages. 5 cashew nuts beverages blended with Brazil nuts, coconut and cocoa were included in the study. The *myo*-inositol phosphates were extracted from the freeze-dried samples with 2.4 % (w/w) HCl for 3 hours at 22°C. Sample preparation was performed by using anion exchange chromatography using AG 1–X4 100–200-mesh resin. For separation and quantification of the *myo*-inositol phosphates HPLC ion-pair chromatography on a Ultrasep ES 100 RP18 column was applied. No *myo*-inositol trisphosphate (IP3) could be detected in the beverages. IP4 (*myo*-inositol tetrakisphosphate) was only observed in the non-blended cashew nut beverage (2.12 ± 0.05 µmol/g dm). The concentrations of *myo*-inositol pentakisphosphate (IP5) ranged from 3.64 ± 0.38 to 6.86 ± 0.81 µmol/g dm in the different beverages studied, The non-blended beverage had the lowest and the beverage blended with cocoa the highest IP5 content. A *myo*-inositol hexakisphosphate (IP6, phytate) between 4.4 ± 0.54 and 11.02 ± 1.17 µmol/g dm was obtained for the beverages included in the study. The lowest concentration was found for the beverage blended with cocoa and the highest for the beverage blended with Brazil nut. In summary, all cashew nut beverages contained an amount of *myo*-inositol phosphates capable of interfering with mineral absorption from gut. Therefore, the substitution of milk for cashew beverages should be carefully evaluated by determining the bioavailability of minerals such as iron, zinc and calcium.

Keywords: *myo-inositol phosphates, cashew nut beverage, minerals, anti-nutritional factor*

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H37

MULTIVARIATE OPTIMIZATION OF THE HS-SPME ISOLATION OF VOLATILE ORGANIC COMPOUNDS FROM EXTRA VIRGIN OLIVE OIL

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Virgin olive oil is very appreciated not only by its nutraceutical benefits, but also due to its unique, delicate flavor. A wide variety of compounds from distinct chemical classes is responsible for its aroma, such as alcohols, aldehydes, esters, ketones and hydrocarbons. The composition of the volatile fraction of the oil depends on the characteristics and growing conditions for the olives, as well as to the processing and storage conditions of the olive oil. A method combining Headspace Solid Phase Microextraction and Gas Chromatography with Mass Spectrometric Detection (HS-SPME-GC-MS) to assess the composition of the aroma-related volatile fraction of Brazilian extra virgin olive oils (EVOO) was developed using multivariate optimization approaches. Extraction conditions were optimized using a Plackett−Burman (P&B) design for selection of variables such as sample mass (g), incubation time (min), sample agitation speed (rpm), extraction temperature (°C), and time (min), and fiber desorption time (min). After selection of the relevant parameters, their optimum values were determined by experiments arranged according to a central composite rotational design (CCRD) using 2^2 factorial design with four axial points and three repetitions of the central point (eleven experiments); the number of detected peaks was considered as response. The levels for those variables on the CCRD study were 40 to 60° C and 20 to 60 min, respectively. EVOO from Arbequina cultivar supplied by EPAMIG (Minas Gerais Agricultural Research Company) was used as sample. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 µm fiber and 20 mL vials were used in the experiments. The chromatographic analysis was performed on a QP-Ultra GC-MS (Shimadzu, Kyoto, Japão) using a DB-WAX capillary column. The selection of variables by P&B using ANOVA ($p > 0.10$) indicated that temperature (T / °C) and extraction time (t / min) were the most relevant parameters, with p-0.0007 and p-0.0073 respectively. The model correlation R^2 was 0.7019 and error was not significant (Ftab = 9.29 for Fcalc = 1.8). These results point out that the method is reliable but the variation of responses between tests performed was low. After the multivariate optimization, the optimum extraction conditions were found as: sample volume = 2 g, incubation time = 10 min, agitation speed = 200 rpm, extraction temperature = 60°C and time = 50 min, respectively. This method allowed the identification of 47 to 51 volatile compounds in Arbequina EVOO depending on the sample, the majority being C6 alcohols and aldehydes such as 1-hexanol, (E)-2-hexenal, n-hexenal, (E)-2-hexenol and (Z)-3-hexen-1-ol, whose aroma notes are associated with green leaves, green bananas and almonds. These attributes are associated to superior olive oils.

Keywords: *SPME, GC-MS, Brazilian EVOO, CCRD*

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H38

IDENTIFICATION OF MECHANICALLY SEPARATED MEAT (MSM): THREE DIFFERENT INNOVATIVE TECHNIQUES DEVELOPED BY THE "MPSQA" PROJECT

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In 2013, the European Food Safety Authority published a Scientific Opinion focused on mechanically separated meat (MSM). In this document, the EFSA solicited the Organisms in charge of food inspections developing new approaches for identifying MSM, since the method adopted for this purpose (calcium quantification) needs complementary tools for results confirmation. During the activities of "MPSQA" Project, financed by the Italian Ministry of Health, three different innovative approaches were developed for identifying MSM. The approaches were based on: 1) sample irradiation and Electronic Spin Resonance (ESR) analysis; 2) Total reflection X-ray fluorescence (TXRF); 3) multivariate approach based on ^{90}Sr, ^{88}Sr, Ca and ash amounts. The presence of bone micro-fragments in MSM was exploited, since the chemical composition of the matrix showed some modifications if compared to fresh meat. The interaction irradiation/bone fragments produced six characteristic signals on the ESR spectrum, useful for identifying MSM. The quantitative determination of bone fragments was also proposed through method validation (LOD and LOQ: 16 and 48 mg/100g (w/w f.w.), respectively; accuracy (CV% = 12.8% - error% (trueness) = 9%; n=18); measurement uncertainty: 14.6% [1]. Regarding TXRF, Triton X-100 0.1% and PVA 0.6×10^{-3}% was selected as the best procedure for sample preparation. The results showed that K, Ca and Fe are the most important markers to distinguish fresh meat from MSM. Chemometrics-based on PCA clearly distinguished different types of meat, and a limit of 40% MSM was set to distinguish fresh meat from MSM. The method accuracy was also evaluated comparing the obtained results with those obtained after acidic digestion and ICP-MS analysis. The comparison confirmed method reliability for MSM identification [2]. Finally, the multivariate approach based on ^{90}Sr, ^{88}Sr, Ca and ash amounts has proved to be more precise if compared to the single determination of calcium, with a precision of 87%, evaluated by analyzing 100 samples of meat products composed of different percentages of MSM and not composed of MSM [3].

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Keywords: *food safety, irradiation, meat quality, mechanically separated meat, TXRF*

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H39

"ALL-IN-ONE" METHOD FOR THE ANALYSIS OF MULTICLASS FOOD ADDITIVES USING UHPLC-MS/MS

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Food additives (FAs) have been used for centuries to improve shelf-lives, appearance and taste of food products. Although they are regulated by European legislations, FAs remain a matter of controversy. Consequently, consumers behavior is changing, yearning for more additive-free foodstuffs. Moreover, given increasing global trade with countries subjected to different regulations, focusing only on authorized food additives may not be enough to guarantee food safety. To protect the consumers, national authorities have to monitor the FAs levels and also perform exposure assessments because the food market is very versatile
In this context, developing an analytical method that enables simultaneous determination of authorized and non-authorized FAs from different technological classes is essential. Contrary to most reported methods, the high-throughput multi-class method developed in the present work covers a large number of additives in a single analysis. This is challenging as FAs include molecules with different physico-chemical properties that are used in a wide range of concentrations in various type of food matrices. Seventy-six additives from four different technological classes (food colors, sweeteners, preservatives and antioxidants) are covered by the method with two thirds of these compounds not authorized in Europe.
A QuEChERS based extraction has been devised using acidified acetonitrile as extraction solvent, followed by a C18 and PSA dispersive solid phase extraction step. For the development of the LC-MS method, ten RP-C18 UPLC columns and different mobile phases were tested. The best result was achieved with a Shim-pack GISS C18 (100x2.1 mm, 1.9 µm). Multiple reaction monitoring is used in both positive and negative ESI modes in order to achieve sensitive and targeted analyses. For each ionization mode, an elution gradient was optimized, yielding a total run time of 22 minutes for one sample.
The results will show the recoveries obtained for each group of FAs associated with the limit of detection for the non-authorized compounds and the limit of quantification for the FAs. Feasibility of this approach will be illustrated with the analysis of 10 highly consumed foodstuffs from dairy products.
In conclusion, the developed method offers the possibility to detect banned substances while determining accurately the actual levels of FAs from different technological classes in one single analysis.

Keywords: *multi-class food additives, foods, UHPLC-MS/MS, QuEChERS*

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H40

SIMPLE LC-MS METHOD FOR SYNTHETIC FOOD COLORANTS DETERMINATION

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Food colorants represent a group of food additives, compounds intentionally added to foodstuff to fulfill a certain technological purpose. For colorants, this purpose is to modify final product color or renew color lost during a processing. According to their origin, we can distinguish either natural or synthetic colorants. It is the second group, which can be the source of concern for people suffering from specific health disorders.
Food colorants are widely used in confectionery, they are used in production of many desserts with purpose of attracting customers. Coloring products are commonly available even for home usage, but they are not covered by any legislation. Regulation (EC) No 1333/2008 sets many limits for food additives in foodstuff, but not in coloring supplies since they are not food themselves. Coloring label declaration is often approximate and sometimes does not include any dosage instructions. Therefore it can represent a considerable risk in exceeding of ADI for used synthetic colorants.
Presented study demonstrates the use of simple LC-MS method for synthetic food colorants determination using ultra performance liquid chromatography and a simple quadropule mass spectrometer (U-HPLC-MS). Analytes were separated by reversed phase column. Presented method was validated for the determination of 10 food colorants (tartrazine, sunset yellow SY, azorubine, ponceau 4R, allura red AC, patent blue V, brilliant blue FCF, brilliant black BN, green S and erythrosine). Analysis of confectionary coloring supplies from 3 different companies revealed a wide use of synthetic food colorants. The same color may be mixed from different colorings by different companies. Some samples even exceed the declared color content by more than twenty percent.

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

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H41

QUANTITATIVE DETERMINATION OF ETHOXYQUIN AND DIMER IN FOOD AND FEED USING LC-ESI-MS/MS

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Ethoxyquin (ETQ) is a quinoline-based antioxidant that was used as a pesticide until 2011. Today, it is mainly used in fishmeal to protect unsaturated fatty acids from oxidation. Other applications include adding to apples and pears to prevent their brown discoloration, to poultry feed to alter skin and egg pigmentation, and to cow feed to increase the stability of the milk. By transferring such antioxidants to the fat and meat tissues of animals, the issue is gaining importance in terms of food safety. The health effects are controversial, as the metabolite ethoxyquin quinone imine is likely to be carcinogenic and mutagenic. The procedure presented here describes the development and validation of a method approach for the extraction of ETQ and its dimer ETQD from food and feed as well as its measurement by LC-ESI-MS/MS. Data acquisition was carried out by MassHunter Workstation Data Acquisition, data analysis was done by MassHunter QQQ Quantitative Analysis. The aim was the development of a simple and uniform sample preparation procedure, which could be applied to all matrices. Furthermore a low limit of quantification (10 µ/kg) should be achieved.

Keywords: *ethoxyquin, UHPLC, LC-MS/MS, chromatography, antioxidants*

H42

SULPHONATE FUNCTIONALIZED COVALENT ORGANIC FRAMEWORK-BASED MAGNETIC SORBENT FOR EFFECTIVE SOLID PHASE EXTRACTION AND DETERMINATION OF FLUOROQUINOLONES

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Most of the reported covalent organic frameworks (COFs) are hydrophobic, limiting their adsorption application in sample pretreatment field. In this work, sodium 3-mercaptopropanesulphonate functionalized magnetic covalent organic frameworks (COFs) composites were first synthesized by loading gold nanoparticles on Fe_3O_4@COF(TpBD) surface and then functionalized by sodium 3-mercaptopropanesulphonate immobilization via Au-S bonding formation (denoted as Fe_3O_4@COF(TpBD)@Au-MPS nanocomposites), which were further utilized as adsorbents for magnetic solid-phase extraction (MSPE) of fluoroquinolones. Compared with Fe_3O_4@COF(TpBD), the composites exhibited higher affinity to fluoroquinolones due to their excellent hydrophilicity and electrostatic interactions. Under optimized conditions, the developed MSPE method coupled with HPLC-MS/MS showed good linearity (R^2 ³0.9989) and low limits of detection (0.1-1.0 mg kg^{-1}) for fluoroquinolones. Moreover, the proposed method was successfully applied for extraction of fluoroquinolones from spiked meats (pork, chicken and bovine). The satisfactory recoveries were in the range of 82-110.2% with the relative standard deviations (RSDs) lower than 7.7%. These results indicated that the Fe_3O_4@COF(TpBD)@Au-MPS is a promising magnetic adsorbent for trace fluoroquinolones determination in meat samples. This work not only provided a facile strategy for functionalizing COF, but also developed an efficient method for the detecting fluoroquinolones in foodstuffs.

Keywords: *fluoroquinolones, covalent organic framework, magnetic solid phase extraction, food analysis, HPLC-MS/MS*

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H43

EUROPEANS HEALTH CONSEQUENCES OF ANTIMICROBIAL AGENTS USAGE IN FOOD ANIMALS

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The global antimicrobial resistance crisis has been the driver of several international strategies on antimicrobial stewardship. The application of antimicrobial agents in poultry, pigs, and ruminants has caused concern as to the health implications of these uses on human health through the eggs, meat, and milk. Concerning the emergence and dissemination of resistant bacteria is the result of the use of antimicrobial agents in animals and consequently in humans. Resistant bacteria from animals may pass through the daily nutrients to humans with the result of resistant infections. Increased resistance to fluoroquinolones or cephalosporins that are important in the treatment of human infections, caused by Salmonella and Campylobacter, has significant public health impact in Europe. Efforts to reduce the impacts of increased resistance require multiple partners to work closely together on the same issue. A significant effort in collaboration with zootechnicians, veterinarians, medical doctors, biochemists, microbiologists, and other scientific groups are more than necessary. Nevertheless, the paper will recommend priority research areas for future optimization of antimicrobial treatment in animals, and develop a roadmap outlining how European countries can advance towards a common high level of veterinary antimicrobial stewardship.

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H44

FATTY ACID PROFILING OF ROYAL JELLY BY LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY

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Royal jelly (RJ), a secretion from the hypopharyngeal and mandibular glands of worker bees, is a white-yellowish, gelatinous, acidic colloid, which contains 3-8% lipids.[1] RJ has been used since ancient times in traditional medicine, and it is currently used as a functional food and as a component of cosmetics. The major fatty acids of RJ are unusual medium-chained (8–12 carbon atoms) free fatty acids, terminally and/or internally hydroxylated, with terminal mono- or dicarboxylic acid functionalities, either saturated or monounsaturated at the 2-position. *trans*-10-Hydroxy-2-decenoic acid (10-HDA) is the predominant fatty acid in RJ and its content has been adopted as a marker of RJ reflecting the quality of RJ. RJ is an important functional food because of its human health promoting properties. It has been demonstrated to possess a variety of bioactive properties such as antibacterial, immunomodulatory, wound-healing and growth promoting activities. Many of the biological properties of RJ are attributed to its unusual bioactive fatty acid components, in particular to 10-HDA. HPLC or UPLC methods as well as capillary zone electrophoresis have been used for the determination of 10-HDA. However, the major RJ fatty acids are usually determined by gas chromatography-mass spectrometry (GC-MS), requiring the conversion of free fatty acids into the corresponding trimethylsilyl derivatives.[2]
The aim of our work was the development of a lipidomics approach able to characterize the full profile of fatty acids in RJ. We present here a method for the determination of twenty seven free fatty acids by liquid chromatography-high resolution mass spectrometry (LC-HRMS) avoiding any derivatization step. LC-MS/MS measurements were performed with an ABSciex Triple TOF 4600 combined with a micro-LC Eksigent and an autosampler. Electrospray ionization in negative mode was used for the MS experiments. Halo C18 2.7 μm, 90 Å, 0.5 × 50 mm^2 (Eksigent) was used as a column and the mobile phase consisted of a gradient (A: acetonitrile/0.01% formic acid/isopropanol 80/20 v/v; B: H_2O/0.01% formic acid). The method was validated and applied in eight samples of Greek RJ. Apart from the major unusual fatty acids of RJ (namely 10-HDA, 10-hydroxy-decanoic acid, decanedioic acid, 3-hydroxy-decanoic acid), palmitic acid, stearic acid and oleic acid were found as components of RJ, although in minor quantities in comparison to 10-HDA.

1. Bioactive compounds and health-promoting properties of royal jelly: A review. M. F. Ramadana, A. Al-Ghamdi *J. Funct. Foods* 2012, S4, 39-52.
2. Gas chromatographic–mass spectrometric investigation of volatile and extractable compounds of crude royal jelly. V.A. Isidorov, S. Bakier, I. Grzech *J. Chromatogr. B* 2012, 885–886, 109– 116.

Keywords: *LC/MS, fatty acids, royal jelly*

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H45

DEVELOPMENT OF A LIQUID CHROMATOGRAPHY-HIGH RESOLUTION MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF FREE FATTY ACIDS IN MILK

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The presence of free fatty acids (FFAs) in milk is of high importance, because FFAs have strong sensory properties contributing to the flavor and aroma of milk. FFAs are primarily formed in milk through the breakdown of triacylglycerols due to the enzymatic hydrolysis by lipoprotein lipase and other lipolytic enzymes. Elevated levels of FFAs are responsible for rancidity in milk and are generally unacceptable. On the other hand, some of the FFAs, for example butyric acid and conjugated linoleic acid, have shown to exhibit beneficial health and nutritional effects. Therefore, the determination of FFAs in milk is of importance for quality control, legislative purposes, authentication and product development. The most common approach to quantify FFAs is the use of gas chromatography flame ionization detection (GC-FID) involving the conversion of fatty acids into corresponding methyl esters (FAME).[1] Most recently, a butyl ester method was reported, where extracted free fatty acids are converted to butyl esters prior to gas chromatography flame ionization detection.[2]
In the present work, we present a convenient method for the rapid determination of FFAs in milk avoiding a tedious sample preparation procedure. A simple liquid/liquid extraction protocol for sample preparation was followed and a liquid chromatography-high resolution mass spectrometry method for the determination of FFAs avoiding a derivatization step was developed and validated. LC-MS/MS measurements were performed with an ABSciex Triple TOF 4600 combined with a micro-LC Eksigent and an autosampler. Electrospray ionization in negative mode was used for the MS experiments. Halo C18 2.7 μm, 90 Å, 0.5×50 mm^2 (Eksigent) was used as a column and the mobile phase consisted of a gradient (A: acetonitrile/0.01% formic acid/isopropanol 80/20 v/v; B: H_2O/0.01% formic acid). The present method allows the simultaneous determination of twenty two fatty acids, including the polyunsaturated ones, in a 10-min single run. Ten cow's milk and five goat's milk samples from the local market were analyzed.

1. "Free fatty acids quantification in dairy products" D. T. Mannion, A. Furey, K. N. Kilcawley *Int. J. Dairy Technol.* 2016, *69*, 1-12.
2. "Development and validation of a novel free fatty acid butyl ester gas chromatography method for the determination of free fatty acids in dairy products" D. T. Mannion, A. Furey, K. N. Kilcawley *J. Agric. Food Chem.* 2019, *67*, 499-506.

Keywords: *LC/MS, fatty acids, milk*

Acknowledgement: *The research presented was carried out within the framework of a Stavros Niarchos Foundation grant to the National and Kapodistrian University of Athens.*

H46

A STUDY ON THE CHANGE OF QUALITIES ACCORDING TO THE STORAGE METHOD OF THE OIL-TREATMENT SEASONED LAVER

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Acid value (AV), peroxide value (POV) and the composition of fatty acid were investigated to find out the quality changes according to the storage method and time span of oil-treatment seasoned laver products distributed in Korean market. After opening the product, each the contents of the product were sealed and stored in the room temperature (natural light & darkroom), refrigeration (5°C) and frozen (-20°C) states. Acid value (AV) after storage for 30 days in each storage condition changed little from 0.28 mg KOH/g to 0.39 mg KOHg/g. For storage at room temperature (natural light), peroxide value (POV) gradually increased from 1.7 meq/kg to 10.6 meq/kg for eight days and then rapidly increased to 64.4 meq/kg by 20 days, but decreased to 13.9 meq/kg after 30 days. Peroxide value (POV) of samples stored in room temperature (darkroom), refrigeration, and frozen state showed a tendency to increase slowly, with 22.0 meq/kg, 14.2 meq/kg and 9.3 meq/kg respectively for 30 days. The composition of fatty acid in oil extracted from seasoned laver products was contained in the order of Linoleic acid (51.93 ± 0.13%) > Oleic acid (30.99 ± 0.08%)> Palmitic acid (12.33 ± 0.07%)> Stearic acid (2.48 ± 0.04%)> Arachidonic acid (1.28± 0.07%)> Linolenic acid (0.99 ± 0.03%) in all samples, and there were few changes in composition according to the storage method and period. The sensory parameter for the occurrence of deodorization after the opening of seasoned laver products suggests 10 meq/kg of peroxide value (POV). After opening the product, the expectable edible period was 30 days (frozen, -20°C), 16 days (refrigeration, 5°C), 8 days (darkroom, room temperature), 6 days (natural light, room temperature) depending on the storage method.

Keywords: *seaoned laver, acid value, peroxide value, fatty acid*

H47

EVALUATION OF ARTIFICIAL RADIONUCLIDES IN BERRIES

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To ensure food-safety of berries from radioactive contamination, radioactivity monitoring were conducted with total 258 samples of the berries and processed berry products (Blueberry, Grape, Strawberry, Aronia, Cranberry, Lingonberry, Rubus Coreanus, Black currant, Billberry, Mulberry, Fig, Blackberry, Rubus crataegifolius, Raspberry, Acaiberry) distributed in Gyeonggi-do, Korea from 2016 to 2018. The concentration of artificial radionuclides, ^{131}I, ^{134}Cs and ^{137}Cs, was analyzed using gamma-ray spectrometry. ^{131}I and ^{134}Cs were not detected from any samples. The range of radioactivity concentration of ^{137}Cs were 0.69~808.90 Bq/kg in 39 cases. 0.70~3.29 Bq/kg of ^{137}Cs were detected in 6 cases of domestic berries,which manufactured by imported raw materials. In 33 cases of imported berries, 0.69~808.90 Bq/kg of ^{137}Cs were detected. The concentration of ^{137}Cs in 1 case of blueberry powder product(808.90 Bq/kg) and 2 cases of lingonberry powder products(103.93, 188.46 Bq/kg) exceed domestic maximum radioactivity limits, all of which are the berries from Poland. These results suggest that monitoring system for imported berries and processed berry products should be continuously intensified to secure food-safety.

Keywords: *radioactivity, cesium, berries*

H48

FLOW MODULATED GCXGC COUPLED TO TOFMS FOR NON-TARGET PROFILING OF FOOD, FLAVOR, AND FRAGRANCE SAMPLES

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Determining individual analytes by mass spectrometry within a complex sample matrix is facilitated with high-resolution chromatographic separations as more analytes reach the detector individually. Gas chromatography (GC) has become a routine part of mass spectrometric analyses and two-dimensional GC (GCxGC) extends the benefits of GC by adding a complementary second dimension of separation. Analytes that co-elute in the first dimension can often separate in the second. Modulation is the heart of this separation technique as it controls how the effluent from the first dimension is sampled and reinjected to the second dimension. In this work, a novel, routine, and robust flow modulator is used for a wide variety of analytical challenges in food, flavor, and fragrance applications.

Keywords: *GCxGC, flow modulation, TOF MS, GC/MS, flavours*

HUMAN BIOMONITORING

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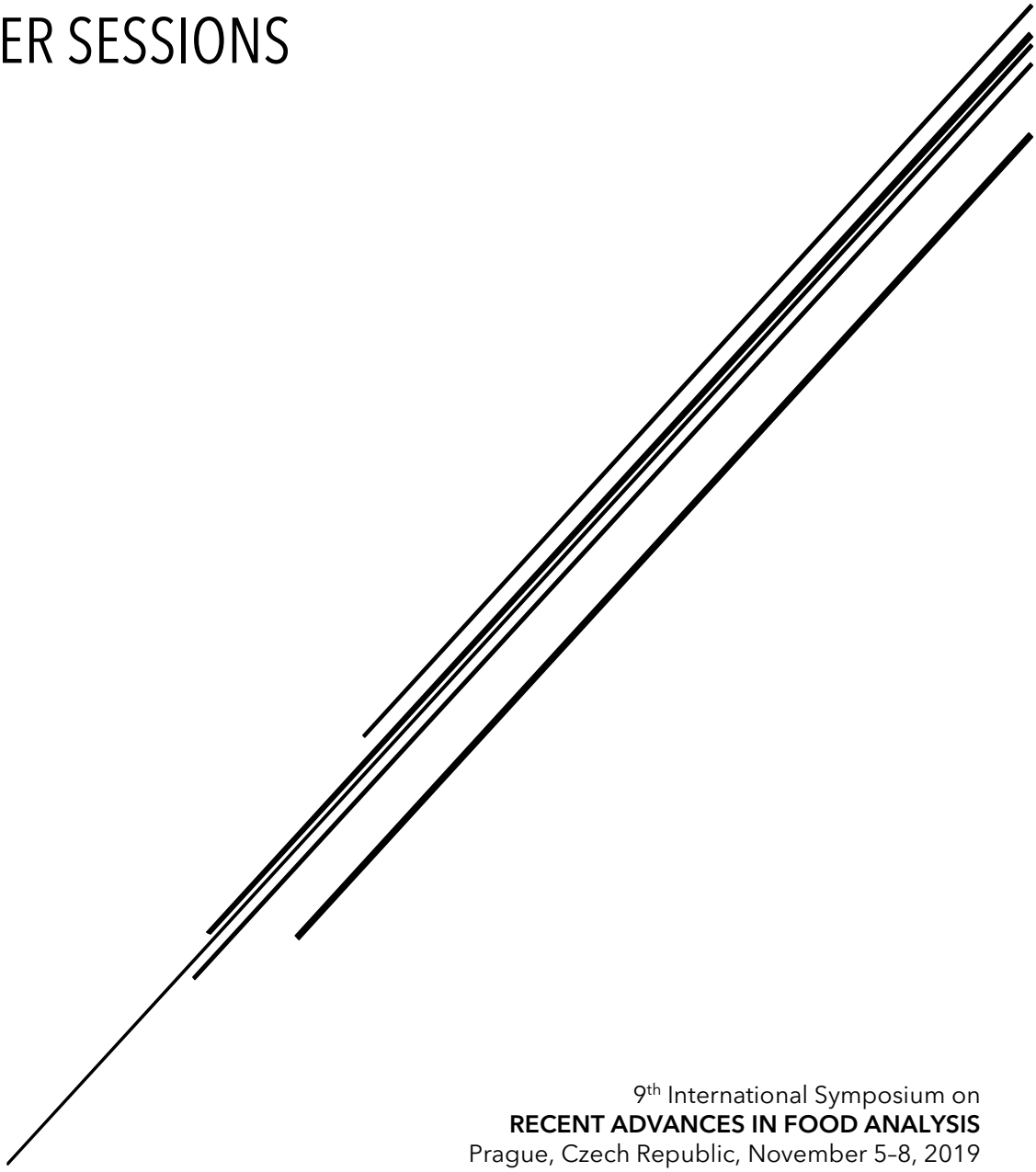

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I1

BISPHENOL ANALOGUES AND THEIR CHLORINATED DERIVATIVES IN BREAST MILK: OCCURRENCE AND PRELIMINARY RISK ASSESSMENT IN CHINA

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Bisphenol A (BPA) has been interdicted to use in the fabrication of baby food containers in many countries since its endocrine disrupting, metabolic disorders, reproductive and developmental abnormal of the offspring. Therefore, its analogues have been produced as a replacement in various applications. Because of the similarity of structure, these analogues exhibited similar toxic effects. Owing to their lipophilic character, they are easily bioaccumulated. Breast milk is one of the significant sources of exposure to these chemicals. However, the level of BPA and its analogues (together referred as BPs) in breast milk is far from known. Therefore, a national survey was conducted in China. Pooled breast milk sample from a national survey in the 4th Chinese Total Diet Study (TDS) was measured to offer a database for the occurrence of the 24 BPs in breast milk in China since it is far less expensive and could reflect the overall exposure level in certain region. Based on the measurements, the dietary intakes of BPs via human milk ingestion for nursing infants were investigated for exposure assessment. BPA, bisphenol F (BPF), bisphenol S (BPS), bisphenol AF (BPAF), mono-chlorobisphenol A (MCBPA), dichlorobisphenol A (DCBPA) and tetrochlorobisphenol A (TCBPA) were detected in the 24 pooled samples from 12 provinces in China. The concentrations of BPs were 0.022-2.740 μg/L. BPA was still the predominant BPs followed by BPF. In rural of Liaoning, Henan and Ningxia provinces, the levels of BPF have surpassed that of BPA. Furthermore, estimated daily intakes for nursing infants (0-6 months old) with breastfeeding only were calculated by multiplying the concentrations of each BPs by the recommended ingestion level of breast milk (750 mL/day) and then divided by the average body weight of infants. The lower and upper bound daily intakes of BPs were 0.002-0.581 μg/kg bw/day and 0.022-0.621 μg/kg bw/day, respectively. The lower level by comparison with the tolerable daily intake indicated little health concern from breastfeeding only. This is the first comprehensive national study of BPs not only BPA in breast milk. The high BPF level found in some areas indicated that concerns should be taken on these BPA analogues, not only BPA.

[1] Andra, S. S., Charisiadis, P., Arora, M., van Vliet-Ostaptchouk, J. V., Makris, K. C.. Environ. Int. 2015, 85, 352-379.
[2] Chen, D., Kannan, K., Tan, H., Zheng, Z., Feng, Y.-L., Wu, Y., Widelka, M.. Environ. Sci. Technol. 2016, 50, 5438-5453.

Keywords: *bisphenol analogues, bisphenol chloride derivatives, breast milk, exposure assessment, infants*

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I2

HUMAN BIOMONITORING OF LEGACY AND EMERGING PER- AND POLYFLUORINATED SUBSTANCES

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Per- and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals that are widely used in consumer and industrial products. This class of compounds includes a large number of chemical structures, including perfluorocarboxylic acids (such as perfluorooctanoic acid, PFOA) and perfluorosulfonic acids (such as perfluorooctanesulfonic acid, PFOS).
Today, PFASs are global ubiquitous environmental contaminants and are found in the environment, food and drinking water. Many of these compounds are toxic, resistant to degradation and bioaccumulate. Due to their persistence the production of main PFASs like PFOA and PFOS has been phased out by producers in North America and Europe and polyfluorinated substances with similar chemical properties but probably less persistence, like ADONA, GenX and F53 B (brand names) are used instead.
This raises the question at which levels legacy PFASs as well as emerging PFAS are currently present in humans in Switzerland. Serum biomonitoring was chosen since it is a useful tool for the assessment of human exposure from the environment as well as from diet.
Here, we present a simple method for the analysis of PFASs in human serum: Acetonitrile and internal standards are added to the samples and the substances are quantified in the supernatant by UHPLC-MS/MS with negative polarity electrospray ionization.
The following compounds are analyzed: PFOA, PFOS, perfluorononanoic acid, perfluorohexanesulfonic acid, ADONA, GenX and F53 B at a limit of quantification of 0.1 µg/kg serum.

Keywords: *perfluoroalkyl substance, PFOA PFOS, ADONA GenX, F53 B, human serum, biomonitoring*

I3

ASSESSMENT OF BODY BURDEN OF CZECH POPULATION TO VARIOUS GROUPS OF POPS

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Blood is one of the most important and most commonly applied biological material for measuring the values of various biomarkers of human exposure, such as persistent organic pollutants (POPs): polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), perfluoroalkylated substances (PFASs), brominated flame retardants (BFRs) and novel flame retardants (novel FRs). Within the human biomonitoring, the use of blood is unique and still preferred because it is in contact with the whole organism (all organs and tissues – storage of substances of interest), thus very well reflects the relationship between exposure and the total body burden. This study is focused on the determination of all aforementioned groups of contaminants (n=81) in plasma samples (n=142). Within March-April 2019, the plasma samples were obtained from police officers living in three big cities in the Czech Republic, namely Praha (n=68), Ostrava (n=56) and Ceske Budejovice (n=18). Due to typically small amounts of plasma sample for the analysis, only one weighed matrix (3 ml of plasma) was used for the determination of all different substances.The sample preparation procedure for non-polar compounds (PCBs, OCPs, BFRs and novel FRs) was based on three-step solvent extraction with *n*-hexane:diethylether (9:1, v/v) mixture followed by the purification step using Florisil® column. The rest of the sample after removing of nonpolar solvent was further extracted by the modified QuEChERS extraction using acetonitrile for the isolation of more polar compounds (PFAS and some BFRs). The identification/quantification of non-polar analytes was realized by gas chromatography coupled to (tandem) mass spectrometry with electron ionization for PCBs, OCPs and negative chemical ionization in case of BFRs. PFASs and some BFRs were analysed by ultra-high performance chromatography coupled with tandem mass spectrometry with electrospray ionization.
The total concentrations of PCBs, OCPs, BFRs and PFASs in all samples ranged from <0.01 to 653 ng/g lw; <0.01 to 587 ng/g lw; <0.1 to 252 ng/g lw and <0.01 to 15.2 ng/mL. From OCPs; PCBs and PFASs, the most abundant analytes were HCB and *p,p'*-DDE; CB 138, CB 153, CB 170 and CB 180 and PFNA, PFDA, PFOA, PFOS, PFHpA, PFUdA, PFHpS and PFHxS quantified in all samples. The major BFR analyte BDE 47 was determined in 81 % of samples.

Keywords: *plasma samples, POPs, body burden, police officers, Czech Republic*

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I4

URINARY METABOLITES OF ORGANOPHOSPHORUS FLAME RETARDANTS: A PILOT STUDY ASSESSING EXPOSURE OF CZECH POPULATION

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Organophosphorus flame retardants (OPFRs) belong to the currently most widely used flame retardants in electronics, materials and consumers' products. Considering their potential harmful effects on humans, e.g. potential carcinogenic and neurotoxic effects, their presence in the environment as well as in human body fluids is needed to be monitored. Unlike well-known brominated flame retardants (BFRs) which are persistent and are slowly excreted in an unmodified form, OPFRs undergo to rapid metabolism by hepatocytes to yield more polar derivatives: diesters, hydroxylated triesters or conjugates with glutathione and with glucuronic or sulfuric acid, which are easier excreted from the human body via urine.

The main aims of this survey was (*i*) to develop a simple, high-throughput method for the determination of six diesters of the most significant OPFRs, namely diphenyl phosphate (DPhP), bis(1,3-dichloro-2-propyl) phosphate (BDClPP), bis(2-butoxyethyl) phosphate (BBOEP), di-*n*-butyl phosphate (D*n*BP) and two isomers of dicresyl phosphate (D*p*CP, D*m*CP) and (*ii*) to apply the new validated analytical approach within the pilot study on the analysis of their concentration in urine of the Czech population (n=142) from three Czech cities, specifically Praha (n=68), Ostrava (n=56) and Ceske Budejovice (n=18). Ultra-high performance liquid chromatography interfaced with tandem mass spectrometry with electrospray ionization in negative mode (UHPLC-ESI$^-$-MS/MS) was utilized for the instrumental analysis of target compounds. The separation was performed on a reverse phase C18 analytical column.

The tested sample preparation procedures were extraction with acetonitrile and a simple "direct injection" approach. The recoveries were in the ranges of 80-115% with repeatability below 8% for both tested procedures. Although the latter procedure was easier, it was not used on the analysis of the real samples due to higher method quantification limits (MQLs) and more intensive clogging of the LC-MS/MS system. The MQLs for method based on extraction into acetonitrile ranged between 0.003-0.06 ng/ml urine. The newly developed and validated method was used for the analysis of urine samples within the pilot study in the Czech Republic. DPhP was the predominant OPFRs metabolite which was found in all samples with concentrations ranging between 0.10-2.89 ng/ml urine (median: 0.45 ng/ml urine). Compared to that, DnBP and DCP isomers were found on 16% and 9% of samples, respectively and BDClPP and BBOEP were not found in any sample. Our results were lower compared to the studies from China (Zhang et al., 2018) or USA (Hoffmann et al., 2017) where the DPhP concentrations were up to 43 and 110 ng/ml urine, respectively.

Keywords: *organophosphorus flame retardant metabolites, Czech population, exposure assessment, urine, UHPLC-MS/MS method*

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.*

I5

ANALYSIS OF POLYCHLORINATED NAPHTHALENES IN HUMAN SERUM USING GC–EI–MS

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Polychlorinated naphtalenes (PCNs) form a complex mixture of up to 75 congeners containing from one to eight chlorine atoms per naphthalene molecule. PCNs were recently added as persistent organic pollutants (POPs) on the list of the Stockholm convention.[1] They can be found in various environmental compartments, including atmosphere, food and biotic matrixes. Air pollution might be a significant source of human exposure to these contaminants. Several of the PCNs congeners exhibit dioxin-like toxicity and may cause toxicological symptoms like hepatotoxicity, carcinogenesis, immunotoxic effects and skin defects, such as chloracne.
Aim of this study was (i) to develop chromatography – tandem mass spectrometry electron ionization (GC-EI-MS/MS) method for analysis of 11 PCN congener with emphasis on a suitable type of an injection which enable to achieve the lowest quantification limits; (ii) to implement the analytical procedure for solation of PCNs from human serum samples and (iii) to apply a newly validated method in a pilot study assessing the exposure of Czech police officers in three regions of the Czech Republic: Prague, Ostrava and České Budějovice. Extraction of target compounds was carried out by liquid-liquid extraction (hexan:diethylether, 9:1, *v/v*) and followed by purification on the Florisil column (analytes eluted by hexane:dichlormethane, 3:1, *v/v*). PCNs were consequently analyzed by GC-EI-MS/MS).[2]
Limits of quantification (LOQs) for individual PCNs were determined as 0,5 – 50 ng/g lipid weight (lw). Analyzed samples were spiked at concentration levels 15, 30 and 700 ng/g lw. Recoveries determined from spiked samples ranged from 85 % to 148 %. Repeatability was 7-19 %. As part of a pilot survey on human biomonitoring, the newly developed method will be applied to a set of 142 human serum samples of Czech Police officers from three locations in the Czech Republic (Prague, Ostrava, České Budějovice) in the age range 25-45 years.

Keywords: *polychlorinated naphtalenes, human biomonitoring, gas chromatography*

Acknowledgement: *This work was financial supporteded from a specific university research (MSMT No 21-SVV/2019), which we are gratefully acknowledged.*

I6

BIOMONITORING OF PHTHALATE AND DINCH METABOLITES IN URINE OF THE CZECH POPULATION

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Esters of phthalic acid (phthalates), mainly used as plasticisers, are also ubiquitous contaminants of the environment. Humans can be exposed to these compounds via various pathways (ingestion, dermal absorption or inhalation). After they enter the human body phthalates are rapidly metabolised and excreted via urine. Due to the adverse effects of phthalates on human health (disruption of the endocrine system and carcinogenicity) they are being replaced with non-harmful alternates such as di-iso-nonyl cyclohexane-1,2-dicarboxylate (DINCH). The aim of this study was to evaluate concentration of 18 phthalate and 4 DINCH metabolites in urine samples collected from mothers and their newborns living in two localities of the Czech Republic (2016-2017). Due to the requirement of the highest sensitivity of the measurement two different ultra-high performance liquid chromatography (UHPLC) systems coupled to tandem mass spectrometers (MS/MS) were compared (Xevo TQ-S and QTRAP® 6500+).
The validated method (limits of quantification 0.15-0.70 ng/mL urine; recovery 79-105 %, repeatability 2-24 % (concentration level 0.7 ng/mL urine) and recovery 71-110 %, repeatability 1-11 % (concentration level 7 ng/mL urine)) was used for the analysis of 630 urine samples. After the enzymatic hydrolysis the samples were diluted with methanol and centrifuged. Identification and quantification of target compounds was performed using UHPLC–MS/MS.
The concentration of all target analytes was 2× lower in the urine samples collected from newborns compared to their mothers. The analyte at the highest concentration in mothers' and newborns' urine samples was MBP (median concentration 22.1 ng/mL urine and median 21.6 ng/mL urine), it was also the most often detected analyte (97 % of the samples). Another often detected compounds at high levels were, MiBP and MEP. Parent compounds of all these metabolites belong to a group of low molecular weight phthalates and thus it could be assumed that this contamination could origin from cosmetic products.

Keywords: *human biomonitoring, UHPLC-MS/MS, urine, mothers, children*

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.*

I7

BIOMONITORING OF 20 ELEMENTS IN URINE OF SPANISH CHILDREN

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Introduction: In this study, we assessed levels of 7 essential (Vanadium, Manganese, Cobalt, Copper, Zinc, Selenium and Molybdenum) and 13 toxic (Beryllium, Aluminum, Nickel, Arsenic, Cadmium, Antimony, Cesium, Barium, Platinum, Thallium, Lead, Thorium and Uranium) metals in urine of 604 samples children, participating in the BIOVAL programme. BIOVAL programme is a cross-sectional Human BioMonitoring (HBM) study carried out by the Health Department of the Regional Government of Valencia (Spain). The objective of BIOVAL was to assess the population exposure to food contaminants, focusing in vulnerable population such as children.
Methodology: A total of 666 children, aged from 6 to 11, were recruited from 25 primary schools located in 16 cities around the Valencian Region (Spain). Out of 666 participating children, 604 donated urinary samples. Samples were analyzed by Inductively Coupled Plasma with Mass Spectrometry (ICP-MS).
Results: Essential elements were highly detected, ranging from 73% (Mn) to 99% (Co), and showing frequencies of detection of 100% for Zn, Se and Mo. Toxic elements were also highly detected, with frequencies of detection from 59% (Be) to 99% (Ni), presenting As and Cs 100%. The geometric mean (GM) for essential elements ranged from 0.085 µg/L (Mn) to 389.454 µg/L (Zn), and for toxic elements from 0.005 µg/L (Pt) to 32.983 µg/L (As).
Conclusion: Urinary levels were similar to other studies in the literature, however, for some essential (Co and Se) and toxic (Ni and Cs) elements higher concentrations were found in our study. In a risk assessment context and taking into account the calculated GM, no risk was found for elements with established risk-based benchmark values. Only As presented a Hazard Quotient above 1, indicating a exposure above their Biomonitoring Equivalent (BE), and consequently higher priority for risk assessment studies should be considered for this metal.

Keywords: *biomonitoring, urine, metals, children, risk assessment*

Acknowledgement: *We want to acknowledge the effort of all members of the BIOVAL task force and the IBSPCV BioBank (PT13/0010/0064).*

I8

DIOXINS, FURANS AND DIOXIN-LIKE POLYCHLORINATED BIPHENYLS IN HUMAN MILK

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Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) are Persistent Organic Pollutants of great concern because of their toxicity and tendency to bioaccumulate through the food chain. Therefore, these compounds enter the human body and accumulate in fatty tissues. They may also concentrate in human milk, which is the main food source for infants.
A total of 120 breastfeeding women were recruited in Valencia (2015) as part of the BETTERMILK project. Samples were collected in prolypropylene containers and stored at -80°C until analysis. The breast milk (25 g) was lyophilized and spiked with $^{13}C_{12}$-labeled internal standards, blended with diatomaceous earth and extracted with an accelerated solvent extractor (ASE). After solvent evaporation, fat content was determined gravimetrically. Power-Prep/6 (Fluid Management Systems) automated lipid removal and enrichment procedure using multilayered silica gel column (acidic, basic, and neutral silica) and alumina column coupled to an AX-21 carbon column was used for the clean-up. Resultant fractions containing PCDD/F or dl-PCBs were evaporated under nitrogen stream and redissolved with the corresponding recovery standards. The analysis was performed on a *DFS*Magnetic Sector GC-HRMS (*Thermo*Scientific), using selected ion monitoring (SIM) at 10000 resolving power (10% valley).
Each set of samples was analysed under QA protocols, including procedural blanks and standard reference materials (SRM1953 and SRM1954, corresponding to organic contaminants in non-fortified and fortified human milk, respectively).
The upper-bound concentrations for the sum of PCDD/F and dl-PCBs ranged from 1.56 pg TEQ_{2005} g^{-1}lipid to 13.48 pg TEQ_{2005} g^{-1}lipid with a mean value of 4.82 pg $TEQ_{2005}g^{-1}$lipid.
The PCDD/F and dl-PCBs levels found in the human milk of mothers from Valencia are similar to those reported in Italy, Canada, France and Sweden (6-30 pg TEQ_{1998} g^{-1}lipid, 2.2-27, 2.55-51.84, 3.7-56 pg TEQ_{2005} g^{-1}lipid, respectively).
Although recent biomonitoring data of PCDD/F and dl-PCBs in human milk seem to indicate a global decline, efforts should be maintained in order to reduce dioxin emission and human exposure to protect the environment and human health in this region. Future studies can help to evaluate the effectiveness of measures adopted.
Action co-financed by the European Union through the Operational Program of the European Regional Development Fund (ERDF) of the Valencian Community 2014-2020

Keywords: *dioxins, furans, dioxin-like polychlorinated biphenyls, human biomonitoring, GC-HRMS*

I9

BIOMONITORING OF URINARY PHTHALATE BIOMARKERS IN LACTATING WOMEN

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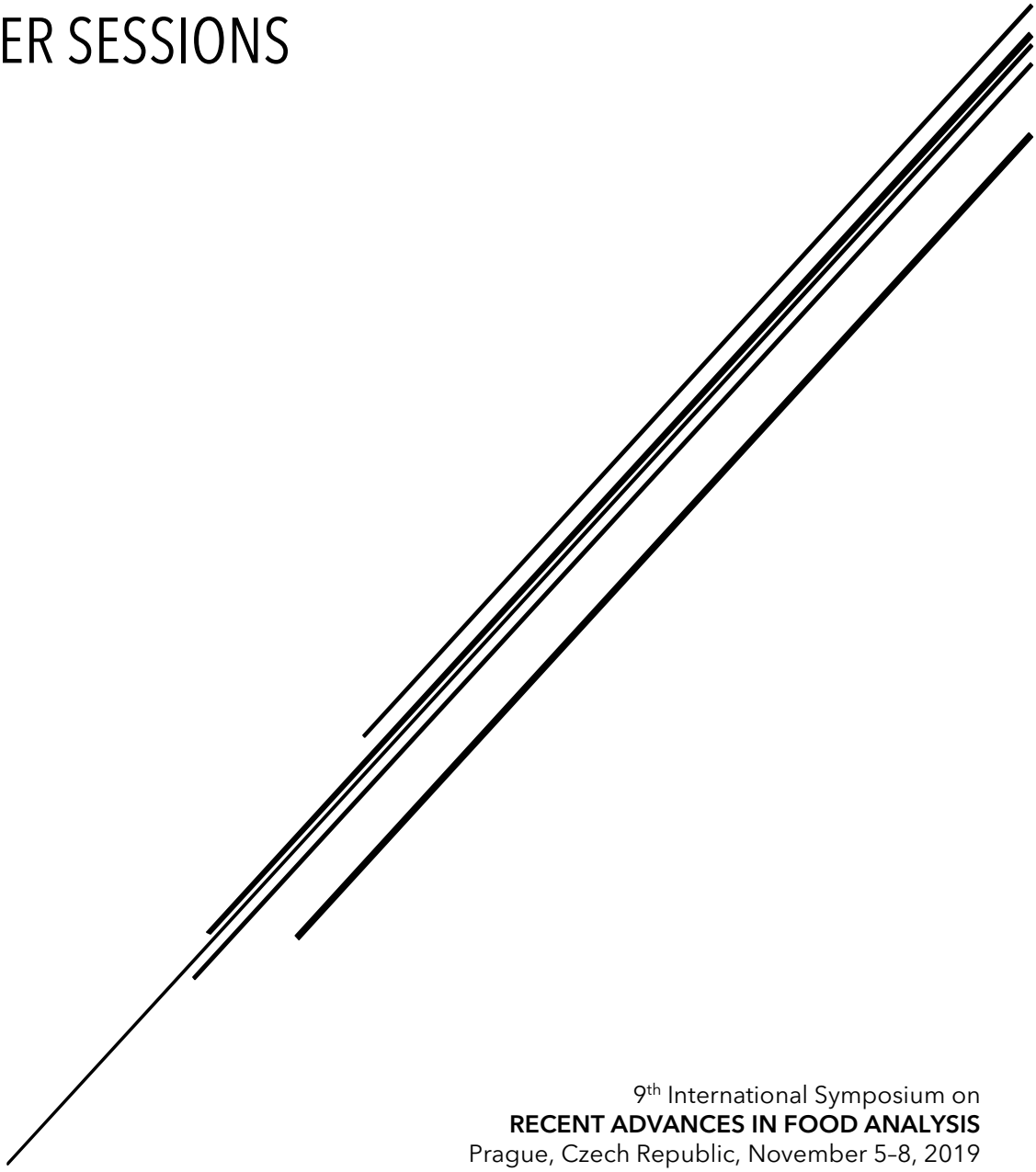
The phthalates are high volume produced chemicals used as plasticisers in a wide range of applications. Phthalates are frequently released into the environment by leaching and migration from the products to the air, food, water and dust. Therefore, general population is continuously exposed to phthalates through ingestion, inhalation or dermal exposure.There is a high concern in exposure to phthalates since they are suspected endocrine disruptors for humans.
After entering in the human body, phthalates suffer metabolism. During the first metabolic step, phthalate monoesters are formed by hydrolysis. Moreover, biotransformations, such as oxidation and hydroxylation, of the formed monoesters are common. A high percentage of the absorbed dose is excreted in urine during the first 24 h as free or conjugated metabolites.
The objectives of the present study are: i) Determine the urinary levels of phthalate metabolites in a population of valencian mothers ii) Estimate the risk assessment of the studied population to phthalates.
Women during breastfeeding period (n=104) provided urine samples from the 2ndto the 8thweek after delivery as part of the project "BETTERMILK" implemented in Valencia (Spain) during 2015.
Sample treatment included an enzymatic hydrolysis followed by ultracentrifugation and injection of the supernatant in a LC-MS/MS(QqQ) system for the analysis of 14 urinary phthalate metabolites.
In order to evaluate the risk assessment, urinary levels were compared with the biomonitoring equivalent (BE) guidance values present in the literature (Aylward et al. 2009a; Aylward et al. 2009b; Hays et al. 2011)
Nine phthalate monoesters presented detection frequencies higher than 80% being MEP and MECPP the metabolites which presented the highest detection frequencies (100%). Levels of phthalate metabolites ranged from <LoQ to 1291 ng/mL, being MEP the metabolite which showed the highest levels (geometric mean (GM) = 34.9 ng/mL).
None of the phthalates at the 95thpercentile level presented concentrations higher than their BE. DEHP expresed as the sum of 5 metabolites (MEHP, MEHHP, MEOHP, 5cx-MEPP, and 2cx-MMHP) presented a 95thpercentile level 2 times lower than the BE guidance value derived from the USEPA RfD (430ng/mL). The rest of analytes were far below to their respective BE values.
The study shows that the participating women exposure to phthalates is below the BE guidance values. However, in order to achieve a best approach to DiNP exposure, other DiNP metabolites which present higher levels in urine, such as mono(hydroxyl-isononyl) phthalate (OH-MINP), mono(oxoisononyl) phthalate (oxo-MINP) and mono(carboxy-isooctyl) phthalate (carboxy-MINP), should be introduced.
Furthermore, in the future, a comparison of the phthalate levels in urine with life style and dietary habits of the participants should be implemented.

Keywords: *phthalate metabolites, urine, human biomonitoring*

MAJOR NUTRIENTS & VITAMINS

POSTER SESSIONS



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J1

UHPLC-PDA-MS ANALYSIS OF VITAMIN B12 IN INSECTS

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Edible insects have become a research object of great interest over the past few years, since they provide – beside proteins and fat – an important source of vitamin B_{12}. Recently, these minor nutrients came into the focus of breeders and consumers. Especially vitamin B_{12} is of utmost importance for a balanced diet and of great concern to vegetarians and vegans as it is produced by microorganisms and therefore almost merely found in food of animal origin.
The buffalo worm (*Alphitobius diaperinus*) used to be known mainly as the cosmopolitan storage pest and pathogen reservoir. However, recent intensive research and subsequent legislative changes on alternative sources of food and feed in EU have changed the perspective on this insect from the annoying vermin to a promising nutrient source. Regarding chemical composition, they are a good source of high-quality protein, lipids as well as other macronutrients and micronutrients. Moreover, buffalo worms are unable to fly, cannot climb on smooth surfaces, and might be fed on various substrates of organic origin (including those low in nutrients and waste or by-products from food industry). Due to their very high reproduction rate and quick life cycle, the biomass gain is even higher than in other edible *Coleoptera*. Therefore, rearing of such insects is assumed to be a non-complicated, environmentally friendly, cheap and effective way of producing insects as food or feed. Other insects of interest are cricket (*Gryllus assimilis*) and cockroach (*Shelfordella lateralis*).
To evaluate the samples' content, an ultra-high performance liquid chromatography approach with preceding immuno-affinity chromatography sample preparation was used. Based on a recently published ultra-high performance liquid chromatography method (Schmidt et al. 2019), the results confirm that whole insects show high levels of vitamin B_{12}. The content of total vitamin B_{12} was measured as cyanocobalamin. In buffalo worms it was determined to be 0.5 µg/100g using photodiode array detection. Surprisingly, the confirmation of this result based on mass-spectrometry using a comprehensive UHPLC-PDA-MS method yielded a content of 0 µg/100g of vitamin B_{12} active compounds. False-positive results in the studied samples are likely caused by pseudocyanocobalamin. Therefore, the presence of biologically none-active pseudoforms of cobalamin must be considered.

Schmidt A., Call L.-M., Macheiner L. & Mayer H.K.: Determination of vitamin B_{12} in four edible insect species by immunoaffinity and ultra-high-performance liquid chromatography. Food Chemistry 281, 124–129 (2019). https://doi.org/10.1016/j.foodchem.2018.12.039

Keywords: *UHPLC-PDA-MS, (pseudo)cyanocobalamin, bufallo worm, cricket, cockroach*

J2

LC-MS/MS DETERMINATION OF MONO-GLUTAMATE FOLATES AND FOLIC ACID IN BEER

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A reliable LC-MS/MS method was developed for the determination of folates in beer. Clean-up steps and chromatographic conditions were optimized to remove interferences and decrease the matrix effect. An immuno-affinity column designed for folic acid (FA) was also suitable for the oxidized forms 10-formylfolic acid (10-HCO-FA) and 7,8-dihydrofolic acid (DHF), obtaining clean extract and improving the limit of detection; for the other vitamer forms, an Oasis MAX column was used. For chromatographic conditions, a RP-18 column with low ligand density showed satisfactory retention times; moreover, the use of methanol instead of acetonitrile lowered the limit of detection up to 0.1 μg l^{-1}.
A total of 80 samples of small-scale (SS) and large-scale (LS) brewed beer (40 and 40, respectively) were collected. The samples were selected based on the most popular beers in Italy and represent about 75% of the total volume of sold beers in Italy. Mainly oxidized mono-glutamate forms, in particular 10-HCO-FA and FA, were found, at levels from 12.4 to 98.6 μg l^{-1} and 1.4 and 11.9 μg l^{-1}, respectively. The other vitamers 5-formyl-tetra-hydrofolate (5-HCO-THF), 5-methyl-tetra-hydrofolate (5-CH3-THF) and di-hydro-folate were found in 39.7, 41.0 and 8.5% of the samples, respectively. The vitamers 5,10-CH2-THF and THF were never detected. Total folates concentration ranged from 15.5 to 104.8 μg/L with a mean of 54.0±19.7 μg/L; the average value was 61.4±20.8 and 46.4±15.3 μg/L for SS and LS brewed beer, respectively. As regards the beer styles, a higher mean level of folates in red and wheat beer compared to regular beer was observed (average value: 74.3±15.7 vs 69.0±18.4 vs 47.8±15.2 μg/L, respectively). A significantly higher average level was observed for SS brewed beers ($P<0.001$); a percentage of 52.4% (22 samples) and 14.6% (6 samples) of SS and LS brewed beers, respectively, showed a folate concentration higher than 70 μg/L. Considering only regular beer, a statistically significant difference ($P=0.010$) between SS and LS beer was found with the average values being 54.1±15.7 and 43.7±13.4 μg/L, respectively.The content of 10-HCO-FA and FA, not naturally occurring forms in raw materials, is probably due to the oxidation of 10-HCO-THF and THF during the brewing.

Keywords: *folates; beer; LC-MS/MS*

J3

SIMULTANEOUS DETERMINATION OF WATER AND FAT SOLUBLE VITAMINS IN TABLETS AND ENERGY DRINKS BY USING A NOVEL UHPLC SYSTEM

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Vitamins can be classified as water-soluble vitamins (WSV) or fat-soluble vitamins (FSV). The differing hydrophobicity of these compounds means that simultaneous determination via liquid chromatography typically requires complex hardware and software solutions. In this work, we introduce an effective workflow that overcomes these challenges based on a novel Vanquish Flex Duo system for Dual LC. This system enables the independent and simultaneous use of two independent columns and methods to optimize for FSV and WSV. Compared to other solutions, this approach is simple to implement, allows the use of more optimized methods, and increases throughput thanks to the simultaneous use of two columns with two methods and faster analysis cycles. Moreover, potential issues such as incompatibility of the mobile phases in the shared flow path, such as buffer salt precipitation in high-organic solvent, are eliminated. In this instance, the Acclaim PolarAdvantage II (PA2) column is used for both vitamin classes. The PA2 column features a polar-embedded stationary phase that effectively operates over a wide range of mobile phase conditions and is highly suitable for the separation of components with very diverse hydrophobicity, such as vitamins.

Keywords: *vitamins, UHPLC, dual LC, water soluble vitamins, fat-soluble vitamins*

J4

FORTIFICATION OF FLOUR AND BREAD PRODUCTS WITH VITAMINS AND MINERALS. QUALITY CONTROL.

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Fortification of flour and bread with premixes containing fat-soluble and water-soluble vitamins (A, B1, B2, B3, B6, B9) and minerals (iron, calcium, zinc, etc.) is one of the simplest and most common ways for overcome micronutrient deficiencies. The problem of fortification of food is quite relevant for Ukraine. At 2020, the law on mandatory fortification of premium and first-grade flour with folic acid enters into the force.
Usually premixes contain stable complexes of vitamins and minerals. The stability of the premix is determined by the shelf life of the vitamins because the minerals are stable. Therefore, minerals can serve as markers to analyze the quality of the process at all stages of fortification.
The aim of the work was to develop a fast and accurate method for the routine analysis of vitamins and elements in flour and bread.
Quantitative analysis of water-soluble B vitamins was performed by high performance liquid chromatography using a triple quadrupole mass spectrophotometric detector. HPLC-MS-MS parameters were optimized for simultaneous analysis of all analytes in the single ingection. The method was validated for linearity, precision, LOD, LOQ, specificity, stability and robustness. Calibration curves presented good linearity (r^2 < 0,992) over the concentration range of 5-100 mkg/L, with LOD and LOQ lower than 2 mkg/L and 6 mkg/L respectively.
The method of optical atomic emission spectrometry with inductively coupled plasma was used for determination of the mass particles of the elements. Microwave mineralization of the samples was used. Regarding validation, trueness, linearity and repeatability were estimated. This method is suitable for monitoring study in fortification of flour. Validation study was applied in three matrices (flour, muffin and sweets). Recoveries were 97-108 %, 95-109 %, 86-115% in flour, muffin and sweets. The optimal analytical condition of these methods are suitable for to control vitamins and elements in routine analysis in flour and bread.
These methods are currently included in the scope of accreditation of the analytical laboratory «L.I. Medved's Research centre of preventive toxicology, food and chemical safety, ministry of health of Ukraine» following ISO/IEC 17025 requirements.

J5

OPTIMISATION OF THE METHOD FOR DETERMINATION OF B-COMPLEX VITAMINS IN INFANT FORMULA

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B-complex vitamins (thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9), cobalamin (B_{12})) represent a group of chemical individuals with different structure, stability, solubility in different solutions and mainly occur in a various amount in food commodities. These hydrophilic vitamins can also occur in various form as a free or bound on proteins, phosphorylated beside that some of them must be quantified as a sum of several substances (e.g. B_9 (folic acid and 5-methyltetrahydrofolate) or B_6 (pyridoxal, pyridoxine and pyridoxamine)). All these factors should be taken into account during choosing an appropriate analytical method for both isolation and determination of these compounds in food samples including infant formula.
The aim of this study was to optimize and validate a method for simultaneous determination of 8 hydrophilic vitamins and their forms in infant formula. Two different sample preparation approaches were tested (with and without enzymatic hydrolysis). For the purification of the obtained extracts solid phase extraction (SPE) was employed. Identification and quantification of target analytes was performed using high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The performance characteristics of the validated method obtained by the analysis of the SRM 1849a (infant/adult nutritional formula, milk-based) in six replicates, for the procedure without the hydrolysis step, were as follows the recovery 42-103 % (for nine analytes) with repeatability 4-15% and the limits of quantification 10-25 ng/ml. For three of the target analytes (B_1, B_9 and B_{12}) the method was not validated.

Keywords: *LC-MS/MS, hydrophilic vitamins, infant formula, B-complex*

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J6

SIMULTANEOUS DETERMINATION OF LIPOPHILIC VITAMINS IN MILK BASED BABY FOOD

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The composition of infant formula is critical for a correct infant development, particularly if the mother is unable to breast feed her baby and formula is the primary source of nutrition. To meet the nutritional needs of infants, the dried milk must be fortified by various nutrients, resulting in very complex composition. Fat-soluble vitamins, including vitamins A, D, E and K are frequently used, as the additives. Requirement on a sufficient consumption intake of vitamins likewise the content check in order to ensure correct label statements, results in a need for an accurate quantitative method for the determination of these compounds in food. Analysis of fortified foods such as baby food, can be particularly challenging due to the wide range in vitamins concentration (µg/g – mg/g). Therefore, any quantitative analytical procedure must be able to accommodate this high variability in concentration of targeted compounds.
The aim of this study was to optimize and validate a method for simultaneous determination of 4 fat-soluble vitamins occurring in different forms (altogether 11 analytes) in infant formula. Three different sample preparation approaches, direct solvent extraction and extraction folloving after saponification and enzymatic hydrolysis were tested. For identification and quantification of target analytes high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was used. The performance characteristics obtained for the solvent extraction by the analysis of spiked samples of powdered milk in six replicates, and the SRM 1849a (infant/adult nutritional formula, milk-based) were as follows: the recovery 39-83 % (for 8 analytes) with repeatability 5-19% and the limits of quantification 100 ng/g. The method was not suitable for δ-tocopherol, menaquinone and retinyl-acetate.

Keywords: *vitamins, LC-MS/MS, inflant formula, lipophilic, milk*

Acknowledgement: *This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, also supported from specific university research (MSMT No 21-SVV/2019), which we are gratefully acknowledged.*

J7

VITAMIN K-BIOFORTIFICATION OF EGGS

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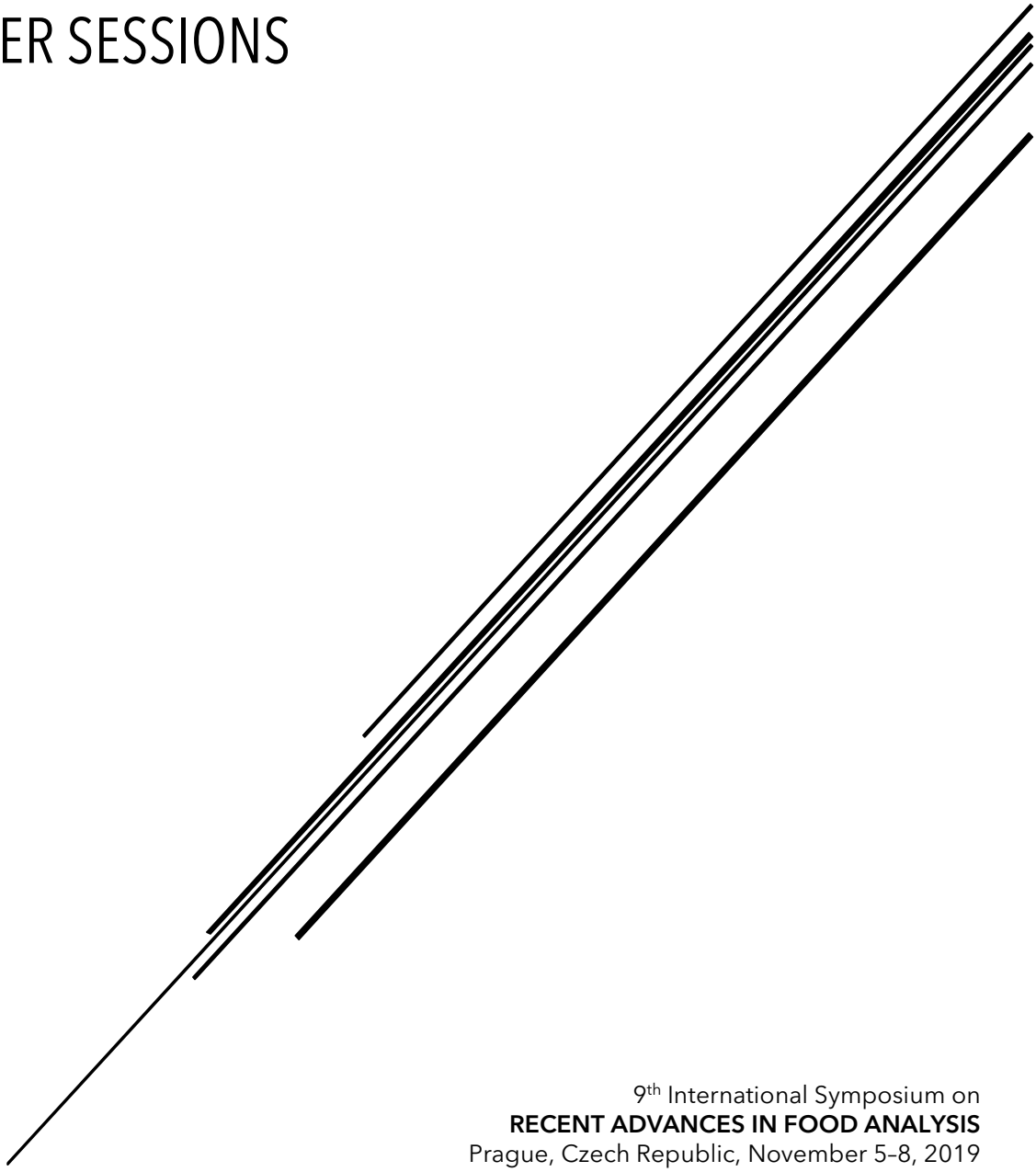
There are two forms of naturally occurring vitamin K, phylloquinone (PK) and menaquninones (MKn). The literature has mainly focused on the study of PK. However, recent evidence for the role of individual vitamin K vitamers in cell survival and cardiometabolic health has generated interest into the study of MKn. National survey data in Ireland has shown that over half of all adults have PK intakes below the Adequate Intake (AI) (<1 µg/kg body weight/d). No AI has been established for MKn. Our study investigated the potential of increasing the vitamin K content of eggs (by increasing the vitamin K_3 content of hen's diets), and to examine any effects on hen performance or egg quality. A 12-week hen feeding trial was conducted with Hyline chickens (n=128), randomised into 4 treatment groups (n=32 hens/group), and fed diets containing 3 (T1), 12.9 (T2), 23.7 (T3), and 45.7 (T4) mg vitamin K_3/kg feed. Egg quality and vitamin K content was assessed at week 0, 4, 6, 8, and 12. A validated liquid chromatography tandem mass spectrometry method was established to determine the vitamin K content (PK, MK-4, MK-7, and MK-9) of composite samples (n=12 eggs/treatment). Total vitamin K content in whole egg samples increased from 22.4 µg/100g in T1 (industry standard feed) to 57.8 µg/100g in T4. MK-4 was found to be the most abundant form of vitamin K in eggs. In conclusion vitamin K-biofortification of eggs significantly increased the total vitamin K content without producing negative effects on egg quality or hen performance.

Keywords: *vitamin K, eggs, LC-MS/MS*

Acknowledgement: *This research was funded by the Irish Department of Food and Agriculture under project contract 15F670.*

METALS & METALLOIDS

POSTER SESSIONS



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Prague, Czech Republic, November 5–8, 2019

K1

SPRINKLER IRRIGATION: AN "HERETICAL" METHOD AIMED TO MINIMIZE THE BIOACCUMULATION OF HARMFUL ELEMENTS IN RICE GRAIN

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Among the factors affecting the bioaccumulation of toxic elements (like As and Cd) in rice, a key role is played by the nature of the irrigation methods. Sprinkler irrigation, optimized for rice in Sardinia [1], Italy, and applied to several tens of rice genotypes over many years, has produced no significant differences in yields, exhibiting also several eco-friendly features. In addition, our previous studies have demonstrated that this water management system allow to reduce, in comparison to the amounts measured in the same conditions using continuous flooding irrigation, the concentration of As (-98%) [2] and Cd (-20%) [3] on rice grain. The principal aim of this contribution is to illustrate the changes in the bioaccu-mulation phenomena of 15 elements of potential health concern (i.e. Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Tl and Zn) in rice (grain, roots, stems, leaves and panicles) at varying of the nature of irrigation method. In addition, also the effect of further agronomic variables (i.e. the genotype effect and the nature and the pollution level of the soil) have been considered in this study. For accomplishing these results, ICPMS, GFAAS and FAAS methods of analysis have been developed and validated. Experimental data supported different dynamics of the translocation of each element from soil to the different parts of the rice plant. Generally, the adoption of intermittent irrigation methods (i.e. sprinkler and/or saturation) in place of the conventional one (i.e. the continuous flooding irrigation) provides a reduction of the total amount of the harmful elements in rice grain. In particular, sprinkler irrigation ensures to produce – also on soils heavily polluted by As and/or Cd (ca. 50 mg kg^{-1} each), a safe rice grains, with concentrations of both elements well below the very strict limits posed by EFSA and EC (0.2 mg kg^{-1} for both elements).

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[3] A. Spanu, M. Valente, I. Langasco, F. Barracu, A.M. Orlandoni, G. Sanna, Sprinkler irrigation is effective in reducing cadmium concentration in rice (Oryza sativa L.) grain: A new twist on an old tale? Sci. Tot. Environ. 628-629 (2018) 1567-1581.

Keywords: *rice, sprinkler irrigation, arsenic, cadmium, bioaccumulation*

K2

ELEMENTAL IMAGING OF DIFFERENT TYPES OF RICE USING LA-ICP-MS

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Rice is one of the most popular foods around the world and in some regions the main source of nutrition. While, rice offers nutritional value it also contains harmful elements such as arsenic. Arsenic can be taken up from the soil, however rice fields are flooded with water (typically contaminated with arsenic) which allows more arsenic to be accumulated in rice as compared to other food products. Arsenic can mimic phosphorous and/or silicon and enter the plant, where the accumulation is typically seen in the bran of the rice grain.
Doing traditional elemental analysis would involve digestion of the rice grains but this would only give an average value per rice grain. Laser Ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) offers a technique that can offer spatial resolved elemental images of rice grains. Using LA-ICP-MS rice grains of various types (organic vs non-organic, white vs brown, long grain vs short grain) will be analyzed for P, K, Mn, Cu, Zn, and As distribution within the cross-section of the rice grain.

Keywords: *rice, arsenic, LA-ICP-MS, elemental imaging*

K3

AUTOMATED LASER ABLATION SAMPLING FOR FOOD SAFETY

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Laboratories around the world test food related products to ensure the general public is provided with safe to consume goods. As it relates to elemental testing, liquids and solids are typically analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). For liquid analysis the process is straightforward, however for solid samples it requires some type of digestion or extraction process to get the sample into solution prior to analysis. Some elements are difficult to get into solution requiring HF acid to complete the digestion process. An alternative approach is to use laser ablation as a solid sampling introduction source, which can eliminate the need for the digestion process. For traditional LA-ICP-MS analysis the throughput has always been an area for concern, however automating this process greatly enhances the ability for high-throughput. The following presentation will focus on automating the LA sampling process for food and food products.

Keywords: *LA-ICP-MS, rice cereal, bulk analysis, toxic elements*

K4

FROM ROUTINE MULTI-ELEMENT ANALYSIS TO DETECTING NANOPARTICLES: USING ICP-MS TO FULLY CHARACTERIZE INFANT FORMULA

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As the market for infant formula continues to grow worldwide, advances are being made in the methods used for elemental analysis of commercial products. Many countries regulate the composition of infant foods and formulas. Typically, the regulations state minimum and maximum levels of minerals, including Na, K, Cu, Mg, Fe, Zn, Mn, Ca, P, I, Cl, and Se. Producers must also adhere to regulations for maximum concentrations of potentially toxic elements, including As, Cd, Hg, Pb, Ni, Sn, and Cr. Since toxic elements are only likely to be present in food samples at low concentrations, ICP-MS is increasingly used for the high throughput, routine analysis of infant formula. ICP-MS is suited to the application since it is a fast, multi-element analysis technique with the necessary sensitivity and dynamic range to measure nutrient and toxic elements in the same sample.

ICP-MS can also be used to measure nanoparticles (NPs) using a single particle ICP-MS (spICP-MS) method. spICP-MS is a powerful technique that is used increasingly to detect metal-containing nanoparticles in various matrices, including foods.

In this study, a simple and robust quantitative workflow was developed using ICP-MS for the simultaneous analysis of 28 nutrient and toxic elements in fully digested infant formula samples. A qualitative multi-element workflow using spICP-MS was also used to scan for 13 major and trace element-containing NPs in the same samples.

The accuracy of the multi-element quantitative method was evaluated by analyzing 13 nutrient elements in a NIST 1849a Infant/Adult Nutritional Formula SRM. The mean measured concentrations were in excellent agreement with the certified values, with recoveries well within ±10% of the expected value. Eight of the most commonly regulated elements in food were spiked into three off-the-shelf infant formula samples. The recoveries of the spiked elements were between 95 and 109%, which further validated the accuracy of the method for the analysis of commercial infant formula samples.

To detect unknown NPs in infant formulas, five off-the-shelf products were prepared according to the label and diluted 1:50 with de-ionized water. Each sample was scanned for 13 major and trace element containing NPs. The results showed the likely existence of NPs containing Al, Ca, Fe, Zn, and Ba in some of the analyzed samples. The study demonstrates the potential of using spICP-MS for the routine analysis and monitoring of unknown metal containing NPs in foods.

Keywords: *infant formula, elements, ICP-MS, nanoparticles, sp-ICP-MS*

K5

EVALUATION OF A COMPLETE WORKFLOW FOR THE DETERMINATION OF ARSENIC SPECIES IN FISH AND RICE

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In addition to the determination of the total Arsenic content, the speciation analysis of Arsenic compounds has become more and more common in quality control laboratories in the food industry. Beverages like apple or orange juice, wine and others can be diluted for simple and fast sample preparation. For groceries such as fish or rice a more complex sample preparation is necessary. Besides dissolving the sample to completeness it is crucial not to change or convert the species and by this lose this information.
Here we compare different sample preparation procedures with respect to digestion quality, recovery of the arsenic species and sample throughput. The samples were either mechanically destroyed using the SpeedMILL PLUS or digested using the TOPwave Microwave. By adding Proteases (Proteinase K) lysis of the samples can be forced. HPLC ICP-MS was used to analyse the arsenic species with ppt limits of detection. A precise comparison of the different workflows proves the applicability of the SpeedMill PLUS for easy, fast and accurate routine analyses.

Keywords: *species, LC-ICP-MS, ICP-MS, arsenic, fish*

K6

ANALYSIS OF INORGANIC ARSENIC IN FEED AND FOOD

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Arsenic occurs in many different forms in various sample types, as inorganic arsenic or organic arsenic compounds. Inorganic arsenic is more toxic than organic forms of arsenic [1]. Some food may contain elevated levels of inorganic arsenic, e.g. rice and rice products, and seaweed. Feed ingredients of marine origin are typically sources of inorganic arsenic in animal feed.
Total arsenic and inorganic arsenic are included in the European Union (EU) feed and food legislation. There are maximum level (ML) for inorganic arsenic in rice and rice products [2], but not in feed or feed ingredients [3]. However, a footnote to ML for arsenic states, "Upon request of the competent authorities, the responsible operator must perform an analysis to demonstrate that the content of inorganic arsenic is lower than 2 ppm". Hence, there is a need for robust and standardised methods for the analysis of inorganic arsenic in feed and food.
The European Committee for Standardization (CEN) has validated methods for the analysis of inorganic arsenic in food [4] and feed [5]. The methods are based on the same principle; determination of inorganic arsenic (as sum of arsenite, As(III), and arsenate, As(V)) using HPLC-ICP-MS following acidic extraction in a water bath.
The European Union Reference laboratory for metals and nitrogenous compound in feed and food (EURL-MN) included arsenic and inorganic arsenic in their proficiency tests (PTs) in 2018 and 2019 in order to evaluate the capability of national
Reference laboratories (NRLs) to analyse these analytes.
The PTs materials were a mixed corn poultry feed, chili powder, and a seaweed meal, and part of the scope was to assess the performance of the NRLs in determining the mass fraction of arsenic and inorganic arsenic in feed and food. The PTs were conducted according to ISO 13528:2015. The assigned values were consensus values based on the results of the participants, and the performance of the NRLs were evaluated using z and ζ scores.
Overall, the performance of the NRLs was very satisfactory. For arsenic and inorganic arsenic, the performance of participants was very good to excellent. Although for inorganic arsenic less than half of the members of the EURL-MN network participated.
The results of the PTs will be discussed in detail, with focus on the methods used by the NRLs to determine arsenic and inorganic arsenic in food and feed.

[1] EFSA 2009, Scientific opinion on arsenic in food, EFSA Journal, 7(10), 1351
[2] EU Commission regulation 1881/2006 and amendments
[3] EU Directive 2002/32 and amendments
[4] EN 16802:2016 Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS
[5] prEN 17374 Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICP-MS

Keywords: *CEN, EURL-MN, HPLC, ICP-MS, proficiency test*

K7

DETERMINATION OF INORGANIC SELENIUM IN SELENIUM-RICH FOOD MATERIALS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO ATOMIC FLUORESCENCE SPECTROMETRY

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Selenium is one of the essential nutrient for human and animals. It has significant physiological functions in humans and plays an important role in the prevention of myocardial necrosis, immune deficiencies, and certain types of cancer. Selenium deficiency results in serious diseases in humans such as Keshan disease and Kashin-Beck disease .However, the safety threshold of selenium is very narrow. The toxicity, bioavailability and reactivity of Selenium depend on its chemical forms and concentration. Selenium has many species, including selenite(SeIV), selenocystine(SeCys2), selenomethionine(SeMet),se-methylselenocysteine(MeSeCys), and selenate(SeVI). Excessive inorganic selenium (e.g. SeIV and SeVI) intake will cause safety issues. Plant-derived selenium-rich food raw materials are used in various food process. Therefore, it is very important to determine the concentration of inorganic selenium in selenium-rich products.

In this study, selenium species in selenium-rich food materials was tested by high-performance liquid chromatography coupled to atomic fluorescence spectrometry（HPLC-AFS）.Sample were extracted into pure water by ultrasonic assisted extraction at room temperature. Inorganic and organic selenium was separated within by using 40 mmol/L diammonium phosphate (pH=6.0) and 60 mmol/L diammonium phosphate (pH=6.8) as mobile phase with gradient elution with Hamilton PRP-X100 anion exchange column (250 mm×4.1 mm, 10 μm). The detection limits were 7.5 μg/kg and 15 μg/kg for Se(IV) and Se(VI), respectively. The relative standard deviation (RSD%) was less than 6.5%.The average recoveries for the spiked samples were in the acceptable range of 80.0% and 120.0%.

This method was successfully applied for the determination of inorganic selenium in selenium-rich food materials. Analysis of diverse selenium-rich food materials indicates that the content of inorganic selenium varies in different that materials. The proportion of inorganic selenium in most samples is less than 10% while few samples containing as high as 25% inorganic selenium. So the content of inorganic selenium in selenium-rich raw materials should be monitored in order to ensure the materials related safety issue

K8

SELENIUM SPECIATION IN FEED INGREDIENTS AND IN SALMON FEED

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Selenium (Se) is an essential element for fish, including Atlantic salmon (*Salmo salar*). The element exists in different chemical forms, or Se species. The chemical properties of the Se species, combined with the concentrations of Se, affect the uptake and metabolism of the element. For Atlantic salmon, the major source of Se is the diet. The average Se concentration of selected fish feed produced in Norway in 2018 contained a concentration of 0.7 mg/kg and was ranging from 0.24 to 2.3 mg/kg (n= 76) [1]. The level of Se in complete feed depends on the feed composition and the natural levels of Se in the feed ingredients used. Traditionally, fish feed mainly composed of marine feed ingredients, whereas today`s feed contains a higher inclusion of plant-based proteins. Currently, there is also a focus on new feed materials, such as insects. Insect meal was recently approved as a feed ingredient for aquaculture production in the European Union (EU) [2]. Se is regulated as a feed additive in EU. The supplementation of Se is regulated with an established maximum level of 0.5 mg/kg feed [3], and the addition of organic selenium (Se-yeast) is limited to a maximum level of 0.2 mg/kg feed [4,5].
To control whether feed comply with the legislation there is a need for an analytical method that can discriminate between inorganic and organic selenium species in feed. Furthermore, few data exist on the Se concentrations and Se species present in feed ingredients, both marine, plant-based and insects. Speciation data on Se in feed ingredients can provide information on the natural composition of Se originating from the feed ingredients. In the present study, Se species were determined using high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICPMS). The Se species were separated using ion-exchange chromatography, and different extraction procedures were applied to evaluate the extraction efficiency of inorganic and organic Se species in the feed ingredients and in the complete feed. Results from analysis of feed ingredients and fish feed will be presented and discussed with regards to analytical challenges.

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[2] EC, Regulation (EC) No 2017/893 of 24 May 2017. Official Journal of the European Union L138/92 (2017).
[3] EC, Regulation (EC) No 1831/2003. Official Journal of the European Union L268/29 (2003)
[4] EFSA, Scientific Opinion on the safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast Saccharomyces cerevisiae NCYC R645 (SelenoSource AF 2000) for all species. EFSA J. 9 (2011) 15.
[5] EFSA, Scientific Opinion on Safety and efficacy of Sel-Plex® (organic form of selenium produced by Saccharomyces cerevisiae CNCM I-3060) for all species. EFSA J. 9 (2011) 52.

Keywords: *selenium, speciation, feed, insects, HPLC-ICPMS*

K9

METHOD OPTIMIZATION USING FRACTIONAL FACTORIAL DESIGN FOR ARSENIC SPECIATION IN MARINE SAMPLES

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Marine organisms from lower trophic levels, such as marine algae, blue mussels, and mesopelagic organisms, are recently being considered as novel feed ingredients in aquaculture production. Within the European Union, maximum limits are established for undesirables in feed and feed ingredients, including heavy metals and arsenic (Directive 2002/32 EC and amendments). Arsenic is a trace element that is known for its complex chemical nature, with over 100 naturally occurring species identified in the marine environment [1]. Inorganic arsenic is classified as carcinogenic substance by the International Agency for Cancer Research (IARC), while the organic compound arsenobetaine is considered non-toxic to both human and animal [2]. The European Food Safety Authority Panel on Contaminants in the Food Chain highlighted the need for arsenic speciation in different foodstuffs to prevent overestimation of health effects related to dietary arsenic exposure.
Arsenic speciation requires extraction under mild conditions to preserve the original chemical properties of the species. As a single extraction procedure for all foodstuffs is not possible, a targeted sample preparation is recommended [3]. In the present study, a fractional factorial design was applied to optimize the extraction of water-soluble arsenic species in blue mussels. The experimental procedure was performed according to 2^{7-3} fractional factorial design (16 experiments). The tested factors were sample amount, type of extraction solution, amount of extraction solution, addition of hydrogen peroxide, extraction temperature, extraction time, and use of ultrasonication. Analysis of variance (ANOVA) was used to test for the statistical significance of main effects at a confidence level of 95%. The optimum extraction condition was evaluated based on arsenic recoveries in the extracts and the stability of arsenic species. Inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the total arsenic, whereas arsenic speciation was carried out via high performance liquid chromatography with both anionic and cationic exchange coupled to ICP-MS detection (HPLC-ICP-MS). The optimised extraction and chromatographic conditions were applied to certified
Reference materials and different marine samples. The proposed method is hereby shown useful for the determination of arsenic species in marine samples.

[1] A. H. Petursdottir, J. J. Sloth and J. Feldmann, *Analytical and Bioanalytical Chemistry* 2015, *407*, 8385-8396.
[2] T. a. S. F. Kaise, *Applied Organometallic Chemistry* 1992, *6*, 155-160.
[3] K. A. Francesconi, *Applied Organometallic Chemistry* 2003, *17*, 682-683.

Keywords: *arsenic, speciation, fractional factorial design, HPLC-ICP-MS*

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K10

DETERMINATION OF INORGANIC ARSENIC BY FAST ANION EXCHANGE HPLC-ICP-MS

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It is well known that the toxicity of the inorganic arsenic (iAs) forms (arsenite, As(III) and arsenate, As(V)) is much higher compared to the rest of the (organo-)arsenic species. That is why regulations in many countries specify the need of determination of iAs content in food- and feeding stuffs, e.g. European regulation 1881/2006 includes maximum levels of iAs in rice and rice products and directive 2002/32/EC on iAs determination in animal feed.[1,2,3] It is expected that the European Commission will consider establishing maximum levels for iAs in other commodities in the future.
This work focused on optimization of an HPLC-ICP-MS method for rapid analysis of inorganic arsenic in different food and feed matrices. The chromatographic method is based in the one developed by Jackson[4] and further modified by Gray et al.[5] and successfully applied to rice and wine samples already. The method presented here is compliant with the current European regulation and follows the requirements of the European standard methods for determination of iAs in food[6] and feed[7].
Acidic extraction as sample preparation followed the guidelines stated in the European standard methods EN16802:2016 and prEN17374.[6,7] During the extraction procedure oxidation of As(III) to As(V) is performed, which simplifies the determination of iAs to just one species (As(V)), and therefore chromatographic conditions can be modified accordingly to achieve selective determination of this species. Determination of iAs was performed with an Agilent 1260 HPLC with binary pump coupled to an Agilent 8900 ICP-QQQ. The latter ran in single quadruple mode with He as a collision gas to remove $^{40}Ar^{35}Cl$ interference, which makes it possible to use the same method with single quadrupole instruments.
By the optimised method, it was possible to separate iAs from other As compounds such as dimethyl arsenic acid (DMA) and methyl arsenate (MA) in less than 2 min. The method is useful for fast quantification of the iAs content in food and feed. The accuracy varied between 82 and 111% for the reference materials. The precision was estimated at 7.5% RSD or less for most of the samples. The estimated LOQ at approx. 0.01 mg/kg is low enough to be compliant with the legislation requirements for iAs determination in rice (LOQ < 0.04 mg/kg; regulation 333/2007[8]) as well as for feed analysis, where the present limit is set at 2 mg/kg[3].

[1] EFSA 2009, Scientific opinion on arsenic in food, *EFSA Journal*, 7(10), 1351.
[2] EU Commission regulation 1881/2006 and amendments
[3] EU Commission directive 2002/32/EC and amendments
[4] B. P. Jackson, *J. Anal. At. Spectrom.* 2015, 30, 1405–1407
[5] P. J. Gray et al., *J. Anal. At. Spectrom.* 2017, 32, 1031–1034
[6] EN 16802:2016, Determination of inorganic arsenic in foodstuffs of marine and plant origin by anion-exchange HPLC-ICP-MS
[7] prEN 17374, Determination of inorganic arsenic in animal feed by anion-exchange HPLC-ICPMS
[8] EU Commission regulation 333/2007 and amendments

Keywords: *Inorganic arsenic, HPLC, ICP-MS, food, feed*

K11

IODINE - A POTENTIAL CHALLENGE FOR SEAWEED INDUSTRY

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Iodine is one of the essential elements for humans and animals. It is important for synthesis of thyroid hormones and hence normal growth and development.[1] The recommended daily intake of iodine for adults is around 150 µg/day.[2] Insufficient intake may result in various health problems, which are well known. Much less emphasis is given on the excessive iodine intake, which can also be harmful especially for people with history of iodine deficiency.[3]
The main source of iodine for humans in the western part of the World is normally iodised salt, but from natural sources, seafood contains the highest amounts of iodine compared to other foodstuffs.[4] Seaweeds have a long tradition in Asian cuisine but their popularity has increased in western parts of the world in recent years. Their high iodine content can be either an advantage in prevention of iodine deficiency, or disadvantage, when the concentration is high enough to result in excessive iodine intake. Some species tend to accumulate extremely high amounts of iodine. Laminaria and Saccharina can contain 8000 µg I/g of dry weight or even more.[5] Consumption of 0.1 g of these seaweed species would not only exceed recommended daily intake, but also upper tolerable limit of 600 µg I/day.[2]
The problem of high iodine content in some seaweed species has also been recognised as a market barrier by the seaweed industry. The possibilities for reduction of iodine content in seaweed and seaweed products therefore need to be investigated.
The results of the iodine reduction experiments in seaweed and seaweed products will be discussed with details on iodine determination and seaweed processing procedure.

[1] Zimmermann, Jooste & Pandav 2008. Iodine-deficiency disorders, *Lancet*, 327, 1251-1262.
[2] EFSA 2014, Scientific opinion on dietary Reference values for iodine, *EFSA Journal*, 12(5), 3660.
[3] Leung & Braverman 2014. Consequences of excess iodine, *Nat. Rev. Endocrinol.,* 10, 136–142.
[4] Osterc, Stibilj & Raspor 2011. Iodine in the environment, *in: Encyclopedia of environmental health*, 280-287.
[5] Sødergreen Hansen & Lewandowski 2014. Determination of iodine and iodine species in marine samples by ICPMS and HPLC-ICPMS, *master thesis*, Technical University of Denmark.

Keywords: *iodine, seaweed, seafood, safety*

K12

SCREENING AND QUANTITATION OF TRACE METALS IN MILK BY USING ICP-MS

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Multi-elemental screening and quantification method of trace elements in milk using microwave assisted closed vessel acid digestion followed by ICP-MS technique using Thermo Fisher Scientific iCAP-RQ ICPMS with Qtegra ISDS software. This method covered 18 elements (Pb, Cd, Hg, As, Cu, Sn, Cr, Ni, Co, Se, Sb, B, Sr, Be, Ba, Mn, V, Al). The milk sample was digested with the tri-acid combination (Nitric acid, Hydrogen peroxide, and Hydrochloric acid). An internal standard mixture (Sc, Ge, Y, In, Tb, Rh, Tl, Ir, Bi) with concentration 20µg/L was used, and gold solution (200 ug/L) is used in sample preparation as a stabilizer for mercury. Isopropanol (2%, v/v) is added as an organic modifier to improve the ionization efficiency of elements. The prepared sample is aspirated and quantified against the calibration curve which is plotted with 5 points (calibration ranges from varies with elements). The linearity offered excellent R^2 values (> 0.995). LOQs (0.5 to 80 ug/L) are verified through spiking and offered >80% recovery with <10% RSD. The method is assessed for its performance in terms of sensitivity, selectivity, recovery, and precision as per the AOAC 2015.01 guideline. An average recoveries were in the range of 80-115% in compliance with AOAC 2015.01 guideline. This method provides a solution to food testing laboratories.

Keywords: *food, milk, metals trace elements*

K13

METALS DETECTION IN LAND SNAILS COLLECTED IN SICILY, SOUTHERN ITALY

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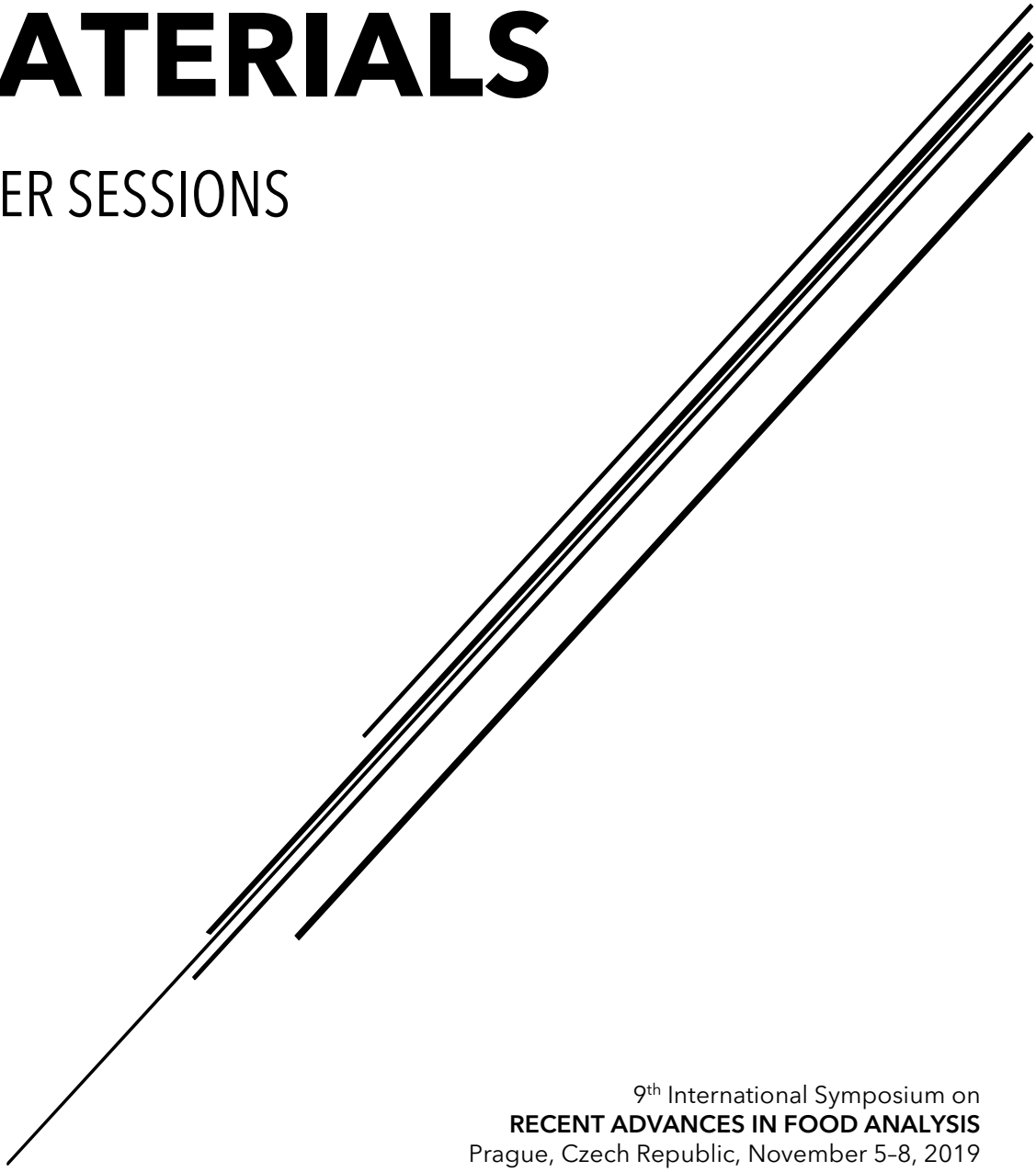
We analyzed the presence of heavy metals and PAHs in 270 samples of terrestrial gastropods belonging to two different species from natural parks (Monti Sicani and Cano Randello) and large industrial and urban complexes (Syracuse and Ragusa), to confirm the possible use of these organisms as environmental bioindicators. Heavy metals such as V, Cr, Cd, Pb, As, Mn and Fe were analyzed through the development and validation of a method based on the ICP-MS. The mercury levels were instead calculated using a direct analyzer. For the detection of PAHs, a GC-MS / MS with QuEChERS extraction was developed and validated. The validation results revealed the high reliability of the methods developed. The results verified significant differences in the values of V, Cr, Pb, As and Fe between urban/industrialized areas and natural parks ($p > 0.05$). No significant differences were found for the values of Cd ($p>0.05$). The Ragusa samples found the highest average values of V, Pb and As (0.18, 0.14 and 0.04 mg/Kg) while the Syracuse samples showed the highest average values of Chromium. The lowest values of heavy metals were found in the samples of Cava Randello. The present study reports for the first time the levels of Fe in terrestrial gastropods highlighting high concentrations (up to 720 mg/Kg). No mercury and PAH levels were detected. The results of this study confirm the use of terrestrial gastropods as environmental indicators for some heavy metals present in the terrestrial environment. Further studies are necessary in order to add the snails among the bioaccumulative organisms into surveillance networks set up by the European territory.

Keywords: *heavy metals, bioindicators, ICP-MS, GC-MS/MS, QuEChERS*

MIGRANTS FROM FOOD CONTACT MATERIALS

POSTER SESSIONS



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M1

TRANSFER OF MELAMINE AND FORMALDEHYDE FROM BAMBOO WARE INTO FOOD AND FOOD SIMULANT DURING MICROWAVE HEATING

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In recent years, light-weight, break-proof and often colorful decorated 'bamboo ware' is being placed on the market. Such articles for the consumption of food are offered in retail and very frequently also in e-commerce. Reusable cups for mobile drinking ('coffee-take-away'), but also drinking bottles and dishes for children are representative for this product category. Although these articles are often advertised as being sustainable, biodegradable and made of 'natural' materials, they are made of plastic to which bamboo fibers are added as an additive (filler). They must therefore comply with the requirements laid down in the Commission Regulation (EU) No. 10/2011. Polymers such as melamine-formaldehyde resins (MFR) are frequently used for the production of 'bamboo ware'. However, the migration of MFR-derived monomers into food and food simulant is regulated by specific migration limits (SMLs, melamine: 2.5 mg / kg food/simulant, formaldehyde: 15 mg / kg food/simulant).
Recent findings by Official Control Laboratories (OCLs) and notifications in the European Rapid Alert System for Food and Feed (RASFF) indicate that the processed materials are not always suitable for the intended use from a health perspective, as in some cases high transfer of formaldehyde and melamine into food simulants was observed. These findings are mainly based on migration tests with acidic food simulants carried out for 2 hours at 70°C (so-called 'hot-fill conditions').
'Bamboo ware' is often labeled to be unsuitable for the preparation of food in a microwave oven. However, data on the transfer of melamine and formaldehyde from 'bamboo ware' into food or food simulant during microwave heating are currently lacking. For this reason, the National
Reference Laboratory (NRL) for Food Contact Materials has carried out investigations to determine the transfer of these monomers under real-use conditions during microwave heating of food. The obtained results are presented and discussed.

Keywords: *melamine, formaldehyde, bamboo ware, microwave oven, migration testing*

M2

TRANSFER OF LUBRICANTS FROM HAND BLENDERS INTO FOOD SIMULANT, WITH A PARTICULAR FOCUS ON THE RELEASE OF CHLORINATED PARAFFINS

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Hand blenders are widely used in many households, i.e. for the preparation of food for population groups, who foreseeably eat their food in a pureed form, e.g. infants and elderly people. In 2015, researchers from the Stockholm University reported the qualitative detection of chlorinated paraffins (CP) in animal feed and explained these findings by transfer of CP from hand blenders used for the comminution of the samples [1].
CP are mixtures of up to 200 congeners of (poly)chlorinated, saturated and unbranched hydrocarbons, which found use e.g. as lubricants in metalworking. They are subdivided into short-chain (SCCP), medium-chain (MCCP) and long-chain (LCCP) chlorinated paraffins.
The intended use of SCCP has been banned since December 3rd 2015 by Reg. (EU) No 2015/2030, amending Reg. (EC) No 850/2004 on persistent organic pollutants. According to REACH Reg. (EC) No. 1907/2006, they were classified as substances of very high concern (SVHC) due to their high persistency, bioaccumulation potential and toxicity (PBT, vPvB). According to CLP Regulation (EC) No 1272/2008, SCCP are classified as carcinogenic (class 2, suspected of causing cancer), Aquatic Acute 1 (very toxic to aquatic life) and Aquatic Chronic 1 (very toxic to aquatic life with long lasting effects). MCCP are included in the Community Rolling Action Plan (CoRAP) according to REACH Reg. Initial grounds for concern are suspected PBT/vPvB properties, high (aggregated) tonnage and wide dispersive use. According to CLP Reg., MCCP are also classified as Aquatic Acute 1 and Aquatic Chronic 1 and labelled as Lact. 1 (may cause harm to breast-fed children).
The National Reference Laboratory (NRL) for Food Contact Materials (FCM) located at the German Federal Institute for Risk Assessment (BfR) has commissioned a study on the release of CP from hand blenders into food simulant. As a first non-representative inventory, a total of 19 randomly selected commercial hand blenders were examined for the release of CP as well as other lubricants into food simulant. The results of this study are presented.

[1] Strid, A., Athanassiadis, J., Bergman, Å., 2014. Hand blenders available on the Swedish market may contaminate food with chlorinated paraffins. In: Annex E submission Pamela Miller, Alaska Community Action on Toxics and IPEN.

Keywords: *chlorinated paraffins, hand blenders, SCCP, MCCP, lubricants, migration*

M3

ANALYSIS OF UNDESIRABLE HYDROCARBONS FROM FOOD CONTACT MATERIALS: IN THE WORLD OF MOSH, MOAH, POSH & CO.

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The contamination of food with different types of hydrocarbons is an on-going topic. The introduction pathways are wide-ranging and can occur at all stages of the production chain from farm to fork. In the past, the main focus was on mineral oil hydrocarbons (MOH), which were observed to migrate from food contact materials, such as jute bags and recycled cardboard, into food. MOH can also enter the food in substantial amounts at earlier stages of the production chain. In 2012, EFSA released an 'scientific opinion on mineral oil hydrocarbons in food', which sets no TDI and recommends further research [1]. EFSA stated also that MOH, especially the aromatic hydrocarbons are of potential concern. Other food contact materials like polyolefins and adhesives can also release aliphatic and aromatic hydrocarbons into food [2] [3]. These migrating synthetic hydrocarbons (oligomers) are also determined during the routine method for MOSH/MOAH (HPLC-GC-FID) and are frequently misinterpreted as mineral oil hydrocarbons. The presentation provides a multiple approach to differentiate mineral oil and synthetic hydrocarbons via multidimensional chromatography (HPLC-GC-FID vs GCxGC-MS/FID). First of all, the retention times and elution locations of analytes in the 1D/2D-chromatograms are compared. Secondly, specific mass fragments to visualize different hydrocarbon types are introduced. Furthermore, specific case studies regarding MOH, PAO, POSH and further oligomers are discussed.

[1] EFSA, EFSA Journal 10 (2012) 2704
[2] Lommatzsch M. et al., Food Addit. Contam. A 33 (2016) 473
[3] Biedermann M. & Grob K., J. Chromatogr. A 1375 (2015) 146

Keywords: *MOSH, MOAH, oligomers, migration, FCM*

M4

TARGETED SCREENING OF 35 ENDOCRINE DISRUPTORS RELEASED FROM PLASTIC BASED FOOD CONTACT MATERIALS

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Over the past decade, the production and use of plastic packaging materials have increased in the wine industry. These containers are made of various polymers containing hazardous chemical components and additives, which are able to leach out from the matrix and may lead to an endocrine disruption effect.
A generic UHPLC-MS/MS method was developed to map out 35 compounds, including alkylphenols, amines, bisphenols, BADGES, benzotriazoles and phthalates without any previous discrimination of the kind of plastic used. To simulate the wine contact, specific migration test were carried out with hydroalcoholic solutions stored at 40°C for several days.
Careful optimizations of the analytical method was performed, the separation of these molecules being critical due to their various structures and chemical properties (logP ranging from -0.4 to 10). Five different stationary phases were evaluated and unequivocal determination of two pairs of isobaric phthalates was ensured using a core-shell polar based support. The method was fully validated using official simulant and satisfying validation results were obtained allowing to reach ppb level for most toxic compounds. Some plastic films used by the wine industry have been tested. A release of 4-nonylphenols (4-NP) at a concentration of 350 µg/kg was observed after only one day of contact in one sample. 4-NP is known to be toxic for reproduction and is therefore banned in such material according to Swiss regulations [1].
A survey dedicated to reusable plastic containers made of various materials, is in progress and showed several contamination with 4-NP, phthalates and Bisphenol A.
These results demonstrated the need to have powerful analytical tools to monitor such contamination and avoid such exposition through foodstuffs absorption.

[1] Swiss Ordinance of the FDHA on articles and materials intended to come into contact with foodstuffs from16 December 2016.

Keywords: *food contact material, endocrine disruptors, migration, packaging, wine*

M5

IDENTIFICATION OF PER- AND POLYFLUORINATED SUBSTANCES IN FOOD CONTACT MATERIALS BY MASS SPECTROMETRIC PROFILING

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Introduction: Food contact materials (FCM) are all types of items intended to be in contact with food fulfilling various tasks like storage or food consumption. Some examples of FCM are coffee filters, pizza boxes, and baking paper. Common materials used to produce FCM are plastic, cardboard, and paper. To improve the functionality of the FCM, chemicals like per-and polyfluorinated alkyl substances (PFAS) are added to these raw materials. The oleophobic as well as hydrophobic properties of PFAS generate a water and grease resistant material. The potential toxicity, strong persistence in the environment and bioaccumulation of PFAS resulted in an increased control of these chemicals in FCM. Nonetheless, the control-procedures only include tests for a limited number of well-studied PFAS. In a survey authorized by the Danish Veterinary and Food Administration, identified PFAS contributed marginally to the total organic fluorine in some analyzed FCM (unpublished). Based on this fact, it is assumed that unidentified PFAS are present. Identification of these components is of high priority for the consumer protection. Therefore, a screening method for PFAS using high-resolution mass spectrometry was developed and applied to FCM.
Methods: A 6 cm^2 piece of each FCM is cut out, placed in an 2 mL Eppendorf-tube and extracted with 1.5 mL of 50% ethanol in water. The mixture is sonicated for 60 min at 60 °C. Afterwards, the extract is transferred into a 0.2 µm filter vial and internal standard (IS) is added. The IS-spiked extract is injected in a liquid chromatography system coupled to a quadrupole-time of flight mass spectrometer equipped with an electrospray ionization source. The chromatographic separation is performed on a C18 Acquity UPLC CSH column using 2 mM ammonium acetate in water (pH 8.5) as mobile phase A and 2 mM ammonium acetate, 15.5 mM ammonium hydroxide in methanol as mobile phase B. The data is acquired in negative ionization mode and screened against a compound library containing multiple PFAS found in industrial products. Additionally, mass defect filtering techniques are employed.
Preliminary Results: The screening revealed multiple PFAS present in FCM. For example in disposable, microwavable plates, the comparison with authentic standards allowed the identification of various PFAS including PFBA, PFPeA, PFHxA, PFHpA, PFDA, and PFOA. For compounds without standards available, such as 1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro-N-(4-hydroxybutyl)-N-methylheptane-1-sulfonamide ($C_{12}H_{12}F_{15}NO_3S$) and 3-Methoxyperfluoro(2-methylpentane) ($C_7H_3F_{13}O$), MS/MS will be employed to confirm their identity.

Keywords: *LC-QToF screening, food contact materials, PFAS, identification*

M6

HEALTH RISK ASSESSMENT OF EXPOSURE TO PERFLUOROOCTANE SULFONATE AND PERFLUOROOCTANOIC ACID MIGRATION OF FOOD CONTACT PAPER AND BOARD UNDER TEMPERATURE VARIATIONS

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Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are widely used in food contact paper and board because of the hydrophobic and lipophobic properties of PFOS and PFOA. They can migrate from food contact paper and board into food and the concentration of migration may increase at high temperature. Moreover, PFOS and PFOA concentrations have been detected worldwide in human blood. A positive association has been observed between concentrations of PFOS/PFOA and total cholesterol. Therefore, the main aim of this study is to assess human health risks of increased total cholesterol associated with long-term exposure to PFOS and PFOA migration of food contact paper and board under temperature variation scenarios in adults. Exposure assessment was carried out by an uptake dose model to estimate PFOS and PFOA uptake doses for the high-, intermediate- and low-exposure scenarios. Then, benchmark dose modelling was carried out to describe the dose-response relationships between PFOS/PFOA concentrations and total cholesterol levels and to estimate the benchmark doses 95% lower bound confidence limit ($BDML_5$) of PFOS and PFOA. Finally, we used a margin of exposure (MOE) approach for the risk characterization. Results of the exposure assessment showed that PFOS and PFOA uptake doses in the high-exposure scenarios were around 1 and 3 order(s) of magnitude greater than the intermediate- and low-exposure scenarios, respectively. Results of the dose-response assessment showed that the $BMDL_5$s of PFOS and PFOA were 1.67 ng kg-bw^{-1} day^{-1} and 1.46 ng kg-bw^{-1} day^{-1}, respectively. The findings of the risk characterization indicated that the MOEs of 95th percentiles of PFOS and PFOA uptake doses were greater than 1 in all of the scenarios; however, the MOEs of maximum PFOS and PFOA uptake doses at high temperature in the high-exposure scenarios should be concerned (lower than 1). This is the first Taiwan study to conduct a systematic health risk assessment regarding the migration of PFOS and PFOA from food contact paper and board. This study provides a methodology to conduct the health risk assessment of exposure to food contaminant migration of food contact paper and board.

Keywords: *perfluorooctane sulfonate, perfluorooctanoic acid, food contact paper and board, migration, health risk assessment*

M7

DETERMINATION OF BROMINATED FLAME RETARDANTS IN CONTAMINATED FOOD CONTACT POLYMERS AND FATTY FOOD SIMULANTS

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Brominated flame retardants (BFRs) are chemical substances commonly employed by industry to prevent or reduce the risk of fire in certain industrial products. They may be divided into families including brominated diphenyloxide, brominated bisphenol-A, brominated phenol, brominated phthalic anhydride, brominated polymers and aliphatic brominated compounds. In the past years, several publications related to plastic food contact articles (FCA) contaminated with BFRs in the European Union were published. It was suggested that BFRs contaminations could come from recycled polymers, particularly from waste electrical and electronic equipment (WEEE). BFRs are not allowed to be used in food grade polymers. If BFRs are present in the final FCA, they may be prone to migrate into food and that may be a food safety issue. We developed analytical methods to analyze BFRs belonging to different families in contaminated food contact materials as well as fatty food simulants by using XRF, GC-MS and LC-MS. The methods were validated in terms of coefficient correlation of the calibration curves, limit of detection, limit of quantification and relative standard deviation, obtaining good results. The first step was to identify BFRs in contaminated FCA. Afterwards, the contaminated samples were placed in contact with fatty food simulants (e.g. 95% ethanol) at a certain controlled time-temperature (e.g. 60 °C for 10 days) in order to perform the migration experiment. Results will be presented and discussed here and will provide valuable information on the migration patterns of BFRs from contaminated polymers into fatty food simulants.

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M8

ADVANCES IN HEADSPACE SAMPLING FOR ENHANCED RESIDUAL SOLVENT ANALYSIS IN FOOD PACKAGING

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Packaging materials and food containers are essential to ensure safety, quality and product shelf-life. Volatile organic compounds (VOC) used in printing inks, varnishes, dyes and adhesive applied to the final package can leach from the surface and contaminate the food product during manufacturing, shipping and storage determining significant health risks and negatively impacting on the taste, aroma and appearance of the product.
Besides the good manufacturing practices, United States and the European Union have implemented regulations to address the use and to quantitate residual solvents in packaging material. Residual VOCs in food packaging are traditionally analyzed by headspace gas-chromatography (HS-GC), representing a fast and simple technique without the need for time-consuming sample preparation.
Innovative design features now available in modern valve and loop headspace autosamplers provide high analytical performance when it comes to routine solvent analysis. This work is focused to highlight performance and benefits of a new valve-and-loop static headspace sampler coupled to a GC-MS/FID dual-detector configuration, according to the regulatory requirements (EN 13628-1:2002). The dual detector GC-FID/MS configuration is easily set
up through a 3-port microfluidic device, allowing for simultaneous identification and confirmation of known and unknown impurities, increasing the confidence in compound identification and solving possible analytes co-elution.
Reliable quantitation through automated Multiple Headspace Extraction (MHE) calibration is
easily achieved and the data processing and reporting easily automated through the chromatography data system software, for a fully automated workflow.
Excellent MHE linearity assures high precision and accuracy in the quantitative determination of residual solvents while significantly reducing the operator workload.
High-throughput requirements are also smartly satisfied for routine operation thanks to the overlapping capability between MHE cycles of the same sample and, as well, between different samples in the sequence, automatically optimizing the overall workflow cycle time.

Keywords: *gas chromatography, VOC, GC, residual solvent analysis, headspace sampling*

M9

MIGRATION OF SULFITES FROM WOODEN FOOD CONTACT MATERIALS INTO A FOOD SIMULANT

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Wooden food contact materials were used widely because these are light, unbreakable, cheap and convenient. These are made by cutting the wood itself. A sulfite solution could be used as a bleach agent. Residual sulfites might be migrated into foodstuffs when food contact materials are used for handling, cooking and eating of them. The purpose of this study was to investigate the amount of sulfites migrated from wooden food contact materials into foodstuffs, which are very important to secure the food safety. Determination of sulfites was performed by ion chromatography (IC). The method validation was accomplished through measurement of the limit of detection(LODs), the limit of quantification (LOQs) and recovery within the calibration curve range of 0.1~10 mg/L, which were 0.01 mg/L, 0.05 mg/L and 97.4~99.0% respectively. The optimized method would be applied to the determination of sulfites migrated from wooden food contact materials. All the samples were wooden articles collected from retailed markets in Korea, such as chopsticks, spoons, bowls, cutting boards and plates. Migration tests were performed at 70℃ for 30 min with water as a food simulant. Monitoring data would be a scientific basis for the safety management of wooden food contact materials.

Keywords: *migration, sulphites, wooden food contact materials, food simulant*

M10

STRATEGIES FOR THE DETERMINATION OF VEGETABLE OILS CONTAMINATION BY MOSH/MOAH USING GC(XGC)-TOFMS AND GC-FID

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Mineral oils represent a complex mixture of saturated hydrocarbons (MOSH, n-alkanes, branched alkanes, saturated cycloalkanes) and aromatic hydrocarbons (MOAH, alkylated aromatic hydrocarbons). MOSH/MOAH can enter the food chain from the environment, food contact materials, various "accidents" during food production (e.g. release of machine lubricants into the food products) or by targeted addition to food (meat products, candies).
MOSH are not carcinogenic, but always contain a certain amount of MOAH, that may be mutagenic to humans, and it is therefore important to monitor their occurrence in food.
In this study, a method for the determination of MOSH/MOAH in vegetable oils using gas chromatography with flame ionisation detector (GC-FID) was implemented in the frame of Recommendation (EU) 2017/84 for the monitoring of mineral oils and the JRC Technical Report, e.g. limit of quantification below 2 mg/kg, recovery in the range 70 - 120 % and repeatability below 20 % for fat and oils [1]). In our procedure MOSH/MOAH were separated from a lipid fraction using solid phase extraction with modified silica gel (0.3 % Ag^+, *m/m*) as a suitable stationary phase. For the elution of targeted compounds a hexane and then mixture of hexane, dichloromethane and toluene were applied. It is even possible to fractionate MOSH/MOAH on this column [2]. Since the FID is a non-selective detector not enabling to distinguish individual substances nor to detect and identify possible interfering compounds from natural sources, other tool for co-eluting MOSH/MOAH separation was needed. Therefore in the new approach two-dimensional gas chromatography coupled with mass spectrometry employing time-of-flight mass analyser (GCxGC-(TOF)MS) applying a mid-polar column (BPX-50, SGE Analytical Science, Australia) in the first dimension and non-polar column (DB-1ht, Agilent Technologies, USA) in the second dimension was tested. MS does not allow quantification of the whole group of substances (MOSH or MOAH), but can be used for identification and determination of individual compounds. These two detection techniques are therefore complementary and can be used simultaneously to best characterisation of mineral oil contamination in food commodities, such as vegetable oil. Finally, both methods will be applied for determination of MOSH/MOAH in 30 vegetable oils obtained from the Czech market (rapeseed n=10, sunflower n=10, olive n=9, coconut n=1).

[1] Bratinova, S. a E. Hoekstra, ed. Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials 2019, Available at: http://publications.jrc.ec.europa.eu/repository/bitstream/JRC115694/kjna29666enn_2.pdf
[2] Liu, L.L., Huang, H., Wu, Y.W., Li, B.N., and Ouyang, J., Offline Solid-phase Extraction Large-volume Injection-Gas chromatography for the Analysis of Mineral Oil-saturated Hydrocarbons in Commercial Vegetable Oils. Journal of Oleo Science, 2017. 66(9): p. 981-990.

Keywords: *MOSH, MOAH, GCxGC-TOFMS, GC-FID*

Acknowledgement: *„Financial support from specific university research (MSMT No 21-SVV/2019)"*

M11

TARGET SCREENING OF ACRYLATES FROM UV-CURING INKS AND COATINGS IN FOOD CONTACT MATERIAL

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Ultraviolet (UV) cured coatings show excellent appearance, durability, and little or no volatile organic compound (VOC) emissions, while enabling increased productivity and lower overall costs. An UV-curable coating is one of the best finishing methods in the paper and packaging industries for protecting ink layers from physical and mechanical defects [1]. Liquid UV-curing printing inks cure within a few seconds by irradiation with UV light.
The majority of commercial light cure resins are based on free radical curing acrylic compounds (acrylates).
In addition to photoinitiators, which start this polymerization, the formulations of printing inks contains monofunctional acrylic esters for viscosity adjustment and as a crosslinker to ensure rapid polymerization. Upon curing, it is possible and likely that this reaction will not be complete and the diluents and crosslinkers used will be unbound in the polymer. The UV-curing printing inks and coatings are widely used, even in the printing of food contact materials.
For the photoinitiator isopropylthioxanthone (ITX) a mass transition from the printed packaging material in the foodstuff was shown [2]. A migration of uncomplete cured acrylate monomers is likely. At least for trimethylolpropane triacrylate (TMPTA), a carcinogenic effect was shown [3].
A sensitive LC-ESI-MS/MS method was developed and validated for the target screening of different acrylate monomers in food simulants used for migration tests.

[1] Soltani, M. et al. (2013) UV-curable coating process on CMYK-printed duplex paperboard, Part 1: Mechanical and optical properties, BioRes. 9(1), 86-92.
[2] T. Jung , T.J. Simat & W. Altkofer (2010) Mass transfer ways of ultraviolet printing ink ingredients into foodstuffs, Food Additives & Contaminants: Part A, 27:7, 1040-1049, DOI: 10.1080/19440041003596543
[3] Kromhout, H. et al. (2018) Carcinogenicity of isobutyl nitrite, β-picoline, and some acrylates. The Lancet Oncology. 19. 10.1016/S1470-2045(18)30491-1.

Keywords: *acrylates, TMPTA, migration, printing ink, food contact material*

M12

ESBO DETERMINATION IN FATS, OILS AND HIGH FAT CONTAINING FOOD BY TRANSMETHYLATION AND 1,3-DIOXOLAN FORMATION, MONITORING

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Epoxidized soybean oil (ESBO) is a fat-soluble substance. In the meantime, ESBO is widely used in gaskets as a plasticizer in twist-off lids [1]. But also a contamination during processing (i.e. conveyor belt) or storage is possible. In the COMMISSION REGULATION (EU) No 10/2011 the specific migration limit (SML) of ESBO is 60 ppm (30 ppm for Babyfood). 1999 the Scientific Committee on Food (SCF) determined a TDI of 1 mg/kg body weight, which was confirmed 2004 by the European Food Safety Authority (EFSA) [2, 3]. At PiCA a wide range of fats, oils and high fat containing food is tested on ESBO. The test method of ESBO at PiCA is based on transmethylation and 1,3-dioxolan formation. There are still food samples exceeding the SML of 60 ppm.

[1] Global Legislation for Food Contact Materials, Joan Sylvain Baughan, Woodhead Publishing Series in Food Science, Technology and Nutrtion: Number 278
[2] SCF, 1999, Compilation of the evaluations of the Scientific Committee for Food on certain monomers and additives used in the manufacture of plastic materials intended to come into contact with foodstuffs until 21 March 1997. Reports of the Scientific Committee for Food (42nd series).
[3] Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food to the use of Epoxidised soybean oil in food contact materials. http://www.efsa.eu.int/science/afc/afc_opinions/467/opinion_afc10_ej64_epox_soyoil_en1.pdf

Keywords: *ESBO, transmethylation, 13-dioxolan formation, GC-MS*

M13

DETERMINATION OF BISPHENOL S AND TEN OTHER BISPHENOL ANALOGUES IN FRESH FISH FROM CANADA

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Bisphenol S (BPS) has been reported as an alternative for bisphenol A (BPA) in thermal papers. However, it is not clear whether BPS may be replacing BPA in other applications such as food packaging. There are also currently far fewer studies on the occurrence of BPS in food as compared to BPA. Multiple reports suggest that bisphenol analogues (e.g. BPS and bisphenol F) act as endocrine disrupters. Thus, it is important to investigate the occurrence of BPS as well as other bisphenol analogues in food to improve current risk assessments. In this study, fish samples were collected from 6 different markets in Montreal (Canada) from 2017 to 2019. The determination and quantification of eleven bisphenol analogues in fish were conducted using QuEChERS extraction followed by high performance liquid chromatography coupled with quadrupole time of flight mass spectrometry (HPLC-QTOF-MS) analysis. Good instrumental linearity (r^2>0.98) and recoveries (76-122%) were achieved for all targeted compounds. The limits of detection for the targeted chemicals was as low as 0.3-0.7 ng/g depending on the fish species. In total, 60 food composites (216 individual fish samples) were analyzed. BPA was detected in one single fish composite (cod, 16.5 ng/g). BPS was detected in 38.3% of the fish composites (not detected-93.8 ng/g). The average level of BPS in fish from Canada are comparable with reports for fish from US and China. Other bisphenol analogues were not detected in the fish composites. This is the first study to determine the level of BPS in fresh fish from Canada.

Keywords: *fresh fish, QuEChERS, HPLC-QTOF-MS, bisphenols*

Acknowledgement: *CIHR Grants: Endocrine disrupting chemicals: towards responsible replacements.*

M14

THE EVALUATION OF MIGRATION OF NON-TARGET COMPOUNDS AND PHTHALATE FROM BABY BOTTLES - AN EXTRACTION METHOD

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A simple and rapid method for the extraction of baby bottle migrants from milk stimulants employing a mixture of ethyl acetate:dichloromethane:hexane, using a simplex centroid design for optimization was developmented. The migration test used 50% EtOH in water at 70 °C during 2 h. Next, extraction and identification of migrants were performed by GC-MS, and the additives in the baby bottle materials were quantified by HPLC-DAD. On the account of the toxicological potential of dibutyl phthalate, the optimized mixture was used for in-house validation by GCMS of the proposed method. Dibutyl phthalate (DBP) migration was detected in three baby bottles with a concentration between of 175 to 235 μg kg^{-1}, which is lower than the specific migration limit determined by the Brazilian Health Regulatory Agency. However, exposure to DBP from baby bottles was estimated, and this was higher than the tolerable daily intake recommended by the European Food Safety Authority, indicatinga potential public health concern.

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M15

USING THERMAL DESORPTION GC-MS FOR THE (SEMI-QUANTITATIVE) EVALUATION OF POLYMERIC FOOD CONTACT MATERIALS

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Polymeric food contact articles are regulated within Europe by the Commission Regulation No. 10/2011 on plastic materials and articles intended to come into contact with food. This regulation describes in its table 1 a whole list of constituents which are allowed to be used if fulfilling the specific migration limit in pre-set food simulants (e.g. 10, 20 50 or 95 (v/v) % ethanol, 3% acetic acid, vegetable oil, TENAX). Such positive list system has the advantage that the analysts already should have received a list with substances which were used to compound the sample. However in certain cases such documentation is lacking or doubts appear in the documentation concerning the trueness of the content. Therefore, thermal desorption coupled to GC-MS is able to screen unknown constituents in polymeric material. Thermal desorption is using heat to extract from the matrix the constituents and therefore cover all food simulant media (polar and non-polar) scanning up to 1000 Da. For identified substances not listed in the positive list a migration limit below 0,01 mg/kg food simulant should be achieved. This evaluation can be performed on a semi-quantitative basis in the material directly by calculating the obtained chromatographical data in relation to the desorbed sample surface/weight, independant on the migration time and temperature of the migration taking into account the worst case migration scenario. As an advantage, several patterns from the not intentionally added substances (NIAS) could be evaluated as well.

Keywords: *thermal desorption, mass spectrometry, food contact material*

M16

COMPREHENSIVE LC×GC WITH FLAME IONIZATION AND VACUUM ULTRAVIOLET DETECTION FOR THE DETAILED CHARACTERIZATION OF MINERAL OIL AROMATIC HYDROCARBONS

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Mineral oil aromatic hydrocarbons (MOAH) are an important group of food contaminants that can end up in foodstuffs via different pathways, e.g. migration from food packaging (jute bags, recycled paper) or as residual contaminants from food-grade white oils used for e.g. coating of fruits and candies. Different methods for the analysis of MOAH have been developed. So far, these all rely on a laborious preseparation of the MOSH (mineral oil saturated hydrocarbons) and the MOAH, either by SPE or normal phase LC, followed by a quantification of the two fractions by GC-FID. In previous work we have studied the use of GC-Vacuum UltraViolet detection (GC-VUV) as a direct method for MOAH quantification without any preseparation.
Here we present an improved comprehensive silver-phase LC×GC-VUV method that uses a silver-loaded silica column for the separation of the sample according to aromaticity, followed by a GC separation based on boiling point (or size). Detection is performed using VUV detection for quantification as well as to provide structural information of the aromatic molecules based on their VUV spectra.
The method showed a better resolution for the MOSH/MOAH separation due to an increase of the peak capacity. Thanks to the use of silver based stationary phases, group-type separation of the mineral oil according to aromaticity (aliphatics, mono-aromatics and poly-aromatics) was achieved, with the VUV detector providing more detailed information on the aromatics. This information is useful for a better optimization of the removal process of the aromatics in white oil preparation.

Keywords: *mineral oil analysis, MOSH/MOAH, comprehensive LC×GC, VUV detection*

M17

DETERMINATION OF POSSIBLE SOURCES OF MOSH/MOAH FOOD CONTAMINATION USING GCXGC-TOFMS

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Mineral oil derives from crude petroleum through distillation processes and various refining steps. It contains saturated hydrocarbons (MOSH) and aromatic hydrocarbons (MOAH). MOSH fraction consists mainly of n-alkanes, isoalkanes and cycloalkanes whilst MOAH fraction is composed of monoaromatic and polyaromatic hydrocarbons with varying level of alkylation.
Mineral oil as food contaminant has received attention due to its possible negative effects on human health. Common sources of contamination are printing inks in recycled cardboard, batching oils in jute bags, machinery lubricating/hydraulic oils, pesticides, exhaust fumes or anti-dusting/anti-caking agents.
To determine the amount of mineral oils in food, an analytical method combining high performance liquid chromatography, on-line coupled with gas chromatography and flame ionization detection (HPLC-GC/FID), was developed by Biedermann und Grob. Mineral oils don't form sharp peaks in a chromatogram, but a so-called hump of unresolved complex mixture (UCM). This UCM is integrated and the total amount of contamination is quantified. A disadvantage of this method is, that it gives no conclusive information about what type of substances form this UCM. This presents a problem, because not only mineral oil can form UCM. Substances from packaging materials as well as natural terpenoids and waxes found in foods can form peak groups in the chromatogram that are similar in appearance to mineral oil contamination.
Comprehensive two-dimensional gas chromatography combined with time-of-flight mass spectrometry (GCxGC-TOFMS) is a technique that gives more detailed information about various contaminants and their chemical structure. With this system, it is possible to differentiate between hydrocarbons from mineral oils and hydrocarbons from other sources. Based on a presence of specific marker molecules together with characteristic signal patterns in the chromatogram and collected mass spectra, it is possible to draw a conclusion about a possible source of contamination and its chemical composition.
Using GCxGC-TOFMS, a method allowing differentiation between MOSH, polyolefin oligomeric saturated hydrocarbons (POSH), polyalphaolephines (PAO) and synthetic resins was developed. To achieve more sensitivity the system was directly coupled to an LC, using the same technology as for LC-GC FID. This enables to get a direct comparison between LC-GC FID data and GCxGC TOF data.

M18

QUANTIFICATION OF PET CYCLIC AND LINEAR OLIGOMERS IN TEABAGS BY A VALIDATED LC-MS METHOD - IN SILICO TOXICITY ASSESSMENT AND CONSUMER'S EXPOSURE

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Polyethylene terephthalate (PET) is one of the most important and widely used polymers as food contact material (FCM). It may contain low molecular weight oligomers which could be formed as side products of incomplete polymerisation during the manufacturing of PET or due to impurities in the raw materials. These are considered as "non-intentionally added substances" (NIAS), with no legal migration limits currently in place.
Several types of such PET oligomers could potentially be formed during production/degradation and are grouped into the so-called 1st, 2nd and 3rd series. The 1st series comprises an equal number of terephthalic acid and ethylene glycol units, whereas in the 2nd series an ethylene glycol unit is replaced by a diethylene glycol and in 3rd series two ethylene glycol units are replaced by two diethylene glycol units. All these oligomers may end up in the final FCM and eventually migrate to food. Their study is currently hindered by the current lack of proper analytical standards.
This work presents the development and optimisation of an UHPLC-qTOF-MS method for the quantification of the 1st series PET cyclic oligomers using commercial standards of known purity for each oligomer. The method has been developed and validated for all analytes in water and food simulants, and applied for the quantification of all series cyclic oligomers in PET teabags after migration. In case of 2nd and 3rd series PET oligomers a semi-quantitative approach has been applied by employing the most structural-similar 1st series PET cyclic oligomer standard as an analytical analogue.
An "*in silico*" genotoxicity assessment using the OECD Toolbox was performed for all the identified linear and cyclic oligomers. No genotoxicity alerts were found for any of these oligomers, but further toxicological examinations are still needed. A first preliminary estimation of the specific consumer's exposure to this group of substances via tea consumption is also presented.
Exposure due to tea consumption did not exceed the respective Toxicological Threshold of Concern (TTC) when a single consumption was assumed, but for multiple consumption this threshold was exceeded in the majority of the samples. Future studies are needed in order to explore further if this exposure is of toxicological relevance.

Keywords: *PET oligomers, migration studies, LC-qTOF-MS, Exposure assessment*

M19

DETERMINATION OF TOTAL MINERAL OIL CONTAMINATION IN MILK POWDERS BY MIXED SOLVENT EXTRACTION

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An efficient extraction method combined with on-line high-performance liquid chromatography-gas chromatography with flame ionization detector (LC-GC) was developed to determine the total mineral oil hydrocarbons (MOSH & MOAH) in complex matrices of foods. Unlike other extraction method, the proposed method was performed in mild conditions and low-boiling-point hydrocarbons were retained to the greatest extent. Since POH often appeared in MOH chromatographic profiles due to the co-eluent of MOH and POH when flowing through LC column. Thus, the integrated LC-GC humps represented the total contents of MOH and POH. Moreover, the efficacy of the new extraction method was validated by recovery and the limit of quantification (LOQ).

Keywords: *mineral oil contaminants; milk powders; extraction; mixed solvent*

M20

ANALYSIS OF BISPHENOL A IN FOODS USING SOLID PHASE MICROEXTRACTION WITH AN OVERCOATED FIBER

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Bisphenol A (BPA) is commonly used for food packaging applications such as polycarbonate bottles, and the linings of metal cans used for soups, juices, etc. It is a suspected endocrine disruptor, and thus low level, long term exposure as a result of migration into food from packaging materials is a concern. Extraction methods for determination of BPA in food include both solvent and solid-phase extraction, with the later more commonly used with liquid samples and the former for solid samples. Analysis can be done by either LC or GC, and both have been used throughout the literature. Solid-phase microextraction (SPME) has been used for the determination of BPA in water but has not been widely used for this application in food matrices due to sensitivity and fiber ruggedness issues associated with exposure to matrix components such as fats and proteins. In this work, the use of SPME was revisited in order to develop a quick, easy and sensitive method for analysis of BPA in a variety of food products. Matrix and fiber durability issues with immersion SPME were addressed through the use of an overcoated (OC) divinylbenzene (DVB) fiber. The overcoating, which consists of polydimethylsiloxane (PDMS), protected the DVB layer from contamination and increased the physical robustness of the fiber. SPME extraction using the OC-DVB fiber was followed by GC/MS/MS analysis for optimum sensitivity. The steps taken in method development and optimization will be described, as well as accuracy in a variety of matrices. Data will also be presented on method ruggedness compared to a standard, non-overcoated DVB fiber.

Keywords: *SPME, food, bisphenol A*

M21

INVESTIGATION OF THE MINERAL OIL CONTAMINATION IN JUTE AND SISAL SACKS BY GCXGC-TOF

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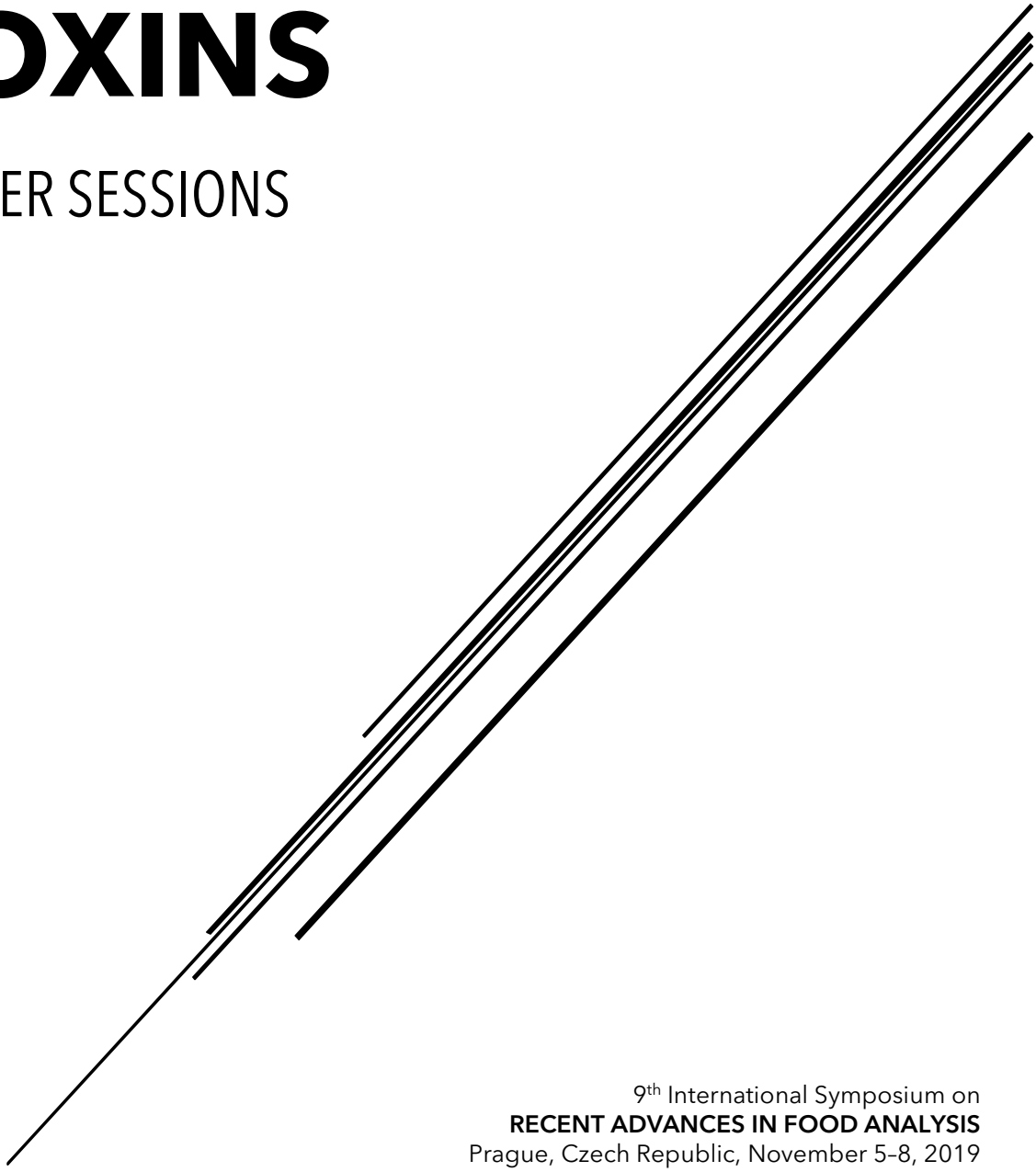
Mineral oil hydrocarbons (MOH) have been much discussed in recent years, and mitigation strategies have already been successfully implemented to protect consumers. The routine monitoring was established by the help of LC-GC-FID analysis prevailing. For this purpose, the MOH are separated by a normal phase LC into a fraction of mineral oil saturated hydrocarbons (MOSH) and another fraction of mineral oil unsaturated/aromatic hydrocarbons (MOAH) fraction and quantified by means of GC-FID, subsequently. [1]
Jute and sisal sacks consist of naturally occurring fibres, which are mainly used for transportation and storage of cocoa beans, coffee beans or spices. During the manufacturing process of these bags, the natural fibres are traditionally treated with petroleum-based oil (e.g. jute batching oil [JBO]) for better processing. In addition to the partially unpleasant odour of JBO, the migration of batching oil is a critical factor for the use as food packaging. [2] Nevertheless, contaminations of cocoa by JBO are very common during routine analysis. However, batching oils are difficult to identify by LC-GC-FID because they do not show a consistent pattern due to the variability of the carbon chain lengths and the variable content of aromatic compounds. A better separation of the analyte mixture and a reliable identification of potential sources is possible with two-dimensional GC (GCxGC) coupled to a Time of Flight Detector (ToF). For this reason, 30 different jute and sisal sacks of different manufacturers and distributors were analysed by GCxGC-ToF. The aim of this investigation was to create an overview of various jute sacks as MOH contamination source and to develop a database for a faster and routine compatible identification of batching oils in different matrices like cocoa beans or coffee beans.

[1] German Federal Institute for Risk Assessment (BfR), 2017
[2] Agarwal, R. et al., Evaluation of carcinogenic effect of jute batching oil (JBO-P) fractions following topical application to mouse skin.", Arch Toxicol. 1988

Keywords: *MOSH, MOAH, GCxGC, mineral oils, two-dimensional chromatography*

MYCOTOXINS, MARINE & PLANT TOXINS

POSTER SESSIONS



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N1

ANALYSIS OF SPICES USING IMMUNOPREP® ONLINE OCHRATOXIN CARTRIDGES

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Ochratoxin A is produced by moulds of the genera *Aspergillus* and *Penicillim*. Such fungi thrive in a warm damp environment. Food and feed are susceptible to fungal contamination during growth, harvest and storage. Ochratoxin can be found in a wide range of food commodities including spices and coffee and European legislative levels are currently in place.

in terms of analysis, immunoaffinity clean-up is already well established in Official Methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all the regulated mycotoxins including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of spice samples as immunuoaffinity clean-up is particularly effective in removing all pigments from the sample to allow accurate quantification by HPLC or LC-MS/MS.

IMMUNOPREP® ONLINE automated affinity cartridges have been developed which offer the same benefits as immunoaffinity column clean-up. Automating analysis offers the added advantage of allowing large scale laboratories to meet increasing pressures with the fastest turn-around times. Sample preparation methods have been validated and tested for the analysis of difficult commodities such as spices and coffee for the automated determination of ochratoxin.

Keywords: *cartridges, hplc, automation, clean-up, mycotoxin*

N2

VERIFICATION OF MULTI-TOXIN IMMUNOAFFINITY COLUMNS IN DETERMINATION OF MYCOTOXINS IN ANIMAL FEED AND SWINE URINE BY UHPLC-MS/MS

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R-Biopharm's new immunoaffinity column, 11+Myco MS-PREP® utilises a single extraction method for the analysis of 11 mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumoninisins B1, B2, deoxynivalenol, zearalenone, T-2 and HT-2) prior to detection by LC-MS/MS. In this study, a bile and urine samples were analysed in order to determine recoveries for legislated recoveries as well as a wide range of non-legislated mycotoxins.
Results demonstrate that the use of an immunoaffinity column enables the concentration of the mycotoxins prior to detection, improving sensitivity and eliminates the use of isotopic labelled standards

Keywords: *multi-toxin, UHPLC-MS/MS, feed, immunoaffinity, clean-up*

Acknowledgement: *National Veterinary Research Institute (Department of Pharmacology and Toxicology) Poland, P. Jedziniak, A. Tkaczyk and L. Panasiuk*

N3

DETECTION AND EVALUATION OF MARINE TOXINS BY ZEBRAFISH AND IN VITRO BIOASSAYS

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Marine toxins are one of the most serious problems in the marine resources' exploitation. These toxins are produced by dinoflagellates, diatoms and cyanobacteria and they are accumulated along the food chain via bivalve mollusk filtration or plankton consumption of several fish and crustaceans´ species.
At first, mouse bioassay was used as
Referencemethod for the evaluation of marine toxins, but new LC-MS/MS methods are now available and are commonly used for regulatory purposes. However, these methods could present limitations when evaluating the potential toxic effects of samples with emerging toxins, metabolites or mixtures of different group of toxins. To address the problem a method based on the evaluation of the effect on the model vertebrate zebrafish (*Danio rerio*) has been developed. Extraction of toxins from shellfish is carried out using standard methods and after that, the extract is exposed to zebrafish embryos during 24-48h. When the set time has expired, toxicity is detected by evaluating embryos' death, malformations and the differential expression of selected genes. The procedure has been tested to be effective for the detection of toxins related to saxitoxin, the okadaic acid group and their metabolites in naturally contaminated mussels, oysters and cockles. Zebrafish embryos provide the response of formed organisms, sharing a high degree of functional homology with all vertebrates including humans. Nevertheless, embryos are not considered animals up to 5 days of life, so the procedure could be an alternative to use of animals.
In addition, toxicity studies with zebrafish have allowed us the selection of a group of biomolecules whose activity is altered by these toxins and the development of an *in vitro* fluorescence assay for the detection of regulated (azaspiracid and yessotoxin) and non-regulated (spirolide and tetrodotoxin) marine toxins. After optimisation the limits of detection were around 1-500 µg/L.
Both methods are based on a detection-by-effect method, they are not able to identify each toxin. However, method based on zebrafish can be useful as a tool to alert for the presence of uncommon and/or unidentified toxins which are not tested in periodical monitoring. Apart from that, the method based on *in vitro* fluorescence assay could be used as a first rapid and easy screening method.

Keywords: *marine toxins, seafood, zebrafish, bioassay*

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N4

MYCOTOXIN BIOMARKERS ANALYSIS OF PIGS URINE SAMPLES BY LC-MS/MS: WHY IT IS SO IMPORTANT TO ADJUST THE CREATININE LEVEL AND CARRY OUT ENZYMATIC HYDROLYSIS?

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Identification of biomarkers in biological matrices is important for the accurate exposure assessment to mycotoxins. The aim of the study was developing the LC-MS/MS method for the determination of 40 toxins (biomarkers of exposure) including deoxynivalenol, zearalenon, ochratoxin A, aflatoxins, T-2 toxin, HT-2 toxin, fumonisin B1 and B2, their metabolites and additionally: citrinin, dihydrocitrinone, nivalenol, fusarenon-X, diacetoxyscirpenol, sterigmatocystin, beauvericin, enniatins and Alternaria toxins in pigs urine. Two sample preparation methods were used: multi-mycotoxin immunoaffinity columns (IAC): AOF MS-PREP® and DZT MS-PREP® (R-biopharm) connected in tandem and liquid-liquid extraction (LLE) with ethyl acetate and salt addition.
The first challenge in the LC-MS/MS multi-mycotoxins methods development is high diversity of validation results for different urine samples, as a consequence of different water content. Biomonitoring data usually are adjusted to a constant creatinine concentration to correct for variable dilutions among samples – so method for creatinine determination in urine samples is needed.
The spectrophotometric method resulted in the high variability of the results for urines with the high creatinine content (CV>20%). Therefore, HPLC-UV method was developed. Chromatographic column was Hypercarb (Thermo Scientific™) and mobile phase consisted of water/acetonitrile/TFA (96.9/3/0.1; v/v). The method was applied for analysis of 30 urine samples (CV<10%). The mean and maximal creatinine concentration was 3.5 and 9 mg/mL. ELISA Kit (Sunlongbiotech) was compared with HPLC-UV method - strong correlation between methods was found.
The second challenge is mycotoxin glucuronides determination. Because of the unavailability of their standards, enzymatic hydrolysis and its optimization is necessary.
Three beta-glucuronidases from different sources (Helix Pomatia, E.coli and Abalone) were tested. Different matrix effects were observed in the urine samples after enzymatic hydrolysis with different enzymes. Only enzyme from E.coli enabled T-2 toxin determination and was chosen for hydrolysis and applied in mycotoxin analysis.
To sum up, the HPLC-UV method for the accurate creatinine determination was developed and successfully applied for the analysis of pigs urine samples, which show high diversity of creatinine level. Adjustment to the constant creatinine level enabled standardization of preliminary validation results for mycotoxin biomarkers determination (apparent recovery: 70-120%). Enzymatic hydrolysis is crucial in biomarkers determination in the urine samples. High glucuronidation rate of zearalenon and other mycotoxins was found in analyzed urine samples. Future optimization of enzymatic hydrolysis conditions is needed.
Above two steps in the sample preparation have a significant impact on reliability of results in mycotoxin biomarkers analysis and should be properly developed and optimized.

Keywords: *enzymatic hydrolysis, mycotoxin biomarkers, creatinine adjustment, LC-MS/MS, biomonitoring*

Acknowledgement: *The study was financed by National Science Centre (Poland) SONATA – BIS project: "Biomarkers of mycotoxins in pigs " No. 2016/22/E/NZ7/00640*

N5

INNOVATIVE MULTIPLEX BIOCHIP-BASED TECHNOLOGY, APPLICABILITY OF MYCO 9 ARRAY TO THE DETECTION OF PREDOMINANT, MASKED AND EMERGING MYCOTOXINS

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Introduction. The continuous development of knowledge and availability of the most up-to-date risk assessments report on co-exposure, effect of low-level cocktails, metabolites and conjugated forms of predominant mycotoxins is of pivotal importance for both the human and animal food chains. The need for testing is consequently important. Maximum limits and/or guidance levels are well established for aflatoxins, fumonisin, deoxynivalenol, zearalenone, ochratoxin and T2/HT2-toxins across various global jurisdictions. Information about currently unregulated emerging mycotoxins, which includes acetylated or glucoside forms of deoxynivalenol have a potential to cause serious health concerns to humans and animals. Generally, commercial technologies available to the industry lack the capacity for multiplex detection, which limits the screening capacity. Therefore, the availability of multi-analytical screening methods is beneficial to maximize mycotoxin detection in testing settings.
This study summarizes the performance data following Commission Regulation (EU) 519/2014 of Myco 9 Array, that by using biochip array technology, allows multi-mycotoxin screening of 22 toxins (including predominant, masked and emerging mycotoxins) from a single sample.
Methodology. Myco 9 Array presented nine simultaneous chemiluminescent immunoassays, defining discrete test regions on the biochip surface. The biochip analyser Evidence Investigator was used, the system enabled the analysis of up to 54 biochips at a time. Mycotoxins were extracted from feed by a single generic liquid/liquid extraction. Screening results were semi-quantitative.
Results. Myco 9 Array presented broad specificity profile allowing detection of aflatoxins (B1, B2, G1, G2), fumonisins (B1, B2, B3), ochratoxin A, paxilline, trichothecenes A (T-2 toxin, HT-2 toxin, diacetoxyscirpenol), trichothecenes B (deoxynivalenol, 3-acetyldeoxynivanelol, 15-acetyldeoxynivanelol, don-3-glucoside), zearalenone (zearalanone, α-zearalenol, α-zearalanol, β-zearalenol, β-zearalanol). The limits of detection ranged from 0.25 ppb for aflatoxin B1 to 175 ppb for fumonisin B1. Ongoing analysis of samples through FAPAS proficiency testing and UKGTN (UK Grain Testing Network) programmes (n=10) showed percentage agreement of 96.6% and the analysis of FAPAS QC samples (n=13) showed percentage agreement of 89%. Out of the 23 samples tested, 19 presented multi-mycotoxin contamination.
Conclusion. Myco 9 Array allows multi-mycotoxin screening of both well-established and emerging mycotoxins from a single cereal or cereal based feed sample and enables the detection of either single or multi-mycotoxin contamination. Myco 9 array provides an analytical solution for the multiple challenges of mycotoxin testing. Innovative multiplex technology is a future of mycotoxin control, which gives an opportunity to adjust to future legislations, including decreasing maximum limits or increasing spectrum of mycotoxins being under control.

Keywords: *mycotoxins, Myco 9 Array, biochip, multiplex, multi-contamination*

N6

DETECTION, STABILITY, AND BIOAVAILABILITY OF ABRIN

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Abrin, one of most potent toxins known and a member of the ribosome-inactivating family, is derived from the rosary pea (jequirity pea) plant *Abrus precatorius* and is a potential bioterror concern. Rapid and sensitive detection assays to abrin in complex matrices are therefore of critical importance to ensure food safety and early diagnosis of intoxication. A panel of monoclonal antibodies (mAbs) to both abrin A- and B-chains were developed and characterized. Two highly sensitive, sandwich enzyme-linked-immunosorbent assays (ELISAs) were optimized for the detection of abrin in both buffer and food matrices. The limits of detection (LODs) for both abrin ELISAs were about 1 ng/mL in buffer and milk, a concentration well-below the concern of consumers. Detection limits of abrin in acidic juices (apple, orange, and carrot) were [endif]--> 40 ng/mL. While detection of abrin in food matrices is of importance, to fully grasp the risks for intentional food contamination, the knowledge of the stability and bioavailability of abrin following food processing is needed. The activity of abrin following inactivation procedures was measured using three different assays: mouse bioassay, cell free translation and Vero cell culture cytotoxicity assays. Thermal inactivation of abrin in buffer required exposure to temperatures ≥ 74 °C for 3 min while pH treatment did not affect abrin toxicity. The effectiveness of selected food processing inactivation conditions against abrin in food matrices (whole milk, non-fat milk, liquid egg, and ground beef) was also evaluated. For both whole and non-fat milk, complete inactivation was achieved at temperatures of ≥ 80 °C for 3 min or 134 °C for 60 s, which were higher than the normal vat/batch pasteurization or the high temperature short time pasteurization (HTST). Toxin inactivation in liquid egg required temperatures of ≥ 74 °C for 3 min, higher than suggested temperatures for scrambled eggs (22% solids) and plain whole egg. These results suggested a protective role that food matrices may have on abrin stability and bioavailability. Sensitive toxin detection coupled with increased knowledge of toxin bioavailability following different storage and food processing conditions will aid in the protection of our food supply from intentional food adulterations.

Keywords: *abrin, detection, ELISA, bioavailability*

N7

RAPID, SENSITIVE AND SELECTIVE DETECTION OF AMATOXINS BY IMMUNOASSAY METHODS

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Amatoxins are potent toxins from a class of RNA polymerase II or protein synthesis inhibitors that are found in some mushroom varieties. Inadvertent ingestion of mushrooms containing amatoxins often result in liver failure (in both humans and animals) and even death. Current methods for the detection of amatoxins require use of expensive instrumentation, such as liquid chromatography/mass spectrometers (LC/MS) and complex extraction protocols using organic solvents. In order to improve the speed and accuracy of amatoxin detection, we have developed novel mouse monoclonal antibodies (mAbs) against α- , ß- and γ-amanitins. These mAbs were then employed in antibody-based bioanalytical test methods such as competitive enzyme-linked immunosorbent assay (cELISA) and lateral flow immunoassays (LFA) to rapidly detect the presence of amatoxins in suspected mushrooms samples and urine specimens from exposed individuals or animals. Both the cELISA and LFA can detect α- and γ-amanitin down to 1 ppb (ng/mL) and slightly less sensitively for ß-amanitins. When paired with a rapid extraction protocol, a qualitative assessment of mushroom samples using LFA can be accomplished in less than 10 minutes. To date, 20 different mushroom species have been tested and all of them have produced accurate test results with no cross-reactivity to mushrooms containing only hallucinogenic compounds or gastrointestinal irritants. Therefore, the new immunoassays are as sensitive and selective as LC/MS methods for amatoxin detection. Simple and field portable detection for amatoxins using LFA would greatly reduce the time and costs for medical or veterinary diagnosis, and could potentially allow convenient home use for mushroom hunters.

Keywords: *amatoxins, detection, ELISA, lateral flow immunoassay*

N8

OPTIMIZATION OF SAMPLE PREPARATION METHOD FOR DETERMINATION ATROPINE AND SCOPOLAMINE BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY IN CEREALS AND CEREAL BASED FOODS

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Plants grow up surrounded by various threats from environment such as microorganisms, insects and predators. In order to existence, many of them produce a wide variety of toxic compounds commonly called secondary metabolites to discourage predators, as well as to dominance competitive organisms in their surroundings. Humans can be exposed to these toxins by consumption of contaminated food like cereals or cereal based products and some of them can be very harmful to human health. The most sensitive groups are infants and young children because of lower body weight toxic substances reach higher concentrations. Atropine and Scopolamine belong to group of natural plant toxins called Tropane alkaloids which occur mainly in *Solanaceae* plants (including mandrake, henbane, deadly nightshade, datura) but also in numerous other plant families such as *Erythroxylaceae* and *Brassicaceae*. These plants are weeds of courtyards and wild habitats but also of cultivated fields like cereal. Coexisting of cereals and plants that produce tropane alkaloids can lead to contamination of cereal grains during harvest. So far, processed cereal-based foods and baby foods for infants and young children are the only products with established maximum permitted levels of atropine and scopolamine, 1.0 µg/kg each. In this work the focus was on simplifying the extraction and clean-up procedures for determination atropine and scopolamine by liquid chromatography tandem mass spectrometry (LC-MS/MS). Investigated matrices were buckwheat, wheat, barley, maize and processed cereal-based foods for infants and young children. Mixtures of acetonitrile, water and formic acid in different percentage and solvent volumes were explored for the extraction of the target compounds. Further optimization of extraction/purification procedure was included two ways of sample treatment. Solid-phase extraction with various adsorbents like HLB, C18, SCX and modified QuEChERS approach based on the use of different salt formulation (magnesium sulphate, sodium chloride and sodium citrate) were tested. Quantification was performed by using isotope labelled internal standards of atropine and scopolamine added to the samples before the extraction. The optimized sample preparation based on QuEChERS approach provided recoveries from 70 to 120% with intra-day precisions ≤ 15% and inter-day precisions ≤ 22%. Limits of quantification were 0.40 µg/kg in baby food and 1.0 µg/kg in cereals for atropine and scopolamine respectively.

Keywords: *atropine, scopolamine, sample preparation, cereals, baby food*

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N9

IMMUNODETECTION OF MICROCYSTINS: COMPARISON OF ASSAY FORMATS AND APPLICATION OF THE DEVELOPED LATERAL FLOW TESTS FOR WATER AND FISH SAMPLES

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Microcystins (MC) are low molecular weight toxic microalgae metabolites which possess adverse effects on human and environmental health. Therefore, their control in drinking water and aquatic products is an extremely important task. Immunoanalytical methods, in particular, immunochromatographic analysis are a promising approach for rapid monitoring of microcystins in different matrixes.

Test systems for controlling the MC content based on the enzyme-linked immunosorbent assay (ELISA) and the lateral flow immunoassay (LFIA) were developed. Concentration and kinetic dependences of the immune interactions were studied and the optimal modes of the assay implementation were determined. Monoclonal antibodies to MC-LR were characterized by high cross-reactivity with respect to its structural analogues (MC-RR, MC-YR, MC-RR, MC-WR, and MC-HtyR). The detection limit of MC-LR by ELISA was 0.013 ng/mL and the working range of detectable concentrations was 0.03–0.51 ng/mL.

Three formats of LFIA were developed and compared. The first LFIA was based on direct labeling of specific antibodies with gold nanoparticles (GNPs). It was characterized by the instrumental detection limit of 0.2 ng/mL, the working range of detectable concentrations of 0.3–1.9 ng/mL, and the visual detection limit of 10 ng/mL. The second LFIA was based on using GNP-labeled streptavidin as well as monoclonal antibodies – biotin and BSA–biotin conjugates. This allowed for formation of the large complex in the analytical zone of the test strip (biotinylated antibodies bind to streptavidin–GNPs conjugates whereas BSA–biotin provides aggregation of several labels). Therefore, the amount of specific antibodies was significantly reduced thus decreasing the visual detection limit down to 1 ng/mL. In spite of this, this test system had low stability and reproducibility. The third LFIA was also based on indirect labeling and used a combination of native specific antibodies and GNP-labeled anti-species antibodies. It allowed for the reduction of the visual detection limit of by an order of magnitude compared with the direct format of the analysis. The assay duration was 20 min in all cases.

The latter test-system was used to testing water and fish samples. Its good applicability for MC-LR detection in fish samples after simple sample preparation was demonstrated. The testing of water samples did not require preliminary sample preparation. The proposed immunochromatographic analysis was characterized by rapidity and simplicity and can serve as an effective tool for the mass screening of water and fish samples for MC-LR.

This study was implemented according to the Complex Plan of Scientific Studies «Priority Scientific Studies in the Field of People Nutrition».

Keywords: *immunochromatography, test strips, microcystin-LR, gold nanoparticles, labeled immune complexes*

Acknowledgement: *This study was implemented according to the Complex Plan of Scientific Studies «Priority Scientific Studies in the Field of People Nutrition».*

N10

PYRROLIZIDINE ALKALOIDS IN OREGANO – AN AROMATIC BUT LOADED CULINARY HERB

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Pyrrolizidine alkaloids (PAs) consist of a large number of structurally closely related secondary plant metabolites produced by *Asteraceae*, *Boraginaceae*, and *Fabaceae* (*Leguminosae*) as a form of natural protection against herbivores. With the exception of borage, PA-producing plants are usually not consumed as food. PA contamination in food is caused rather by the unintended harvesting and processing of weeds or by the transfer of plant nectar and pollen by bees into honey. As they are suspected of being genotoxic and have been shown to cause cancer in animal experiments, particularly 1,2-unsaturated PAs and their corresponding *N*-oxides are undesirable in foods [1]. While PA contaminated (herbal) teas and honey have been identified as main sources of intake for consumers [1], studies by the CVUA Stuttgart have now shown that also culinary herbs can be contaminated with alarmingly high PA levels.
A total of 41 samples of dried oregano (*Origanum vulgare* L.) from German retail, wholesale, and food processing companies were tested for 42 different 1,2-unsaturated PAs. The results revealed consistently high PA levels of up to 32,400 µg/kg dried oregano (mean: 6,160 µg/kg, median: 5,430 µg/kg). This shows that the average PA burden found in oregano was approximately 25 times higher than various (herbal) teas normally considered to be problematic. The majority of samples (51%) showed PA levels of 1,000 – 10,000 µg/kg. PA levels higher than 10,000 µg/kg were detected in 24% of the samples. Only every fourth sample exhibited PA levels below 1,000 µg/kg. In all the contaminated samples, the highest amounts were measured for the alkaloids europine and lasiocarpine, along with their corresponding *N*-oxides. Interestingly, the observed alkaloid pattern was identical for all samples, suggesting that the detected contamination is mainly caused by a widespread, but still unknown, weed of the borage family.
Legal values for the sum of PA or individual PA in food have not yet been established in the European Union. However, in compliance with a recently published risk assessment of the German Federal Institute for Risk Assessment (BfR) [2], around 70% of the oregano samples examined were determined to be unsafe or even injurious to human health as a result of the considerable contamination. In order to reduce PA levels in dried oregano to an acceptable minimum, thorough inspections performed by the food operators prior to processing and marketing as well as causal investigations are urgently required.

[1] Federal Institute for Risk Assessment (BfR), *Opinion Nr. 030/2016,* 2016
[2] Federal Institute for Risk Assessment (BfR), *Opinion Nr. 017/2019,* 2019

Keywords: *pyrrolizidine alkaloids, oregano, official food control, contamination*

N11

CARBON BLACK MODIFIED SCREEN PRINTED ELECTRODES AND MAGNETIC BEADS FOR MASS PRODUCTION COMPATIBLE POINT OF SITE DETECTION OF DOMOIC ACID IN SHELLFISH

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Carbon screen-printed electrodes (SPEs) are an optimum platform for point-of-site electrochemical detection due to their low background currents, wide potential window and chemical inertness. Advancements in screen printing technology has pushed the development of these cost-effective sensors across multiple fields. However, sensitivity of SPEs is suboptimal and improvement of SPE based detection has been accomplished by modifying SPEs with drop-casted nanomaterials such as graphene and carbon nanotubes or electrodepositioned gold nanoparticles. Unfortunately, these modifications are costly and/or time consuming and impair price-competitive mass production. Moreover, biofunctionalisation of the modified SPEs further complicates mass production/storage and is known to be sensitive to matrix effects due to washing difficulties. These facts may account, at least in part, for why SPE based sensors are currently not commercially available for food contaminant detection. In the present study novel solutions were sought to enable the creation of a cost effective, mass production compatible and sensitive SPE based biosensor with reduced matrix effects. Carbon black (CB), (a largely unexplored material with similar electocatalytic properties as graphene and carbon nanotubes but at least 100X more cost effective) and synthesized gold nanospheres or gold nanostars (which were simply drop-cast onto the SPE thus avoiding lengthy electrodeposition steps) were used for the modification of homemade SPEs. The electrochemical performance of the nanomaterial-SPEs was compared to SPEs which underwent classic (time-consuming) pre-anodization in phosphate buffer (Pre-SPEs). A magnetic-bead hapten conjugate was used for the development of a competitive immunoassay based on the Enzyme-Linked-Immunomagnetic-Electrochemical (ELIME) format to allow better washing and avoid SPE biofunctionalisation. Finally, optimized nanomaterial-SPEs and pre-SPEs were used for the detection of the regulated marine toxin, domoic acid (DA), in buffer, spiked shellfish matrix and naturally contaminated shellfish. This comparison showed that CB-SPE had the best performance and enables detection of DA with a LOD tenfold lower as Pre-SPE in buffer (4 ng/ml vs. 0.4 ng/ml). Matrix effects remained limited with a LOD in spiked matrix of 0.7 ng/ml and a final LOD of 0.7 mg DA/kg shellfish (well below the EU action level of 20 mg DA/kg shellfish). Moreover, good agreement with HPLC data for DA quantification in contaminated scallops (R^2=0.965) was obtained. In summary, this work led to the identification of the largely unexplored carbon-nanomaterial CB as highly interesting for SPE modification and showcased that combining CB and the ELIME format has merit for the development of a mass production compatible, robust, cost effective (material cost < €1.0 per assay) biosensor for *in-situ* detection of contaminants in a complex matrix.

Keywords: *marine toxin nanomaterial electrochemistry shellfish domoic acid*

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N12

SYMMETRIC LATERAL FLOW TECHNOLOGY WITH ONE STEP MULTITOXIN AQUEOUS EXTRACTION FOR THE QUANTIFICATION OF ALL MYCOTOXINS

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Introduction: The use of state-of-the-art features for the quantification of all the major mycotoxins (Aflatoxin B1, B2, G1, G2, Deoxynivalenol (DON), Zearalenone (ZON), Ochratoxin A (OTA), Fumonisins, T-2/HT-2) in grains, constitutes the biggest challenge in rapid mycotoxin analysis. In addition, an aqueous extraction solution could eliminate the adverse effects of organic solvents on users involved in this analysis. A Lateral Flow method that uses a multitoxin aqueous extraction, with very high accuracy and repeatability, having also low LOD, LOQ and Coefficient of Variation (CV), is considered to be an essential tool in mycotoxin analysis.
Aim: The aim of this study was to evaluate the recovery levels of Total Aflatoxin, DON, ZON, Fumonisins, OTA and T-2/ H-T2 toxins, in corn and animal feed samples, using one single aqueous extraction and Lateral Flow Symmetric Technology.
Methods: The levels of all Mycotoxins were determined by the 5-minute Symmetric Green Lateral Flow assays (Prognosis Biotech S.A.), using one single aqueous extraction. In detail, the levels of Total Aflatoxin, DON, ZON, OTA, Fumonisins, T-2/HT-2 were determined using the Symmetric Total Green 0-30 / S3448 / S3448004, Symmetric Ochratoxin Green / S6048 / S6048004, Symmetric DON Green / S4048 / S4048008, Symmetric ZON Green / S5048 / S5048004, Symmetric Fumonisin Green / S7048 / S7048005 and Symmetric T-2/HT-2 Green / S8048 / S8048004, respectively.
Results: Different Reference Materials from FAPAS were used, including all mycotoxins. The recovery levels and CV were acceptable. The recovery results were also in agreement with those of different FAPAS multi mycotoxin Ring Tests.
Conclusion: For the first time a single aqueous extraction can be used for all the mycotoxins, providing unique advantages to the users in terms of cost and time-saving while Symmetric Technology signifies the transition of Lateral-flow sticks from being a low-esteemed screening tool into a reliable confirmatory method.

Keywords: *lateral flow, multimycotoxin analysis, aqueous extraction, single extraction, mycotoxin quantification*

N13

DETERMINATION OF CARDIAC GLYCOSIDES BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Cardiac glycosides (CGs) are found in a diverse group of plants including *Digitalis purpurea*, *Digitalis lanata*, *Nerium oleander* and *Convallaria majalis*. Many CG-containing plants are known to be toxic to humans, and their accidental (*e.g.*, via food or beverage) or deliberate ingestion might, depending on the dose, adversely affect cardiovascular, gastrointestinal and central nervous systems or lead to death. In case of poisoning, availability of an analytical method with good specificity and sensitivity is essential to confirm or rule out the ingestion of CG-containing plants/extracts.
The objective of this work was the development of an analytical method for quantification of cardiac glycosides (CGs) in food and human urine. Five relevant CGs have been selected based on previous knowledge on their occurrence and toxicity. The baseline separation of the analytes was achieved over 15 min using an ACQUITY liquid chromatography system (Waters) and an ACQUITY UPLC BEH C18 column (Waters) with a basic mobile phase (pH 9). The detection was performed by a triple quadrupole Xevo TQ-S mass spectrometer (Waters) operated in positive electrospray ionization mode. The sample preparation was designed to provide a fast method suitable for daily high-throughput analysis. Finally, the food and urine matrices were extracted with acetonitrile and/or diluted with an aqueous solvent and subjected to a solid-phase extraction on Oasis HLB cartridges (Waters). After elution of GCs with methanol, the extract was further concentrated by evaporation under a stream of nitrogen and reconstituted in a mixture of water and methanol.
The presentation of this work will describe a step-by-step optimization of the different method parameters as well as data on method validation.

Keywords: *cardiac glycosides, LC-MS, SPE, development*

Acknowledgement: *This research was supported by the Belgian National Reference Laboratory – Mycotoxins, Plant Toxins and Marine Toxins.*

N14

CARIBBEAN CIGUATOXIN-1 STABILITY UNDER ACID CONDITIONS: CHARACTERIZATION OF A NEWLY C-CTX1 METHOXY CONGENER

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The recent emergence of ciguatoxins in the eastern Atlantic, particularly in the Canary Islands (Spain) and Madeira (Portugal) prompted the need of the implementation of analytical methods with ability to characterize this emerging risk. Liquid chromatography tandem mass spectrometry is considered an adequate analytical tool aimed to the mentioned purpose and therefore this method is being implemented in order to make it available for the control of these toxins. The main limitation to advance, not only in method implementation but also in achieving an increased knowledge about the toxicity of these particular compounds, is the lack of
Referencematerials. The present efforts of our research team who is involved in the characterization of the ciguatera fish poisoning (CFP) in these areas through a project partially founded by the European Food Safety Authority, have been hampered by the low concentration of ciguatoxins found in the samples from the above mentioned Atlantic coastal areas. The optimization of sample pre-treatment protocols, as well as the LC-MS/MS methods for confirmation have been critical to progress in the isolation of Caribbean ciguatoxins, in particular C-CTX1 to prepare a laboratory
Referencematerial of this toxin which seems to be the main responsible for the CFP in these geographical areas. This material has been used to assess the stability of this congener under acid conditions and in different solvents commonly used in the LC-MS/MS analysis. The results obtained show that the use of acidic conditions might lead to the transformation of Caribbean Ciguatoxin-1 in a C56 methoxy congener, C-CTX1-Me. C-CTX1-Me was structurally characterized as a C-CTX1 congener with MS/MS experiments and a fragmentation pathway for both analogues has been proposed. The results obtained highlighted the importance of minimizing the use of acidic conditions, to avoid the transformation of Caribbean Ciguatoxin-1 which might lead to false negatives consequently affecting to an inappropriate characterization of the CFP risk.

Keywords: *ciguatoxins, emerging marine biotoxins, ciguatera fish poisoning, LC-MS/MS*

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N15

VALIDATION OF A FIVE MINUTES LATERAL FLOW ASSAY FOR THE QUANTIFICATION OF OCHRATOXIN A IN WINE SAMPLES

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Introduction: In the analysis of wine, there are several ELISA tests that either detect, semi-quantify or quantify Ochratoxin A (OTA) (EU limit is 2μg/kg) with a total procedure time of 60-90 min. Regarding the Lateral Flow technology, the quantification of OTA in wine is quite limited with procedure time of 10 min and most of the products available are only qualitative. Intensely colored matrices like wine require special pretreatment including organic solvents for extraction, laboratory equipment and scientific technicians.
Aim: The aim of this work was to determine the OTA levels in spiked wine samples with a new, simple and innovative 5-minute Lateral Flow assay, comparing the recovery results with ELISA.
Methods: A quantitative 5-minute lateral flow test (Symmetric Ochratoxin Wine, B6148, Lot B6148002) and an ELISA test (Bio-shield Ochratoxin Wine, B6196, Lot B6196004) from ProGnosis Biotech S.A. were used to determine the OTA levels in wine samples. Considering the physical and chemical properties of wine, both methods use a dilution normalization requiring no previous treatment. Twelve OTA-free wine samples originating from different varieties (Sauvignon Blanc, Riesling, Grüner Veltliner, Grenache Rouge, Zinfandel, Cabernet Sauvignon, Pinotage, Merlot, Crianza and the Greek Xynomavro, Agiorgitiko and Lagorthi) were chosen from the global market and were spiked with OTA. Quality control materials were also used and the recovery of samples was calculated.
Results: Using the 5-minute Symmetric lateral flow assay for spiked wine samples of different varieties or for Quality control materials, the recovery and CV% were identical with ELISA's. The level of 1.5 ppb of OTA was easy to be detected visually, making the assay suitable for a qualitative test too.
Conclusion: This innovative lateral flow device constitutes the most simple, rapid and accurate tool in the quantification of OTA in wine samples, providing results comparable to those of a quantitative ELISA.

Keywords: *ochratoxin A, wine, lateral flow, quantification method, 5-minute immunoassay*

N16

EVALUATION OF THE APPLICABILITY OF THE NEURO-2A CELL BASED ASSAY ON THE DETECTION OF CTXS IN FISH SAMPLES

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Ciguatoxins (CTXs) are a group of neurotoxins responsible for the syndrome Ciguatera Fish Poisoning (CFP) as a result of the consumption of contaminated fish. The presence of these toxins has been detected around the Pacific, Caribbean and Indian coasts. Recent reports indicate the emergence of CFP in other geographic areas, in particular in European coasts, Canary Islands (Spain) and Madeira (Portugal). Neuro-2a cell line (N2a), co-incubated with Ouabain and Veratridine compounds, has been applied specifically in the detection of neurotoxins responsible for activating voltage-gated sodium channel (VGSC) of excitable cells. The good potential of N2a assay as a sensitive tool for the CTXs screening has been recognized and therefore this method has been widely applied for the screening of these toxins, allowing their sensitive detection at levels below the ones recommended as guidance levels of security by the FDA (USA). The complexity of the matrix is a critical point on the application of N2a which needs to be evaluated. The aim of this work is providing recommendations to enhance the applicability of N2a assay for detection of CTX-like compounds in complex biological matrices. Furthermore, the effects of different parameters on the toxic potency of CTXs was demonstrated, allowing to conclude that the evaluation of the mechanism of cell death is a critical point to ensure the reliability of the results.

Keywords: *neuroblastoma cell assay, matrix effect, ciguatoxins*

N17

INVESTIGATION OF OCHRATOXIN A AND PHOMOPSIN A IN PEAS DURING PASTA MANUFACTURING AND PREPARATION

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Grain legumes are rich in proteins, consequently, they are regarded as an alternative to animal based products. The year 2016 was declared as "Year of Pulses" by FAO to emphasize their growing significance worldwide. Recently, in popular media, Beyond Burger™ patties that are based on pea and mung bean protein caught significant attention. The use of grain legumes can be further diversified. Apart from meat alternatives, whole pea flour has already been used to create 'low carb' noodles that are available in retail.
Like for any other food commodity, for grain legumes, the contamination with mycotoxins may pose a potential health risk to the consumer. The nephrotoxin ochratoxin A (OTA) is a common mycotoxin in grains and was also shown to occur in grain legumes [1]. The *emerging mycotoxin* phomopsin A (PHOA) and its derivatives are produced by the legume infesting fungus *Diaporthe toxica* and *in vitro*, production of PHOA on peas could be confirmed [2]. PHOA has proven to cause a fatal liver disease in grazing animals. The *European Commission* has recommended the development of quantitation methods as well as further research on its occurrence [3].
In this work, a stable isotope dilution (SIDA) LC-MS/MS multimethod for the quantification of OTA and PHOA was validated on pea flour and verified on pea noodle matrix. The method is based on a modified QuEChERS approach. Following the extraction with 80/20 (v/v) acidified acetonitril/water, the sample is concentrated by evaporation. Phase separation is achieved by a salting-out step with oversaturated $MgSO_4$ solution. The developed method was also applied to investigate the fate of OTA and PHOA in pea noodles during manufacturing and preparation. Pea fusilli noodles were produced through extrusion in a small scale industrial noodle machine from whole pea flour naturally contaminated with PHOA and spiked with OTA. Afterwards, the dried noodles were cooked, collecting ready-to-eat noodles and cooking water.
Data obtained by applying the established LC-MS/MS multimethod will help to derive processing factors for OTA and PHOA in pea noodle production and preparation. As the analytical method is based on a SIDA approach, it will also be feasible to apply the same protocol for the analysis of other legume containing products in the future.

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N18

A RAPID AND SIMPLE LC-MS/MS METHOD FOR THE QUANTIFICATION OF THE EU REGULATED MYCOTOXINS IN CEREAL-BASED PRODUCTS

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Mycotoxins are toxic secondary metabolites produced by various mold species found to infect a variety of agricultural commodities and processed food. Herein we developed and validated a quantitative method for the analysis of aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, deoxynivalenol, toxins T-2 and HT-2, zearalenone, ochratoxin A and nivalenol in wheat grain flour, based on a high sensitivity LC-MS/MS instrument.
The ultimate sensitivity and robustness of the Xevo TQ-XS allowed the extreme simplification of the sample treatment process, which consists in a rapid solvent extraction and dilution of the matrix without the need for time consuming pre-concentration or clean-up steps.
Both external and internal standard calibration methods were evaluated as part of this study. All regression equations showed coefficients of determination (R^2) between 0.9941 and 1.0000, and percentage residuals lower than 20% across the full calibration range. Method LOQs were adopted as the lowest point of the linear ranges, which bracket the EU Maximum Permitted Limits (MPLs). When using the internal standard method, %Recoveries lie within the range 94-105%, whilst RSD% (n=7) were below 10% for all analytes. Thus, trueness and precision of the method were well within the criteria set by the EC Regulation No 1881/2006 and subsequent amendments. Matrix effects ranging from >30% signal suppression for nivalenol, to >1000% signal enhancement for ochratoxin A were encountered. The incorporation of ^{13}C-labelled internal standards within the analytical workflow leads to enhanced method performance and is therefore recommended as an efficient approach to correct for both matrix effects, and the inevitable analyte losses during the sample preparation.
The method performance fulfills the EU Regulations also when applied to oatmeal, and to a mixture of different flours (rice, potato, tapioca, maize and buckwheat), and it appears to be potentially transferable to different types of commodities commonly contaminated with mycotoxins, including dried spices.

Keywords: *mycotoxins, liquid chromatography-mass spectrometry, food safety, cereals, validation*

N19

DOMOIC ACID LEVELS IN MUSSELS FROM BULGARIAN BLACK SEA COAST AND HUMAN EXPOSURE ESTIMATION

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Domoic acid (DA), naturally produced by several diatom species belonging to the genus *Pseudo-nitzschia*, is as a potent neurotoxin. Filter-feeding marine organisms, such as mussels, when exposed to blooms of *Pseudo-nitzschia* spp., accumulate the toxin. Consumption of contaminated mussels could pose serious health risk if the toxin concentration is exceeding certain levels.
The aim of this study is to assess the domoic acid levels in mussels and to estimate the human exposure.
Wild and farmed mussels are sampled from north Bulgarian Black Sea coast in 2017. Sampling strategy includes the seasons of active mussel harvesting. Domoic acid is extracted with methanol solution and concentrations are determined via LC-MS/MS. The limit of detection is calculated 0.4 ng/g.
In total 28 farmed and 26 wild mussel samples were investigated. Domoic acid is detected in spring samples only.
Among farmed mussels investigated, 62% of the spring samples were positive for the toxin. Highest detected concentration in farmed mussels was 1374.3 ng/g and lowest positive concentration- 313.1 ng/g. Analysis of the toxin seasonal variations showed that its level reached a peak in April 2017 and until the end of the season its decreasing.
Studied wild mussels showed lower positive percent and lower maximum concentration. Positive for domoic acid were 45 % of spring wild mussel samples. Highest detected concentration was 919.0 ng/g.
Human exposure is evaluated by calculating acute (AE) and chronic exposure (CE), as well as by hazard quotient (HQ) calculation. Estimated maximum and mean positive values are compared with acute reference dose (ARfD) (30 mg/ kg bw) and tolerable daily intake (TDI) (0.075 µg / kg bw/day) accepted for domoic acid.
Human exposure estimation showed that by maximum contamination level (1374.3 ng/g) of farmed mussels, the calculated AE was 30 times lower and by mean contamination level - 60 times lower than the ARfD. The estimated AE to the toxin if wild mussels was about 45 times lower by maximum contamination and 40 times lower by mean contamination than the ARfD.
Maximum CE calculated as well as HQ were about 4 times lower than resp. TDI and reference value of 1.
This study shows in general low contamination level with domoic acid of investigated mussel samples. Thereon, the estimated exposure of Bulgarian population is also much lower than the regulatory thresholds.

Keywords: *wild mussels, farmed mussels, LC-MS/MS, low contamination*

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N20

ROBUST, HIGH-THROUGHPUT, FAST POLARITY SWITCHING QUANTITATION OF 530 MYCOTOXINS, MASKED MYCOTOXINS AND OTHER METABOLITES USING THE SCIEX TRIPLE QUAD™ 5500+ – QTRAP® READY LC-MS/MS SYSTEM

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Mycotoxins are toxic fungal metabolites, which are derived from certain molds and fungi. The growth of mold can occur before crops are harvested or under inappropriate storage conditions such as warm and humid conditions. Consumption of food products containing mycotoxins can have serious health implications. According to the World Health Organization (WHO), the effects of some foodborne mycotoxins are acute, with symptoms of severe illness appearing quickly after consumption. Others have been linked to long term human health effects, such as cancers or immune deficiency.
The most important classes of mycotoxins including the highly carcinogenic Aflatoxins (e.g. AFB1), trichothecenes (e.g. DON), Fumonisins (e.g.FB1), Ochratoxins (OTA) and Zearalenone (ZEN) and several others are regulated in many countries. In China, GB 2761 regulates mycotoxin limits in certain products; in the EU, mycotoxins in foodstuffs are regulated by the EC1881/2006
A living plant can change the chemical structure of toxins and produce so-called "masked mycotoxins". The plant might modify the chemical structure of the toxin with a glucose or sulfate moeity, which reduces its toxicity to the plant. The plant itself may now contain only the conjugated form of the toxin, but the original mycotoxin may emerge during human or animal digestion if the conjugate functional groups are cleaved, thus exposing the consumer to the dangers of the toxin. The term 'masked mycotoxins' was coined to refer to this group of conjugated or otherwise transformed mycotoxins which become undetectable by targeted methods for the original compounds. Current knowledge of these "emerging mycotoxins" (e.g: NX-Toxins), as well as masked or other modified forms of mycotoxins, is limited but the number of compounds that need to be analyzed is increasing rapidly, requiring more comprehensive analytical LC-MS methods.
Mycotoxin analysis needs to be comprehensive and able to deliver accurate and consistent results across a wide range of matrices. This poster introduces an improved approach to testing Mycotoxins, their metabolites and emerging masked mycotoxin compounds using LC-MS/MS with fast polarity switching.

Keywords: *mycotoxins, 5500+ QTRAP, polarity switching*

N21

VALIDATION OF LC-MS/MS BASED MULTI-ANALYTE METHODS – A SUGGESTION FOR REDUCING THE WORKLOAD AND FOCUSING ON THE DATA THAT ARE ESSENTIAL

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Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has become the most intensively used instrumental technique for the quantitative determination of small molecules attributed to natural and anthropogenic contamination of foodstuffs. However, there is a lack of official guidance for determination of method performance parameters that focus on matrix effects and that at same time scrutinize the applicability of more general guidance documents to multi-target analysis. For instance, the criterion given in SANTE demands matrix matching if the response in matrix is decreased or increased by more than 20% compared to the external calibration based on solvent standards, but it is not clear whether extreme suppression by e.g. a factor of 5 or even 10 is still accepted provided it is properly compensated. In addition, it might not be absolute but rather relative matrix effects that are the main limitation for the performance of an LC-MS based multi-method, particularly as they cannot be compensated by matrix-matched calibration.
It is emphasized that the workload associated with the validation of such a method requires finding approaches to reduce the analytical burden by e.g. pooling matrices for validation. A particular challenge in connection with a method covering several hundreds of analytes is the consumption of time for evaluation of raw data, which is particularly true for concentrations near the LOQ, which requires manual inspection of the chromatograms.
Based on method performance data we have obtained for 550 secondary metabolites in seven different matrices, we have identified validation experiments which can be skipped in order to significantly reduce the workload required for in-house validation and revalidation upon transfer to a new matrix.

N22

LC-MS/MS DETERMINATION OF MONILIFORMIN BY ADDING LANTHANIDE IONS IN MOBILE PHASE

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An innovative chromatographic analysis was developed for the determination of moniliformin (MON). Because of its ionic nature, MON is weakly retained by reversed-phase chromatography and the separation may be tricky; nevertheless, this technique is normally used or with the formation of ion pairs or employing specific RP columns for polar compounds or combining anion exchange and hydrophobic interactions. Hydrophilic interaction chromatography (HILIC) was also used, but satisfactory chromatographic separation was not always obtained. Besides its ionic nature, MON is a di-ketone; these organic compounds, mainly β-di-ketones, can easily form complexes with lanthanide ions. Then, it was evaluated in this work if the presence of lanthanide ions, added in the chromatographic mobile phase, can improve the separation of MON. La^{3+}, Tb^{3+} or Eu^{3+} aqueous solutions were used as mobile phase and MON was chromatographed using a LC-NH_2 column. The probable formation of complexes lanthanide-MON in the mobile phase allowed to obtain a satisfactory chromatographic separation and an easy determination using both UV detector or mass spectrometer (MS/MS). Finally, a suitable extraction and purification method for the MON determination in cereal samples were developed. Then, MON was determined in ten samples of maize and ten of wheat produced in northern Italy; quantification was carried out by LC-MS/MS. MON occurred in 100% and 50% of maize and wheat samples, respectively. The levels of contamination, corrected for the recovery percentage, ranged between 38 and 3629 μg kg^{-1} for maize (in 4 samples MON exceeded 1000 μg kg^{-1}), <10 and 481 μg kg^{-1} for wheat samples.

Keywords: *moniliformin; lanthanides; LC-MS/MS*

N23

DETERMINATION OF MYCOTOXINS IN CEREALS AND CEREAL PRODUCTS FROM HUNGARY

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Cereals and cereal products play important position in human nutrition. Since the 1960s, man had to increase cereal production in order to keep pace with the growing population. The chemical plant protection methods supports this high-scale crop production, but nowadays, the limiting number of the authorized pesticides allows space for pathogens, such as molds. Infection of crops with fungi can result in the production of mycotoxins, with constitute the main concern in food safety issues. Mycotoxins can cause acute or chronic disease based on their carcinogenic, mutagenic, teratogenic, estrogenic, nephrotoxic, hepatotoxic and immunomodulation effect. The most typical mycotoxins in cereals are aflatoxins, deoxynivalenol, T-2 toxin, fumonisins, ochratoxin A and zearalenone. Literature showed that the mycotoxin co-occurrence is also a usual phenomenon in the grain fields; moreover, the infected plant as part of the defence mechanism of the living organisms can biologically transform the native toxin resulting in a modified form of it. These modified forms together with the native toxins should be surveyed to get real picture about mycotoxin contamination of food. Geographical as well as climatic conditions seem to be the most important factors in mycotoxin occurrence.
Few such a survey has been conducted in Hungary, and they inv estigated only a limited numbers of mycotoxins, using different analytical methods. Borbély et al. investigated 284 raw cereals (rice, winter wheat, maize) from eastern-Hungary for the presence of 4 common mycotoxins using HPLC-DAD method. [1] In most of the samples (> 80%) the concentration of mycotoxins were bellow detection limit, noting, that the sensitivity as well as selectivity of such a system is much lower compared to an HPLC-mass spectrometric method. In another work, 116 cereal samples from Hungary were examined for 3 common mycotoxins using ELISA method. [2] A drawback of this method should be mentioned, i.e. that the amount of mycotoxin measured might also respond to transformed forms, making the separation of different species of mycotoxins impossible. [3] We investigated 13 mycotoxins - including some of modified forms - in 37 cereals using dilute-and-shoot extraction method followed by HPLC-MS/MS measuring system. There are several bio-products are between the samples. For quantification of the positive findings standard addition calibration method was used.

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N24

IDENTIFICATION OF TOXIC PLANTS THAT CAUSE SEVERE FOOD POISONING USING REAL-TIME PCR

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Many species of higher plants exist on earth. Some plants contain toxic and deadly compounds. In USA, 10 poisonous plants are known such as Daffodil (*Narcissus poeticus*), White hellebore (*Veratrum album*), and Deadly nightshade (*Atropa belladonna*). Foxglove (*Digitalis purpurea*), Aconite (*Aconitum napellus*), and Rosary Pea (*Abrus precatorius*) are major poisonous plants in the world. In Japan, many food poisoning cases occur every year due to misidentification of toxic plants as edible plants. Four hundred sixty-three food poisoning cases occurred, and 5,699 patients suffered from them for 50 years. Fatal cases were 26 between 1961 and 2010. When focusing on the recent five years, 83 food poisoning cases and 494 patients were reported by the Ministry of Health, Labor and Welfare (2014-2018). Surprisingly, fatal food poisoning occurred in 10 people, eight of who were caused by *Colchinum autumnale*. Remaining 2 cases were from *Narcissus tazetta* and *Veratrum album*. Main causes are misidentification of poisonous plants as edible plants. From this background, we developed an identification method to detect 5 poisonous plants, some of which are deadly toxic (*Colchinum autumnale*, known as autumn crocus or meadow saffron) using real-time PCR. We compared the sequences of toxic plants with those of edible plants in *mat*K, *rbc*L, and *trn*H-*psb*A intergenic spacer regions of chloroplast. Unique sequences were found in *mat*K gene. Therefore, we designed primers and probes on the region. The method we developed can distinguish toxic *Colchinum autumnale*, *Datura metel*, *Narcissus tazetta*, *Veratrum album*, and *Aconitum japonicum* from the corresponding edible plants. Also, the method did not cross-react typical 15 crops such as rice, wheat, maize, soybean, tomato, eggplant, carrot, etc. The LOD was 32 copies or below. Taken together, we have established the specific method to identify poisonous and deadly plants. The method can be applicable to food residues as well as cooked foods. We believe that our method will help to prevent onset of food poisoning cases.

Keywords: *food poisoning, toxic plant, identification method, Colchinum autumnale, Real-time PCR*

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N25

NEW POSSIBILITIES IN EFFECTIVE ISOLATION OF MODIFIED TYPE A TRICHOTHECENES: SURVEY OF SUITABILITY OF COMMERCIALLY AVAILABLE IMMUNOAFFINITY COLUMNS

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In the recent years, growing attention has been paid to 'modified/masked' mycotoxins, i.e. mycotoxin conjugates that are products of either (i) plant/mammalian detoxification processes, or (ii) products of specific food processing technologies. In addition to glycosides of the most well-known trichothecene deoxynivalenol, presence of mono-/oligoglycosides of other trichothecenes has been proven (among others, nivalenol- or HT-2/T-2 toxin-glycosides could be named). The presence of these modified mycotoxins in a human diet has become an issue of health concern, which is underlined also by Scientific opinion issued by European Food Safety Authority in 2014, so the effective strategies for their isolation and detection are of major importance. In our study, we focused on checking the possibilities of immunoaffinity pre-concentration of these compounds, hypothesising the cross-reactivity of antibodies originally developed to parent mycotoxins (depending on the immunization process and particular epitopes, cross-reactivity with structurally similar mycotoxins is possible). We worked with extracts from *Fusarium avenaceum* inoculated barley and malt and beer thereof, where the presence of glycosides of HT-2/T-2 toxin, neosolaniol and diacetoxyscirpenol was proven by the preliminary liquid chromatographic-mass spectrometric screening. Several commercially available immunoaffinity clean-up columns dedicated for HT-2 and T-2 toxins (produced by Vicam, R-Biopharm and Eagle Biosciences, among others) were then tested for pre-concentration of the above mentioned mycotoxins glycosides. Separation of modified mycotoxins was realized by ultra-high performance liquid chromatography using the Acquity UPLC® HSS T3 (100 mm x 2,1 mm, 1,8 µm; Waters) column and buffered aqueous-methanolic gradient, and for detection, high resolution mass spectrometer Q-Exactive Plus™ (Thermo Scientific) was utilized. As the analytical standards of the modified type A trichothecenes have not been available so far, columns cross-reactivities with these compounds were expressed as relative ratios of 'original to modified' mycotoxin forms.

Keywords: *modified mycotoxins, type A trichothecenes, immunoaffinity columns, cross-reactivity, high resolution mass spectrometry*

Acknowledgement: *This work was supported by the Ministry of Education, Youth and Sports (project No 8E18B045).*

N26

ANALYSIS OF PLANT TOXINS IN PLANT-BASED DIETARY FOOD SUPPLEMENTS BY UHPLC-MS/MS

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Within the food industry, the field of dietary food supplements (DFS) is among the fastest-growing ones. This is mainly due to the increasing interest for dietary health aspects, the increasing interest for alternatives to the classical pharmaceutical products, and the easy access and the widespread availability of DFS through several distribution channels including supermarkets, herbalists, pharmacies, bio- and web-shops, etc.
However, this growing interest for DFS generates therefore a significant level of human exposure from a public health point of view. Indeed, the possible presence of plant toxins in plant-based DFS implies health risks for consumers due to their possible toxic effects, side effects, and/or negative interactions with other therapeutic treatments.
To protect consumers, a maximum tolerable daily intake has been set for some plant toxins based on their respective available toxicological data, while for other plants or plant toxins, DFS thereof may not contain "detectable amounts".
To guarantee the safety of plant-based DFS, Liquid Chromatographic – tandem Mass Spectrometric (LC-MS/MS) methods have been described in literature. However, these methods mainly target one specific family of such plant toxins (a method for pyrrolizidine alkaloids (PA), another for naphtoquinones, etc.).
In order to avoid the need of multiple methodologies to ensure the safety of a plant-based DFS, our study concerns the development and the validation of a sensitive and reliable standardized multi-analyte UHPLC-MS/MS method allowing to accurately and sensitively detect and quantify 25 small molecules and polypeptides as well as a protein. The selected toxins represent more than 12 plant toxin families (PA, naphtoquinones, anthraquinones, acetogenins, cyanotoxins, quinazoline alkaloids, (cardioactive) steroid glycoalkaloids, neo-clerodane diterpenoid alkaloids, etc.). After optimization of the mass spectrometric parameters for each plant toxin, as well as the liquid chromatographic conditions, several extraction and purification protocols have been evaluated and optimized. For protein plant toxins, a separate methodology was developed. The validation was performed with several DFS manufactured in various formulations including tablets, capsules, and elixirs. Validation parameters considered were limit of detection and quantification, linearity, specificity, overall recovery, precision (within and between days) and method measurement uncertainty.
Important bottlenecks during method development appeared to be the high chemical diversity of the toxins considered, as well as the scarcity of commercially available toxin standards. Results will be presented during the symposium.

Keywords: *plant-toxins, dietary food supplement, UHPLC-MS/MS, consumer's food safety, multi-toxin*

Acknowledgement: *Project funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment through the contract RT16/5 ANAPLANTOX.*

N27

DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN PLANT MATERIAL USING SFC-MS/MS

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Pyrrolizidine alkaloids (PAs) are potentially carcinogenic plant metabolites. Exposure to PAs in food, beverages or phytopharmaceuticals, is a possible long-term concern for human health.
Based on available data, the Panel on Contaminants in the Food Chain (CONTAM) have proposed a list of PAs to be monitored in foodstuffs.
Since some of the analytes are isobaric, the isomers cannot be distinguished by mass spectrometry; hence, they have to be separated chromatographically. LC-MS/MS is the standard method for determination of PAs. However, separation of these compounds often poses a significant challenge. SFC offers complementary chromatographic selectivity to RP-LC and is an excellent technique for challenging separations such as stereoisomers. This is demonstrated by the separation and determination of 34 PAs including 5 Lycopsamine and 2 Senecionine isomers.
This study describes a novel, fast SFC-MS/MS method for the determination of PAs at trace levels from extracted tea samples. Tea samples were extracted twice with 0.05 M sulfuric acid by sonication. Before centrifugation the pH of the extracts were adjusted with ammonium hydroxide. The raw extracts were cleaned up by solid phase extraction and evaporated to dryness. Samples were reconstituted in pure methanol and injected into the SFC-MS/MS system.
A ternary gradient, using supercritical CO2, methanol and a constant flow of 50 mM ammonium formate, separated the PAs and their N-oxides for quantification. By careful fine-tuning of the chromatographic conditions the separation of all critical pairs could be achieved within 8 minutes runtime.
By using SFC-MS/MS, separation and individual quantification of 18 PAs (Indicine, Trichodesmine, Senkirkine, Senecivernine, Senecyphilline, Senecionine, Retrosine, Monocrotaline, Lycopsamine, Lasiocarpine, Jacobine, Intermedine, Heliotrine, Europine, Erucifoline, Echimidine, Echinatin, Rinderin) and 16 of their related N-oxides (Echimidine-N-oxide, Lasiocarpine N-oxide, Jacobine N-oxide, Intermedine N-oxide, Heliotrine N-oxide, Erucifoline-N-oxide, Europine -N-oxide, Indicine N-oxide, Senecivernine N-oxide, Senecyphilline N-oxide, Senecionine N-oxide, Retrosine N-oxide, Monocrotaline-N-oxide, Lycopsamine N-oxide, Echinatin-N-oxide and Rinderin-N-oxide) could be achieved.
Calibration curves showed good precision and accuracy, and even in a complex matrix like tea we were able to easily quantify the PAs in the range of at least 5 to 200 µg/kg. For all analytes, linear weighted regression resulting in $r^2 \geq 0.99$ could be obtained, with S/N > 10 for LLOQ levels.

Keywords: *SFC-MS/MS, pyrrolizidine alkaloids, tea*

N28

ANALYSIS OF MYCOTOXINS IN CANNABIS AND RELATED PRODUCTS USING MULTI-TOXIN IMMUNOAFFINITY CLEAN-UP COLUMNS IN CONJUNCTION WITH HPLC-FLD

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More than 40 years ago a paper published in the scientific journal *Mycopathologia* showed that under favourable conditions, *Aspergillus flavus* and *A. parasiticus* could flourish and produce aflatoxins on marijuana. Uncured marijuana plant material or inadequately processed marijuana could offer the right conditions for fungal growth. There appears to have been little or no follow-up to these observations, probably as at that time they largely concerned the safety of an illegal substance. However, legalisation of consumption of cannabis products in Canada and also in several States of the USA, for medicinal purposes and in some cases recreationally now brings safety to the fore. Cannabis now needs to be scrutinised for residues and contaminants to the same extent as food or pharmaceutical products. This means applying the same safety standards for levels of mycotoxins as apply to foodstuffs and conducting routine monitoring to ensure standards are maintained for products placed on the market.
Fortunately, in terms of analysis, immunoaffinity clean-up is already well established in Official Methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all the regulated mycotoxins including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of botanical products such as herbal medicines, which have matrix similarities to marijuana. Recent work by R-Biopharm has demonstrated that multi-toxin immunoaffinity columns such as AO ZON PREP® provide excellent clean-up of marijuana samples when spiked and can be used with LC-fluorescence. The use of immunoaffinity columns results in excellent clean-up and better chromatography as well as having the added benefits of improving productivity and lowering overall analysis costs when compared to using single toxin immunoaffinity columns.

Keywords: *cannabis, immunoaffinity*

N29

DESIGN AND OPTIMIZATION OF ANALYTICAL APPROACHES FOR THE DIAGNOSIS OF CIGUATERA IN HUMAN SAMPLES

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Ciguatera is a food poisoning derived from the consumption of fish with an incidence in the world of more than 50,000 intoxicated every year. This condition is endemic to tropical and subtropical regions of the Caribbean and Pacific oceans and is caused by the consumption of some fish species that generate ciguatoxins through metabolic processes of biotransformation of compounds produced by dinoflagellates of the genus Gambierdiscus and Fukuyoa.
In recent decades, the number of ciguatera cases in Europe has increased, specifically in Spain (Canary Islands) and Portugal (Madeira), which has led to the consideration of ciguatera as an emerging risk in Europe by the European Food Safety Authority (EFSA). The dissemination of dinoflagellates as a result of the increase in water temperature due to global warming, together with factors such as maritime transport and the arrival of migrating fish from endemic areas of ciguatera to European coasts could lead to an increase in the incidence of ciguatera in Europe.
The symptoms that characterize this poisoning include gastrointestinal, cardiac and neurological disorders, and the latter may become chronic. Currently there is no antidote that stops the disease, only a treatment that relieves part of the symptoms. It is an underdiagnosed syndrome due to the non-specificity of the symptoms and the absence of sensitive and specific rapid detection methods against ciguatoxin that confirm the intoxication. This project aims to provide an effective analytical procedure that allows rapid diagnosis of ciguatera in humans by analyzing blood samples or other human biological samples. The development of a method of detecting ciguatoxins in human samples will contribute in turn to the development of a specific treatment of intoxication, the detection of future outbreaks of ciguatoxin in Europe and will help to solve unknowns about the characterization of the risk of ciguatera resulting in the implementation of prevention measures against this intoxication.

N30

EFFICIENT AUTOMATED ANALYSIS OF MYCOTOXINS IN CANNABIS AND RELATED PRODUCTS USING ON-LINE IMMUNOAFFINITY CLEAN-UP CONJUNCTION WITH HPLC-FLD

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There is an increasing trend towards the legalisation of cannabis around the world, whether it is for medicinal purposes or recreational use. The techniques employed in the drying of the leaves and flowers could encourage the growth of mould and harmful mycotoxins. The leaves and flowers are dried slowly in the dark with ventilation which can encourage mould growth. Aflatoxins and ochratoxin A are the most prevalent mycotoxins present in cannabis according to the observational data available. Surveillance of aflatoxins and ochratoxin A would therefore be prudent in order to protect public health and ultimately determine maximum levels permissible in cannabis and its related products.
Immunoaffinity clean-up columns with antibodies raised to mycotoxins are routinely used in food analysis and are well established in Official Methods (AOAC International and CEN Standards). These immunoaffinity column methods have been rigorously validated and have been applied to a variety of botanical products such as herbal medicines and other plant-based materials similar to cannabis. Immunoaffinity columns provide the specificity for the compounds of interest while allowing the complete removal of sample matrix, giving clear analyte peaks on the chromatograms when using HPLC-FLD. Increased efficiency and a higher throughput of samples is achieved using the RIDA®CREST integrated HPLC instrument, combined with IMMUNOPREP® ONLINE immunoaffinity cartridges containing highly specific antibodies to aflatoxins and ochratoxin A.
In this study samples of cannabis and its related products were analysed for aflatoxins and ochratoxin A using the RIDA®CREST combined with IMMUNOPREP® ONLINE immunoaffinity cartridges. Two immunoaffinity cartridges were used simultaneously in the dual cartridge mode allowing for increased sample throughput.

Keywords: *cannabis*

N31

THOROUGH EXTRACTION OPTIMIZATION FOR DETERMINATION OF TOXIC PLANT ALKALOIDS IN SPICES AND HERBAL TEAS EMPLOYING U-HPLC–MS/MS

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A wide variety of medical plants and herb species contain alkaloids, the secondary metabolites containing at least one nitrogen atom in their molecules. More than 10,000 compounds of different structures have been identified, and are classified into various groups. From the most widespread ones, the pyrrolizidine and tropane alkaloids produced by *Solanaceae*, *Brassicaceae* and *Fabaceae* species could be named. These substances may enter the human food chain through a variety of contaminated commodities (honey, cereals, pseudocereals, herbs, spices, herbal teas and food supplements). As the toxicity of the majority of plant alkaloids is rather high, and new legislative regulation is in preparation (additionally to tropane alkaloids atropine and scopolamine, new maximum limits for many of pyrrolizidine alkaloids are being prepared now), reliable and sensitive multidetection analytical approaches are of major importance. During our research, the following main obstacles in plant alkaloids (multi)analysis have been identified: (i) inhomogeneity of alkaloids distribution in tested matrices, especially in herbal teas and spices (ii) efficiency of extraction of naturally occurring (non-spiked) analytes, and (iii) matrix effects. The aim of this study was thus to thoroughly optimize the analytical method suitable for the multidetection of 33 pyrrolizidine, 22 tropane alkaloids in herbal tea and oregano matrices. The following steps within the analytes isolation were optimized: (i) the optimal sample weight with respect to the particular investigated matrix, (ii) different isolation techniques and extraction periods, and (iii) different ways of sample extract purification. Within the experiments, the QuChERS-like procedure, as well as solid-liquid extractions with various clean-up arrangements (solid-phase extraction columns (OASIS MCX, Waters) or dispersive solid-phase extraction (C18, PSA, Z-sep) were tested. The separation and detection were realized by the ultra-high performance liquid chromatography with Luna Omega C18 (150 x 2.1 mm, 1.6 µm; Phenomenex) column, coupled with tandem mass spectrometry (U-HPLC–MS/MS) QTRAP 6500+ (Sciex). It is important to notice that a significant part of the work was realized by using of internal
Referencematerials with natural occurrence of alkaloids. It is not as common as using of 'spikes', nevertheless the informational value of experiments is incomparably higher.

Kywords: *plant alkaloids, spices, herbal teas, U-HPLC–MS/MS, extraction*

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N32

ONE STANDARD ELISA WITH A PRECALIBRATED CURVE, USING TOXIN-FREE 5-MINUTE IMMUNOSYSTEM FOR THE QUANTIFICATION OF MYCOTOXINS

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Introduction: The rapid quantification of mycotoxins (Aflatoxins, Deoxynivalenol (DON), Zearalenone (ZON), Ochratoxin A (OTA), Fumonisins, T-2/HT-2) in grains, without handling harmful mycotoxins, constitutes a challenge. It is also important to develop techniques, with high accuracy and repeatability, having also low LOD, LOQ and Coefficient of Variation (CV%). Consequently, the use of Mycotoxin-free 5-minute ELISA system, using one Mycotoxin-free standard and precalibrated curve could be an essential tool for the quantification of mycotoxins.
Aim: The aim of this study was to evaluate the recovery levels of Aflatoxin B1, Total Aflatoxin, DON, ZON, Fumonisins, OTA and T-2/ H-T2, in grains, using a 5-minute ELISA with one mycotoxin-free standard after a single extraction.
Methods: The levels of Aflatoxin B1, Total Aflatoxin, DON, ZON, OTA, Fumonisins, T-2/HT-2 toxins were determined using the One Standard 5-minute ELISA (Prognosis Biotech S.A.) Bio-Shield One Standard B1 (B4948, B4948005) , Bio-Shield One Standard Total (B4348, B4348009), Bio-Shield One Standard DON (B4548, B4548006), Bio-Shield One Standard ZON (B4448, B4448005), Bio-Shield One Standard Ochratoxin (B4248, B4248004), Bio-Shield One Standard Fumonisin (B4748, B4748003) and Bio-Shield One Standard T-2/HT-2 (B4848, B4848005) respectively. All methods were used with or without standards. It was used a single extraction with methanol 70%. Mycotoxin-free samples were chosen and spiked at three levels (including the LOQ), with a mixed mycotoxin solution. Reference materials from FAPAS were also analyzed.
Results: The recovery and CV% in all spiked samples and Reference Materials lied within acceptable range and were consistent either with or without the use of standards. Furthermore, the LOQ did not show significant difference between the paired techniques.
Conclusion: The innovative Mycotoxin-free 5-minute ELISA system, using one standard and precalibrated curve, gives acceptable recovery and CV%. Using a single extraction, the rapid toxin-free methods are effortless, accurate and cost-effective, providing unique advantages in the quantification of mycotoxins.

Keywords: *ELISA, mycotoxins, grains, one standard ELISA, mycotoxin-free method*

N33

DEVELOPMENT OF A NEW ANALYTICAL SERVICE FOR THE QUALITATIVE AND QUANTITATIVE DETERMINATION OF SELECTED MYCOTOXINS IN CEREALS BY LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY (LC/MSMS) USING SHIMADZU LCMS8050

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SASA is a division of the Scottish Government Agriculture and Rural Delivery Directorate and its primary role is to provide scientific services and advice in support of Scotland's agriculture and wider environment. SASA occupies a world class laboratory, glasshouse and experimental farm facility where a community of 100 scientists and their supporting staff work to ensure the quality, safety and security of food supply in Scotland.
In 2018 The Official Seed Testing Station for Scotland (OSTS) and Chemistry Branch - two branches of SASA – collaborated on the development of a screening assay and the setup of a new analytical service to test for the presence of selected mycotoxins in cereal crops. Mycotoxins in the growing crop are produced by fungal pathogens of the *Fusarium* species. The production of mycotoxins by these fungi is often dependent on geographic location and climatic conditions. Taking those two factors under consideration the new method focused on the four mycotoxins: deoxynivalenol (DON), zearalenone (ZON), T-2 and HT-2, and was validated in three types of cereal grain: wheat, barley and oats. Simple extraction protocol of shaking milled grain samples with the extraction solvent combined with use of immuno-affinity clean-up columns, to minimise matrix effects and with sensitive LC/MSMS instrumentation for quantitation of the residues detected will provide reliable information to seed processors, merchants and growers on seed quality.
This poster presents details of the UKAS accredited LC/MSMS method for the fast and affordable quantitative determination of four mycotoxins in three types of cereal grain. The method validation criteria comply with the regulatory limits and our results from the FAPAS proficiency testing scheme quality control samples and reference samples demonstrate the suitability of the method for successful application in routine sample analysis.

Keywords: *mycotoxins. LC-MS/MS immuno-affinity clean-up columns*

N34

IMMUNOAFFINITY SOLID-PHASE EXTRACTION WITH HPLC-FLD DETECTION FOR THE DETERMINATION OF AFLATOXINS B2, B1, G2, AND G1 IN GROUND HAZELNUT

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Mycotoxins are naturally occurring fungal toxins that were first found in fungus aspergillus flavus. Most of them are very stable and are not destroyed during processing or cooking procedures. One common group are the aflatoxins, of which 20 naturally occurring forms are known. Aflatoxin B1 is considered to be the most toxic to human health, but in addition the aflatoxins, B2, G2, G1, and the milk-derived derivatives M1 and M2 also have high importance. The B and G aflatoxins occur in various foods, such as nuts, grains, and spices, while the M derivatives are found in dairy products. The focus of this application is the determination of the toxins B2, B1, G2, and G1 in ground hazelnuts. The European Commission has set various maximum levels of aflatoxins in several foods under consideration of their consumption and use[1]. The maximum level for aflatoxin B1 ranges from 2 to 12 µg/kg for foods used for direct consumption or as an ingredient, with the exception of baby food products, which allow for a maximum level of 0.10 µg/kg. The limit sum of all four aflatoxins varies between 4 and 15 µg/kg. Therefore, a sensitive and accurate analytical method is required to monitor the low levels in various foods. For reliable identification and quantification, high-performance liquid chromatography (HPLC) coupled with fluorescence detection (FLD) is one of the most common techniques.
In this work, we determined and quantified four aflatoxins in ground hazelnuts by using immunoaffinity-SPE for cleanup followed by HPLC-FLD analysis without photo derivatization[2]. The use of a selective immunoaffinity-SPE and fluorescence detection provided sufficient trace level detection performance to determine aflatoxins in this matrix far below the limits defined by the European Commission.

[1] COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006: setting maximum levels for certain contaminants in foodstuffs, ANNEX section 2: mycotoxins (M5).
[2] Thermo Fisher Scientific application note 72686: Determination of underivatized aflatoxins B2, B1, G2, and G1 in ground hazelnuts by immunoaffinity solid-phase extraction with HPLC-FLD detection

N35

MASKED MYCOTOXINS IN CEREALS – DEVELOPMENT AND VALIDATION OF A QUECHERS-BASED LC-MS/MS MULTIMETHOD

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Mycotoxins are low-molecular-weight, toxic secondary metabolites produced by molds. Toxigenic molds can grow on cereal crops and thus contaminate food and feed. Plants can metabolize xenobiotic mycotoxins (and thus change their chemical structure) as part of their defense against pathogens. Conjugation reactions may reduce toxicity and increase water solubility. However, as a result of enzymatic hydrolysis, conjugated toxins can regain their original toxicity during mammalian digestion. These "masked" mycotoxins are currently neither routinely screened for nor regulated by legislation. The European Commission has set maximum residue levels for native mycotoxins in food and feed to protect humans from intoxications. Mycotoxins regulated in cereals include aflatoxin B1; the sum of aflatoxins B1, B2, G1, and G2; zearalenone; deoxynivalenol; ochratoxin A; and the sum of fumonisins B1 and B2[1-3]. Guidance values for the Fusarium toxins T-2 and HT-2 in cereals are recommended[4].

The purpose of this study was to develop a multimethod for quantification of 38 mycotoxins in four matrices (wheat, corn, rice, and barley). The method includes the analysis of 16 modified mycotoxins, of which nine are masked. Within this study, an analytical method based on a modified QuEChERS (Quick, Easy, Cheap, Efficient, Rugged, Safe) extraction and liquid chromatography-tandem mass spectrometry was evaluated. QuEChERS refers to a standardized approach to analyze pesticides that has been adapted for mycotoxins in this work. The method was validated according to performance characteristics established in the Commission Decision EC No 657/2002[5]. Limits of quantification were below the defined maximum residue levels for all regulated mycotoxins. Furthermore, an initial application of the method was carried out on 28 grain samples from local retailers. The analysis primarily revealed contamination with deoxynivalenol, deoxynivalenol-3-glucoside, zearalenone, and tentoxin. Fumonisin concentrations in one corn sample exceeded the maximum residue levels of Commission Regulation EC No 1126/2007[2]. In addition to the masked mycotoxin deoxynivalenol-3-glucoside, zearalenone-14-sulfate was quantified in one corn sample. A continuous extension of the multimethod is required to estimate human exposure to both mycotoxins and masked mycotoxins. In the future, a two-dimensional liquid chromatography method will be developed concerning more complex samples and insufficient peak capacities.

[1] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs.
[2] Commission Regulation (EC) No 1126/2007 of 28 September 2007 amending Regulation (EC) No 1881/2006.
[3] Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006.
[4] 2013/165/EU: Commission Recommendation (CR) No 2013/165/EU of 27 March 2013.
[5] 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC.

Keywords: *mycotoxins, masked mycotoxins, QuEChERS, LC-MS/MS, cereals*

N36

VERIFICATION OF THE PERFORMANCE OF TWO ELISA TEST KITS FOR AFLATOXIN M1 IN MILK AND DAIRY PRODUCTS

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I'screen AFLA M_1 and *I'screen* AFLA M_1 milk ELISA kits have been object of several published studies in the last 15 years, demonstrating accuracy and robustness, both in skimmed and unskimmed milk testing, and therefore they can be considered a reliable screening method for control bodies and dairy industries.
In this work, an overall overview of the kits performances during the years is presented, adding the very latest results obtained for batches produced in different sites. Specificity and sensitivity on raw milk samples were compared among batches produced from 2005 till today, showing high reproducibility of results. Trueness in the analysis of control materials in different batches is compliant and comparable, pointing out high lot-to-lot consistency with mean recoveries ranging from 90% to 100%.
External Quality Assessment during a period of 10 years by Tecna participation to proficiency test organized by Test Veritas, Fapas and AIA, shows 100% of <1.21 z-scores. In 2017, 19 participants to proficiency test Progetto Trieste (Test Veritas), all using Tecna kits, obtained z-score values between -1.11 and +1.14, while the other 21 participants, using other screening methods, had z-score values between -3.22 and +2.23.

Keywords: *aflatoxin M1, ELISA, screening, validation, milk*

N37

DETERMINATION OF SEVENTEEN MYCOTOXINS IN BRAZILIAN BREWING BARLEY

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The present study describes determination of 17 mycotoxins (aflatoxins B1, B2, G1, and G2, fumonisins B1 and B2, ochratoxin A, deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxin, enniatins A, A1, B, and B1, and beauvericin) in 60 Brazilian brewing barley.
The samples were detected using ultra-performance liquid chromatography coupled to mass spectrometry. A modified QuEChERS method was used for extraction.
Eighty eight percent of samples were contaminated with at least one of mycotoxins. None of the investigated samples contained aflatoxins, ochratoxin A, T-2, HT-2 and fumonisin B2. Fumonisin B1 occurred only in one sample. Enniatins were detected in 77% of samples.
Deoxynivalenol was found in forty-one (68%) samples, ZEN in eighteen (30%) samples. The data were compared with the maximum allowable limits for DON and ZEN in unprocessed cereals set by the Brazilian and European Union. Ten samples (17%, n=60) exceeded the maxim allowable limit for DON set by the European Union, thirteen (22%, n=60) by the Brazilian regulation. Eleven samples (18%, n=60) exceeded the maximum allowable limit for ZEN set by the Brazilian and European Union regulation.

Keywords: *Brazilian brewing barley, mycotoxins, LC/MS, QuEChERS*

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N38

COMPUTATIONAL INSIGHTS ON THE "PERSONALIZED" TOXICOLOGY OF MYCOTOXINS - DOES IT MATTER FOR SAFE FOOD AT INDIVIDUAL LEVEL?

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Mycotoxins in food may threat public health at a global scale. However, for most of them, the current body of knowledge does not support a proper risk assessment and more data are needed to better understand the mechanisms of action underlying the toxic effects in living organisms. In particular, the assessment of the so called "personalized" toxicity may result in a better comprehension of the adverse effects on living organisms at an individual level. However, the assessment of "personalized" effects of mycotoxins is still largely overlooked though it might improve the understanding of their toxicity, eventually refining both the design and interpretation of epidemiological studies.
This work dealt with the early analysis of possible "personalized" effects of alternariol and zearalenone, widespread mycotoxins produce by *Alternaria* and *Fusarium* species respectively, when mutations on the androgen or estrogen receptors occur. Specifically, mutations naturally occurring in human population, including some related to malignancies onset or progression, were accounted to study the possible effects of these mycotoxins in vulnerable population groups. To do so, it was validated and applied a computational work-flow relying on docking simulations, pharmacophoric modeling and molecular dynamics that assessed the capability of alternariol and zearalenone to interact with a set of mutated receptors.
The results collected highlighted the likely existence of either mutations able to "protect" from mycotoxin action or to exacerbate the mycotoxin-dependent activation of these receptors. Overall, it has been described: i) the mechanistic basis of the diverse sensitivity of mutated receptors to alternariol or zearalenone activation; ii) the likely existence of inter-individual responses to mycotoxins stimulation; iii) the need of broadly collecting data on the "personalized" toxicology of mycotoxins of food origin to move their risk assessment at an individual level.

Keywords: *alternariol, zearalenone, estrogenic activity, androgenic activity, personalized toxicology*

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N39

HUMAN EXPOSURE TO CYANOTOXINS: EXPLORING IN VITRO DETOXIFICATION USING ATMOSPHERIC COLD PLASMA TREATMENT TO PROTECT HUMAN HEALTH

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Recently, there have been increases in freshwater Harmful Algal Blooms (HABs) globally. HABs are known to contaminate some of the Great Lakes, such as Lake Erie, which supplies over 80% of surface water in North America. HABs can produce cyanotoxins, many of which are hepatotoxins such as the microcystins (MCs) and cylindrospermopsin (CYN). Cyanotoxins have been reported in freshwater systems across the world, with their health effects including; promotion of various cancers, neurotoxicity, genotoxicity and potential carcinogenicity. Human exposure to these occurs through numerous pathways, including the ingestion of contaminated water and recreational use of water bodies. Current methods utilised by water treatment facilities to remove cyanobacteria and cyanotoxins from drinking water can be successful if tailored to individual toxins. However, with certain cyanobacterial species capable of producing more than one class of cyanotoxin, as well as producing numerous congeners, their removal could become more problematic, posing a risk to consumers. Therefore, there is a need to try and effectively detoxify drinking water contaminated with cyanotoxins to safeguard human health by use of new and novel techniques, such as atmospheric cold plasma treatment. To investigate its efficacy, six MCs, nodularin (NOD), cylindrospermopsin (CYN), anatoxin-A (ATX-A) and the marine toxin domoic acid (DA) were subjected to atmospheric cold plasma treatment, comparing helium gas alone against a helium/oxygen gas admixture.
The results of the cold plasma treatment on these cyanotoxins in water indicated effective degradation of the MCs and NOD, whereas the more polar cyanotoxins, CYN and ATX-A, were relatively stable to this technique, which was also found to be more effective when using helium gas only.

Keywords: *cold plasma, cyanotoxins, detoxification*

Acknowledgement: *I would like to acknowledge Rachael Irwin from the Centre for Plasma Physics, QUB, for her guidance with this work.*

N40

DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN TEAS AND HERBAL TEAS BY LC-MS

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Presented is an application of a routine method to determine pyrrolizidine alkaloids in teas and herbal extracts by LC-MS/MS on a Shimadzu Nexera UHPLC coupled to an LCMS-8060 Mass Spectrometer.
Pyrrolizidine alkaloids (PAs) occur in many plants and protect the plant against herbivores. They are particularly common in *asteraceae*, *boraginaceae*, and *fabaceae*. Depending on the plant family, different PAs are formed, covering a multitude of different substances. In plants, PAs occur as N-oxides and in their free form. PAs are of interest because their metabolites are liver toxic and carcinogenic.
The analytical challenges result from a multitude of stereo-isomers having the same mass and often a similar fragmentation pattern. Chromatographically, not all pairs can be separated in a single run. Sum parameters may be problematic, as the fragmentation patterns are similar but not always identical.
An overview is given of some measurements in 2016/2017. Different patterns can be seen in different kind of teas resulting from typical contaminations e.g. from Fen Ragwort (*senecio angustifolia*) in rooibos or common ragwort (*Senecio jacobaea*) in camomile. Fortunately, overall the amounts measured in 2018/2019 are decreasing.
For extraction and clean up, we follow the BfR protocol that includes an extraction with sulphuric acid. An extraction with hot water comparable to a preparation of a cup of tea in the kitchen provided sufficient results for teas as well. In routine analysis, one protocol suitable for as many matrices as possible is required. For other foodstuff, a water extraction may not be suitable, so we keep to the original protocol.
LC separation follows the BfR protocol as well (C18 column, water/methanol gradient containing 0.1% formic acid and 5 mM ammonium formate). If necessary to separate critical pairs of analytes we perform a second run with identical chromatographic conditions using a phenyl-hexyl column as suggested by other labs. Still unseparated pairs are reported as a sum parameter, if the response of the transitions is comparable between the analytes.
Ionisation is carried out in ESI positive mode with an Ion Spray voltage of 4000 V at a temperature of 300 °C. MS/MS Transitions are optimised for the instrument.
The BfR proposes several analytes. Some we discarded from the list when they rarely occur. In contrast, we added analytes that may occur in our samples and that can cause problems, as they are isomers of analytes already on the list.

BfR-protocol, Bestimmung von Pyrrolizidinalkaloiden (PA) in Pflanzenmaterial mittels SPE-LC-MS/MS Methodenbeschreibung, BfR-PA-Tee-2.0/2014
Mulder, P. P. J.; et al., Occurrence of Pyrrolizidine Alkaloids in Food, EFSA Supporting Publication 2015: EN-859, External Scientific Report, 03.08.2015
C. Czerwenka, A. Turkowitsch: Pyrrolizidine alkaloid isomers: Analytical challenges and solutions, poster presentation at RAFA symposium, 2017

Keywords: *pyrrolizidine alkaloids, tea, LC-MS, plants, toxins*

N41

FUNGAL PROTEASES THAT PROMOTE DISEASE AND MYCOTOXIN CONTAMINATION OF CROPS

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Improving plant defenses and preventing fungal diseases in crops results in less mycotoxin contamination of grain at harvest. To improve plant defenses an understanding the molecular interactions that govern plant-fungal interactions is required. Plants detect and respond to fungal infection using protein signaling networks and through activity of antifungal proteins. Successful pathogens evade detection and counteract antifungal proteins by producing specialized proteases that modify the activity of plant immunity proteins. Using an enzyme activity–based approach we identified the corn ChitA chitinase protein as a common target of ear rot pathogens. To determine the identity of the pathogen proteases, their ChitA-degrading activity was used to guide their purification from fungal cultures. Once isolated the proteins were analyzed by tryptic peptide LC-MS/MS and obtained spectra were compared to genomic sequence information to generate a list of candidate proteins. Correct identification of the ChitA-degrading proteases was then confirmed by producing recombinant proteins in the yeast *Pichia pastoris* from fungal cDNAs. Using this methodology, we have identified three distinct, unrelated proteases that cleave corn ChitA at the amino terminus to produce truncated forms. These fungal proteases, termed chitinase modifying proteins, have been shown to contribute to fungal virulence. Understanding the molecular mechanisms behind corn ear rot promises to guide disease resistance breeding and ultimately reduce levels of mycotoxin contamination. Improved disease resistance in corn will improve the safety of corn-based foods and animal feeds and allow producers to avoid economic losses.

Keywords: *mycotoxin, protease, chitinase, effector, disease*

N42

MULTIMYCOTOXINS ANALYSIS METHOD BASED ON MOLECULARLY IMPRINTED POLYMERS SOLID PHASE EXTRACTION NOT ONLY FOR CEREALS BUT ALSO FOR FATTY MATRICES

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Mycotoxins are secondary metabolites of fungi. With regard to the widespread distribution of fungi in the environment, mycotoxins are considered to be one of the most important natural contaminants in foods and feeds. To protect consumers' health and reduce economic losses, surveillance and control of mycotoxins in food and feed has become a major objective for producers, regulatory authorities, and researchers worldwide. In this context, availability of reliable analytical methods applicable for this purpose is essential.
In spite of the broad variety of mycotoxins chemical structures, a solid phase extraction (SPE) clean-up methods based on the use of Molecularly Imprinted Polymers (MIP) could be developed. MIPs are affinity columns made with very stable polymers to aqueous or organic solvents as well as temperature. These cost-effective products are widely used for clean-up and preconcentration applications.
This method enables the analysis of 11 regulated multimycotoxins on cereals and more fatty matrices such as sunflower seeds. Deoxynivalenol (and its derivatives), Aflatoxins, Ochratoxin A, Fumonisins, Zearalenone, HT-2 and T2 toxins could be analyzed by this fast, robust, efficient and cost-saving clean-up process prior a LC-MS/MS analysis. High recovery yields were obtained.

Keywords: *multi-mycotoxin analysis, molecularly imprinted polymers, fatty matrice analysis, cereal analysis, clean-up method for mycotoxins*

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EMERGING MARINE BIOTOXINS"TTXS AND PLTXS": A THREAT FOR FOOD SAFETY IN ITALY?

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Palytoxins (PlTXs) and Tetrodotoxins (TTXs) are Emerging Marine Biotoxins (EMBs) of particular interest for the Mediterranean Sea. In the last years *Ostreopsis ovata*, a known PLTXs producer dinoflagellate, periodically bloomed in some coastal areas of the basin affecting environmental and human health. PlTXs intoxication in human can cause neurological and gastrointestinal symptoms. From the 2003 pufferfish and other TTXs bearing organisms entered the Mediterranean Sea through the Suez Canal and successively TTXs were reported in shellfish in Greece.TTXs are potent neurotoxins produced by bacteria mainly belonging to *Vibrio* genus, responsible for fatal pufferfish poisoning. PlTXs and TTXs are not yet regulated in Europe but EFSA suggested a Referencelevel of 30 µg/kg [1] for PlTXs and of 44 µg eq/kg for TTX in shellfish meat [2]. Since 2006, every late summer, wild mussels collected on the Conero cliff showed traces of PlTXs-like compounds assessed by mouse and in vitro assays [3]. Recently TTXs have been found in mussels from Italy [4]. Here two in-house adapted protocols for the analysis of EMBs by LC-MS/MS and their application in order to evaluate EMBs presence in wild mussels from Marche coasts are presented. For PlTXs, a method was developed starting from literature data [5]. Mussel tissue was extracted in triplicate with methanol and water and analysed by LC-MS/MS. For TTXs, a method based on the EURLMB HILIC-MS/MS protocol for determination of TTXs was developed. PlTXs method performed very well while for TTXs the method sensitivity was variable and and not sufficiently high (LOD 8-25 µg/kg), mainly as consequence of a dramatic matrix effect. Wild mussels *M. Galloprovincialis* were collected along the Marche coasts from July until October 2016 for PlTXs and from June till August 2018 for TTXs. PlTXs were not detected until September, when levels increased gradually to exceed the guidance level; at the same time a *Ostreopsis Ovata* bloom was observed and OVTX-a was the predominant analogue in the mussel toxin profile. Of 20 samples analysed only 3, harvested in July 2018 from Pesaro wild sites, showed detectable TTXs traces. Literature data support this finding, since Pesaro sites have some environmental features, which may promote TTXs accumulation [6]. PlTXs can be a threat for consumers health and therefore a problem for shellfish industry even if the phenomenon is limited both temporally and geographically. TTXs are not yet a problem but monitoring is very important to be able to prevent eventual future large-scale mussel contamination.

1 Palytoxin group *EFSA Journal* 7(12):1393 2009
2 TTX in marine bivalves and gastropods *EFSA Journal* 15(4):4752 2017
3 S. Bacchiocchi et al. *Proc. International Symposium on Marine Biotoxins* Trieste 2007, 27-29 May
4 C. Dell'Aversano et al. *Chemophere* 215 2019, pp 881-892
5 P. Ciminiello et al. *Analytical Bioanalytical Chemistry* 401 2011, pp 1043-1050
6 D. Turner et al. *Marine Drugs* 15, 277 2017, pp 1-18

Keywords: *palytoxins, tetrodotoxins, LC-MS/MS, wild mussels*

Acknowledgement: *Authors acknowledge Italian Ministry of Health and Marche Region for funding support (IZSUM RC 0072016, PAM12016)*

N44

QUANTITATION OF MYCOTOXINS IN FOUR FOOD MATRICES COMPARING STABLE ISOTOPE DILUTION ASSAY (SIDA) WITH MATRIX MATCHED CALIBRATION METHODS BY LC-MS/MS

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Mycotoxins are secondary fungal metabolites produced by mold that may be found in food or feed. They can cause severe health problems in humans and animals, and can result in significant economic losses. Among the hundreds of toxic mycotoxins, aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, HT-2 toxin, zearalenone, and T-2 toxin are considered as a major concern for corn, wheat, peanuts and other agricultural products. LC-MS has become the standard and is now widely used for routine mycotoxin analysis and identification. One of the challenges faced by LC-MS techniques is the matrix effects caused by the use of electro-spray ionization (ESI). Generally, sample preparation, chromatographic and calibration techniques are the common strategies for reducing the negative effects of matrix effects. Standard addition, matrix matching, and stable isotope dilution assay (SIDA) are all possible calibration solutions. In this work, a quick "dilute-filter-shoot" method was used for sample preparation. A seven-minute LC-MS/MS method using a biphenyl phase column was developed and verified for quantifying twelve mycotoxins in four commodities: corn, peanut butter, brown rice, and corn & wheat mixed. Both SIDA and matrix matched calibration methods were applied, compared, and evaluated in terms of recovery, efficiency, advantages, and limitations.

Keywords: *mycotoxin, grain, peanut, corn, SIDA*

Acknowledgement: *We acknowledge Dr. Kai Zhang, (U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition) for his support.*

N45

VALIDATION OF A QUECHERS APPROACH FOR THE ANALYSIS OF CYTOCHALASIN E IN URUGUAYAN MALTED BARLEY BY LC-ESI-MS/MS

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Cytochalasin E is a metabolite produced mainly by the fungus *Aspergillus clavatus*, responsible for disrupting cytoskeletal actin organizations. It is very important to detect its presence in food matrices, because it is harmful to humans as it inhibits the formation of blood vessels (angiogenesis). In the malting industry, the barley undergoes a malting process, which under certain conditions of humidity and temperature, can favour the development of this fungus, being able to produce this type of mycotoxins. The Uruguayan industry set an internal limit of 2 μgkg^{-1} for Cytochalasin E in malted barley.
In this work a methodology based on the QuEChERS approach for the determination of Cytochalasin E in malted barley was validated. It briefly consisted of an hydration step of a 10 g portion of homogenised sample, followed by an acetonitrile extraction. Afterwards, a salting out with $MgSO_4$ and NaCl was done. The crude extract was then subjected to a dispersive solid phase extraction (d-SPE) clean-up with $MgSO_4$ and PSA prior to the injection in the analytical system.
The analytical determination was performed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) in Scheduled MRM™ mode, available on the hybrid quadrupole – linear ion trap (QLIT) instrument.
The validation of the developed methodology was carried out following the European SANTE Document guidelines in terms of accuracy and precision (recovery percentage), limit of quantification (LOQ), linearity and matrix effect. The recoveries were assayed at three levels (1, 2 and 10 μgkg^{-1}) by quintuplicate with results in the range 86 - 96 %. Precision, evaluated as RSD was between 6 and 14 % for the three levels. LOQ was determined as the lowest fortification level with acceptable precision and accuracy results, and defined as 1 μgkg^{-1}.
The validated methodology was successfully applied to the analysis of 16 malted barley samples provided by the local industry. Ten samples out of the 16 were below the LOQ. Positive samples were in the range 2.9 - 17.1 μgkg^{-1}. This development generated a service of great value for the local industry.

Keywords: *cytochalasin E, malted barley, QuEChERS, LC-ESI-MS/MS*

Acknowledgement: *This proyect was funded by UTEC with the IDEI 1605 proyect. We also would like to thank Cympay S.A. Malting Company currently belonging to AB-Inbev for the provided samples. This proyect could not have been done without the support of Departamento de Química del Litoral (DQL) - Universidad de la Repúblca (UdelaR), they provided us with the LC-MS.*

N46

IMPROVED MASS SPECTROMETRIC DETECTION OF MASKED FUMONISINS FROM MAIZE

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Fumonisins are a group of mycotoxins that are routinely found worldwide in commodities such as maize. The group of toxins interacts with matrix components non-covalently, and forms covalent products with matrix constituents, such as carbohydrates and proteins. These covalent modifications to the fumonisins make it more difficult to assess the total amounts of the toxins that may be present in a commodity. We have developed an liquid chromatography – mass spectrometry (LC-MS) method for the determination of a known product of the reaction of fumonisin B1 (FB1) with glucose: N-(1-deoxy-D-fructos-1-yl) fumonisin B1 (NDFrc-FB1). The method was further developed to determine products from similar reactions that produce fructosyl-analogs of fumonisins B2 and B3. The fumonisin analogs were determined from naturally contaminated maize samples. To improve the analytical performance of the method isotopically labelled standards of the modified fumonisins were synthesized. Further, an ambient ionization technique was evaluated for the analysis of the modified toxins without need for chromatographic separation. These results demonstrate the potential to isolate and detect modified fumonisins and will facilitate efforts to determine the frequency of the occurrence of these compounds in maize.

Keywords: *mycotoxin, fumonisin, masked toxin, maize*

N47

SENSITIVE AND RAPID ANALYSIS OF MULTIPLE MYCOTOXINS WITH SIMPLE SAMPLE PRETREATMENTS USING LC-MS/MS

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Introduction: Mycotoxins may contaminate food and feed and are known to give serious damages to health of human and animals. For food safety, manufacturers should monitor their products to avoid contamination based on analytical data. Simultaneous analysis of mycotoxins has been important due to increase of regulated mycotoxins, analysis efficiency, or synergistic effect caused by mycotoxins. From these backgrounds, we investigated to build simultaneous analytical methods of multi-mycotoxins with simple sample pretreatments for efficient inspections.
Materials and Methods: 18 mycotoxins (Nivalenol, Patulin, Deoxynivalenol, Fusarenon-X, 3-Acetyl-Deoxynivalenol, 15-Acetyl-Deoxynivalenol, Aflatoxin B1, B2, G1, G2, Diacetoxyscirpenol, Fumonisin B1, B2, B3, T-2 toxin, HT-2 toxin, Ochratoxin A, Zearalenone) were used for evaluation of matrix effect and recovery rates in wheat.
Analytical samples were prepared through the extraction protocol of MycoSpin™400 (Romer Labs), which is a very convenient method without evaporator nor nitrogen purge procedures. The operation of MycoSpin™400 was completed within 5 minutes.
Analysis was performed by a LCMS-8050 which was equipped with a Nexera™ X2 UHPLC. Pentafluorophenyl (PFP) bound column was used to separate the regioisomeric pair (3-AcDON / 15-AcDON, FB2 / FB3) by gradient elution with a series of mobile phases containing ammonium acetate, acetic acid and methanol. Quantitative limits had been deemed to be less than or equivalent to the minimum values specified in EC/1886/2006. The developed method achieved the simultaneous determination of mycotoxins in 15 min analytical cycle. This analytical method was developed by the modified LC/MS/MS Method Package for Mycotoxin (Shimadzu).
Results: All mycotoxins were well separated in just 15 mins by the developed method regardless of samples, wheat, corn powder, peanut powder, and almond powder. Although Fumonisins have coordinating ability to metal ions, which can cause poor peak shape and carryover, the troubles were improved in developed method by applying a metal free column and optimized rinse methods using 4 types of reagents.
MycoSpin™ protocol was convenient in short timescale. However, even after the clean-up, many matrix compounds remained and were affected. We used the standard additive method for quantification instead of the internal standard method. The results indicate that the standard addition calibration method could help correct and improve the recovery rate even under the influence of the matrix effect. To improve the clean-up and quantitative performance, further evaluation of multi-function columns or ion exchange columns would be effective.

Keywords: *LC/MS/MS, simultaneous analysis of mycotoxins, simple pretreatment, elimination of fumonisin carryover, 15 mins*

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IMPACT OF NATURALLY CONTAMINATED SUBSTRATES ON ALPHITOBIUS DIAPERINUS AND HERMETIA ILLUCENS: UPTAKE AND EXCRETION OF MYCOTOXINS

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In the last years, edible insects have been explored as a promising alternative food source in order to overcome the future food demands connected to growing world population, belonging in the European Union to the category of Novel Foods. Insects have the ability to grow on a different spectrum of substrates, which could be naturally contaminated by mycotoxins. Very little it is known about their potential bioaccumulation in insect organism, but some studies suggest their potential detoxification effects. In the present work, the mycotoxin uptake and/or excretion in two different insect species, *Alphitobius diaperinus* (Lesser Mealworm, LM) and *Hermetia illucens* (Black Soldier Fly, BSF), grown on naturally contaminated substrates, was evaluated. As substrates of growth, different organic wastes recovered from cereal and vegetable processing were considered under a circular economy perspective. LC-MS/MS and LC-UV/FLD methods were applied for the detection of common mycotoxins found in crops and/or vegetables. Among all the substrates tested, the Fusarium toxins DON, FB1, FB2 and ZEN were found in those based on wheat and/or corn. No mycotoxins were detected in BSF larvae, while quantifiable amount of DON and FB1 were found in LM larvae, although in lower concentration than those detected in the growing substrates and in the residual fractions. Mass balance calculations indicated that BSF and LM metabolized mycotoxins in forms not yet known, accumulating them in their body or excreting in the faeces. ZEN was detected in BSF residual fractions but not in the starting materials, suggesting a possible hydrolytic activity carried out by larvae upon growing. Our results proved the urgency of better deciphering the ability of insects to uptake, biotransform, and excrete mycotoxins, in view of a safer use of insects as alternative food source.

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N49

SELECTED TRICHOTECENES AND THEIR MASKED FORMS IN WHEAT CULTIVATED IN POLAND DURING 2017 AND 2018 SEASONS

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Wheat is a staple food used in many countries around the world. Poland, with approximately 11 million metric tons cultivated each year, is the fourth largest producer of this crop in the European Union. Cereal plants are vulnerable to fungal infections, including Fusarium head blight (FHB) which is induced mainly by *Fusarium graminearum*. FHB leads to economic losses caused by the decrease in crop yields, but also can have an impact on food safety as it is connected with an accumulation of mycotoxins in grains. The most important *Fusarium* toxins are nivalenol (NIV), deoxynivalenol (DON), and their masked forms.

For the study 300 samples of wheat grain were collected in Poland during 2017 and 2018 cultivation seasons. The grains were grounded, homogenized with water, and purified using DON-NIV WB immunoaffinity columns. Cross-interaction of antibodies within the columns allowed the simultaneous determination of NIV, DON, their glucosides (NIV-3G, DON-3G), and 3-acetyl-deoxynivalenol (3-AcDON). The samples were analyzed using an H-class liquid chromatograph coupled with a mass spectrometer with a time-of-flight analyzer.

DON was the most frequently found mycotoxin in the tested samples; the percentage of DON-positive grains was 92% in 2017 and 61% in 2018. Additionally, the concentrations in 2017 samples (5.2–1670.7 µg/kg) were higher than in 2018 samples (5.0–461.7 µg/kg). The similar relationship was found for DON-3G; however, no statistically significant differences were found between the seasons for other three mycotoxins. Strong correlations were found between content of primary mycotoxins and their glucosylated forms.

The concentrations of mycotoxins in tested wheat samples were affected by weather conditions during cultivation period, as these conditions are influencing a development of toxin-producing fungi. The warm and dry spring of 2018 in Poland was not favorable for *Fusarium* growth, thus 2018 samples were less contaminated than 2017 samples. The maximum DON levels specified in 1881/2006 Commission Regulation were exceeded in 3 of samples harvested in 2017, but in none of 2018 samples. The glucosides accompanying DON and NIV can increase the risk connected with the food contamination, thus legislation updates concerning the occurrence of masked forms of mycotoxins should be implemented.

Keywords: *deoxynivalenol, wheat, nivalenol, nivalenol-3-glucoside, deoxynivalenol-3-glucoside*

N50

CHANGES OF THE SELECTED FUSARIUM TOXINS LEVEL DURING MALTING OF WHEAT GRAIN

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Malt production is one of the methods of wheat utilization. The malt is used in baking and pastry production, but it is also a material for production of beer. The malt quality depends on, among others, contamination of grain by fungal spores and their secondary metabolites.
The aim of experiments was to determine an impact of process of malting of wheat grain on its content of deoxynivalenol (DON), nivalenol (NIV), and their metabolites - deoxynivalenol-3-glucoside (DON-3G) and nivalenol-3-glucoside (NIV-3G). Two wheat cultivars used during the research - *Legenda* and *Pokusa* were grown in Poland in 2018. Concentrations of mycotoxins in *Legenda* cultivar were 1355 µg/kg of DON, 208 µg/kg of DON-3G , 382 µg/kg of NIV, and 210 µg/kg of NIV-3G; in case of *Pokusa* cultivar the concentrations were 1904 µg/kg of DON and 514 µg/kg of DON-3G, while levels of NIV and NIV-3G were below the limit of quantification. The grain was soaked twice for 7 hours with 17 hour air rest in a temperature of 14 °C; the final humidity of grain was 44%. Subsequently, the grains with newly grown sprouts and root ovules was placed on perforated plate with constant ventilation with air of humidity of over 95% and temperature of 14 °C; the process lasted 7 days. The malting grain was mixed in order to aerate the material and to gather the samples for analysis once each 24 hours.
Steeping of grain caused significant decrease of DON content. The levels after the steeping were: 551 µg/kg of DON, 159 µg/kg of DON-3G, 289 µg/kg of NIV, and 123 µg/kg of NIV-3G (*Legenda*) and 921 of DON and 335 µg/kg of DON-3G (*Pokusa*). Continued malting (particularly since fourth day) caused significant increase of analyzed compounds. At the last day of malting, the mycotoxin levels were: 4679 µg/kg of DON, 3141 µg/kg of DON-3G, 551 µg/kg of NIV, and 583 µg/kg of NIV-3G (*Legenda*) and 2884 of DON and 1608 µg/kg of DON-3G (*Pokusa*).
The malting process led to the increase of DON and NIV content, as a result of *Fusarium* growth, which was visible at macroscopic level. Observed increase of glucoside level was a result of activity of glucosyltransferase enzymes which are responsible for detoxication of xenobiotics. The limit of DON content in unprocessed grain is 1250 µg/kg (Commission Regulation (EC) No 1881/2016), thus using contaminated grain for the malting process carries the thread for food safety. Secondary growth of fungi during the malting leads to increase of mycotoxin level, therefore it is important to utilize grain of good quality for this process.

Keywords: *fusarium, malting, trichotecenes, wheat, food safety*

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THE EFFECT OF GRIND AND EXTRACTION SIZE ON ZEARALENONE RESULT VARIABILITY

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In the 2018 corn crop, zearalenone was found in several areas of corn growing regions in the United States. In many years, zearalenone is detected at low levels in areas of the US, however in 2018 the contamination levels were found to be higher than in typical years. These types of crop years highlight the importance of proper sample preparation for zearalenone in corn. Sample preparation of products being tested for Zearalenone is a critical part of the total analytical process. The difference in sample grind size, as well as the amount of sample extracted can also contribute to the overall result variability. An evaluation was conducted to compare the extraction of corn naturally contaminated with Zearalenone utilizing different sample grind and different sample extraction weights. The naturally contaminated corn was ground to various mesh sizes, homogenized and various sample sizes where extracted. The extractions were performed using acetonitrile/water (84/16) with a 1 hour on Eberbach shaker. The extracts were then analyzed by LC-MS/MS. Data presented shows the effect grind size and sample extraction size has on Zearalenone result variability.

Keywords: *zearalenone*

N52

THE EFFECT OF GRIND AND EXTRACTION SIZE ON DEOXYNIVALENOL RESULT VARIABILITY

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Deoxynivalenol (DON) is a common problem in areas growing barley in North America. Each year levels fluctuate from low to high. One thing remains constant however, sample preparation for this commodity is critical for obtaining accurate DON results. This study evaluates sample preparation of barley containing DON. Sample preparation is a critical part of the total analytical process. The difference in sample grind size, as well as the amount of sample extracted contributes to the overall result variability. An evaluation was conducted to compare the extraction of barley naturally contaminated with DON utilizing different sample grind and different sample extraction weights. The naturally contaminated barley was ground to various mesh sizes, homogenized and various sample sizes where extracted. The extractions were performed using acetonitrile/water (84/16) with a 1 hour on Eberbach shaker. The extracts were then analyzed by LC-MS/MS. Data presented shows the effect grind size and sample extraction size has on DON results variability.

Keywords: *deoxynivalenol*

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HUMAN BIOMONITORING TO ESTIMATE EXPOSURE TO DEOXYNIVALENOL AND ZEARALENONE: A COMBINED 24-HOUR DUPLICATE DIET – 24-HOUR URINE STUDY

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Human exposure to mycotoxins is mostly based on food analysis data combined with food consumption data [1]. Human biomonitoring is an alternative way to assess exposure [2,3]. Mycotoxins can exist in several modified forms which are often not covered in food monitoring. Biomonitoring might be beneficial to assess the overall internal exposure. However, for use in risk assessment in food safety, it is necessary to link internal exposure data from human biomonitoring with external exposure through food. To gain insight in such relationship, a pilot study was conducted in the Netherlands in 2018 in which duplicate diet samples and 24-hour urine samples* (until next morning's first void) were collected from the same 35 persons on the same day. In addition, food items consumed were recorded. Duplicate diets were analysed for deoxynivalenol (DON) and related compounds (3- and 15-acetyl-DON, DON-3G), and for zearalenone (ZEN) and related compounds (ZEN, a/b-ZEL, a/b-ZAL, ZAN), using LC-MS/MS based methods. Urine was analysed using dedicated methods involving enzymatic deconjugation and immuno affinity cleanup in order to reach appropriate LOQs (< 0.25 ng/ml for DON biomarkers, <0.02 ng/ml for ZEN biomarkers).
DONs and biomarkers were found in all duplicate diet samples (2-16 ng/g) and urine samples (3-32 ng total-DON/ml). ZENs could also be detected in most of the samples, but at much lower concentrations (0.2-10 ng/g, and 0.02-0.5 ng/ml, respectively). For each subject, the ratio urinary excretion of the biomarker vs dietary intake was determined. The median ratio for DON was 84% which corresponded reasonably well with that reported in literature [4]. For ZEN the median was 67%. Especially for ZEN much higher and lower ratios were observed at an individual level, indicating that excretion may take longer than 24 hours. The data indicate that human biomonitoring can be a valuable alternative to food monitoring, but also that more data on toxicokinetics (especially for ZEN) are required for adequate establishment of urinary biomonitoring equivalents related to health-based guidance values.

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* Ethical approval was obtained through the METC of Wageningen University.

Keywords: *mycotoxins, exposure assessment, human biomonitoring, duplicate diet, urine*

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INTERLABORATORY VARIABILITY IN QUANTITATIVE DETERMINATION OF MYCOTOXINS

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Quantitative analysis of samples for mycotoxins by different laboratories inevitably results in variation of results, even when analysing portions of the same very well homogenised sample, and even when using the same or equivalent methods of analysis.
In the field of mycotoxins, it is still common to use the (modified) Horwitz equation [1,2] to predict the achievable interlaboratory variability. In its modified form [2] the predicted interlaboratory variability expressed as relative standard deviation (RSD_R) is 22% for levels ≤120 µg/kg, and then decreases with increasing levels (e.g. 18% at 500 µg/kg and 11% at 10,000 µg/kg). In part, performance criteria embedded in EU legislation for mycotoxin analysis [3] have been based on the Horwitz approach.
In this work, the actual interlaboratory variability of quantitative determination of mycotoxins was investigated. This was done using the robust standard deviations as observed in proficiency tests (PTs) organised between 2013 and 2018. For this, PTs from the European Union ReferenceLaboratory for mycotoxins and from FAPAS were used. Since PT data were used, analysis methods used for analysis varied and included both LC-MS/MS based methods as well as classical LC-UV-based methods, reflecting current routine practices. The data set contained more than 750 PT RSD_R-values from a wide variety of mycotoxin/matrix/level combinations. Mycotoxins were mostly the regulated ones, matrices varied from wheat to black pepper and milk, and levels from 0.03 to 10,000 µg/kg.
An assessment was made to reveal any dependencies of the RSD_R on the concentration, the mycotoxin, or the matrix. In contrast to the Horwitz prediction, no relationship of the RSD_R with concentration was observed. In addition, there was also hardly an effect of the mycotoxin or the matrix. The median RSD_R was 22%, the 75th percentile 26%. Apparently, when suitable validated methods are used, and laboratories are experienced, similar method performance can be obtained irrespective the mycotoxin, matrix or concentration. Based on this outcome, it would make sense to set a fixed generic method performance criterion for the RSD_R of 25%, rather than the mycotoxins/concentration dependent RSD_R's from current legislation. In line with this, for evaluation of PT results, the use of a fixed fit-for-purpose target standard deviation for proficiency assessment of 25% is proposed instead of the Horwitz-based values.

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Keywords: *mycotoxins, analysis, performance criteria, interlaboratory variability, horwitz*

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THE FORMS OF OCHRATOXIN A IN SOLUTIONS AT DIFFERENT PH

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The forms of ochratoxin A in solutions at different pH
Ochratoxin A (OTA) is a potent toxin that causes negative effects on animal and human health. Produced by secondary metabolisms of Aspergillus and Penicillium species, it affects a large variety of foodstuffs. It is considered one of the most important chronic dietary risk factors. In fact, the European Food Safety Authority (EFSA) set a tolerable weekly intake and the regulation (EC) n. 1881/2006 established maximum levels of OTA in different foodstuffs. Immunochemical methods and liquid chromatography coupled to fluorescence spectroscopy or to mass spectrometry are the commonly selected techniques for OTA determination.
In aqueous solution, OTA shows three levels of proton dissociation in the pH range 1.0 - 8.0 (Figure 1) ruled by the characteristic acid dissociation constants ($pK_{a1,2}$). In this work, thanks to the high molar absorptivity and the high intensity of the fluorescence emission of OTA, the pK_a values of this toxin were rigorously established by UV-Vis spectrophotometry and by fluorescence spectroscopy coupled with a stoichiometric and a chemometric approach, respectively.
Under alkaline conditions, the reaction products of OTA were identified by fluorescence spectroscopy. The experimental findings were flanked by density functional theory calculations, which were carried out for the first time to provide the relevant energy barriers and therefore to reveal the most probable reaction products of the degradation mechanism of OTA. At pH 12.5, hydrolyzation of the lactone ring starts in less than one hour, but only after two hours the degradation process leads to fragmentation, which is the condition of toxicity deletion of the toxin. Moreover, the reaction products occurring when the solution is reported back under acidic conditions were investigated by fluorescence spectroscopy. OTA degradation is still reversible if acidic conditions are promptly restored, yielding again a hazard to humans. Irreversible fragmentation can be completed only after a very long time (more than one week) in strongly basic solutions. These conditions are necessary to achieve an effective OTA inactivation.
The aim of this work is to investigate the conditions upon which the different forms of OTA are stably present in aqueous solution, which would allow obtaining aqueous solutions containing mainly one specific form of OTA. This paves the way towards the development of certified Reference materials of OTA and consequently specific toxicological studies of one of the most dangerous and diffused natural toxins of foodstuffs in different protonation and hydrolyzation conditions.

N56

ANALYSIS OF 11 LEGISLATED MYCOTOXINS IN ANIMAL FEED SAMPLES USING A MULTI-ANALYTE IMMUNOAFFINITY COLUMN CLEAN-UP

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Animal feed matrices tend to be complex in their formulation and present challenges in the extraction, isolation and analysis of mycotoxins. The maximum levels permitted in animal feed for each mycotoxin varies according to the type of animal (e.g. bovine, porcine), its age (e.g. calves, lambs) and its intended place in the food chain (e.g. beef cattle, dairy cows). Therefore, an analytical method needs to be flexible across a range of required control levels. Detection and quantitation of mycotoxins by LC-MS/MS is an increasingly popular technique as it allows several analytes with diverse physical-chemistry properties to be measured in a single injection. While LC-MS/MS as a technique provides increased specificity for each analyte, methods may be subject to signal suppression or enhancement due to the presence of sample matrix in the ion source if the sample extraction and clean-up procedure is insufficient. Methods based on simple dilute & shoot extractions, QuEChERS and solid phase extraction columns (SPE) have been reported, however, these may give rise to signal suppression of the analytes as the sample matrix remains in the final solution for injection, leading to a loss of method sensitivity. Complete matrix removal is desirable which is achieved using immunoaffinity clean-up columns. The 11+ Myco MS-PREP® column consists of antibodies raised to the analytes of interest ensuring excellent recovery of multiple mycotoxins and complete matrix removal in a single extraction of the sample. Matrix effects (signal suppression/enhancement) are determined by comparing the response of the mycotoxins spiked onto the "blank" sample matrix taken through the analytical method, with the equivalent levels spiked onto a solvent-based equivalent. Differences of less than 10% indicate that matrix effects are not present, and that correction with matrix-matched calibration standards or stable isotope labelled internal standards is not required. Thus, a single sample extraction, with clean-up using a multi-analyte immunoaffinity column allows for the use of external solvent-based calibration standards and ensures the accuracy and sensitivity of the analytical method.

Keywords: *mycotoxins, lc-msms, multitoxin, immunoaffinity colums*

N57

QUICK AND EASY DETERMINATION OF AFLATOXINS IN FOOD MATRICES

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Aflatoxins are the best-known group of mycotoxins produced as secondary metabolites by fungi, mainly by Aspergillus flavus and Aspergillus parasiticus, but to a smaller extent also by other strains. This origin is also where the name Aspergillus flavus toxin comes from. Since they can easily enter the marketplace and be hazardous to public health it is important to develop effective analytical methods for the identification and quantification of mycotoxins. The big challenge thereby is that already minimal amounts are toxic and need to be detected reliably. Aflatoxins occur in different food- and feedstuffs e.g. cereals, nuts, and milk products. Unfortunately, these substances can persist long after the fungi have been killed and therewith contaminate foods. Most mycotoxins are stable compounds that are also not destroyed during food processing or cooking. Although a large number of Aflatoxins exist, only a limited number is important in analytical practice and several analytical methods for the determination of the recommended limits does exists. Most of them deal with toxic mediums or the cost intensive MS detection. In this case analysis is performed with an HPLC system that contains an UVE photochemical reactor located right after the column and a fluorescence detector. With this reactor the so called photochemical derivatization of the non-visible Aflatoxins to their fluorescent derivates is realized. The described method is compared to the other existing methods and the complete sample preparation for a fast and non-toxic determination of the Aflatoxins with respect to the regulations of the FDA and the European commission will be described.

Keywords: *aflatoxins, mycotoxines, cereals, HPLC, toxines*

N58

ANALYSIS OF CUCURBITACINS IN CUCURBITACEAE

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Cucurbitacins are toxins that are produced by plants in order to protect themselves against herbivores. It is an heterogeneous group of more than 40 substances out of approximately 18 structure groups (cucurbitacin A to T, without M and N)[1], that are commonly glucosides[2]. They are mainly build under stressful conditions, like dryness and heat. They can also be found in some brassicaceae and specific mushrooms.
The climate is changing quickly since the beginning of this century, more and more very dry periods are observed in many parts of Europe, but even in a relatively mild region like Germany enough stress can occur for the plants to start producing cucurbitacins, that already caused health issues and even death[3]. Those effects should not occur using certified seeds, but nevertheless consumer complaints are reaching the retailers. Ornamental gourds or self-grown pumpkins and courgettes from non-commercial seeds can cause severe health issues.
Nevertheless, there are complaints by consumers about commercially purchased vegetables, in most cases they detected a bitter taste in the raw product or in the meal prepared from them -toxins in most cases cannot be reduced through cooking - or even felt bad after eating a meal containing cucurbitaceae. Those health issues can be stomach cramps or even an haemorrhagic gastroenteritis. The official authorities of some federal states (CVUA Stuttgart, LGL Bayern) advise not to eat bitter tasting meals and raw cucurbitaceae, the federal institute for risk assessment (BfR) shared this opinion in its publication „Mitteilung Nr. 027/2015 des BfR vom 4. September 2015"[4].
Fortunately, only very few cases of death were reported until now.

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Keywords: *cucurbitaceae, plant toxins, natural toxins, pumpkins, courgettes*

N59

DETERMINATION OF DON IN WHEAT, WHEAT FLOUR AND ITS PRODUCTS

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Current situation in the region, regarding mycotoxin Deoxynivalenol (DON) in wheat, flour and its products, is causing the higher concentration of mycotoxin DON because of the favorable climate for the development of mold in wheat.
Deoxynivalenol (DON), commonly called vomitoxin, is produced by several molds of the genus *Fusarium*, especially *F. graminearum*, which causes pink scab disease in wheat. It is not possible to completely avoid the presence of DON in wheat. DON is sometimes found in wheat grown under normal weather conditions, however, the fungus thrives in cool, wet conditions. When DON occurs in wheat, the levels are reduced by the processing of wheat into wheat products like flour, but processing does not totally eliminate DON.
This study was performed using an HPLC/PDA Dionex UltiMate 3000, with column Eclipse XDB-C18 5µm, 4.6x250mm, Agilent and sample preparation was performed using immunoaffinity column. The data that we collected were from August and September in 2019year routine samples. The mycotoxin DON was determined in wheat, flour and its products.
We have analyzed 267 samples in the past two months, 133 samples of wheat flour and its products and 134 samples of wheat. 106 samples of wheat, flour and it products did not have DON. 109 samples of wheat had DON in the range from 99µg/kg to 1075µg/kg. 98 samples of wheat flour and its products had DON in range from 101µg/kg to 5474µg/kg. Maximum level for DON according to the Commission Regulation (EC) No 1881/2006 for cereals is 1250 µg/kg, for cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption is 750 µg/kg and for bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals is 500 µg/kg.
According to the results obtained, 18% of wheat samples and 26% of samples of wheat flour and its products, where not in accordance with EU regulation. 56% of all samples that we have analyzed (wheat, flour and its products), were in accordance with the EU regulation.

Keywords: *mycotoxin, liquid chromatography*

Acknowledgement: *SP Laboratory*

N60

HINTS FOR OKADAIC ACID INGESTION MECHANISM OF ACTION TRIGGERING DIARRHOEA

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Harmful algal blooms (HABs) are characterized by the rapid proliferation of toxin-producing microorganisms of the phytoplankton. Phycotoxins produced by the dinoflagellates *Prorocentrum* spp. and *Dinophysis* spp. concentrate in filter-feeding organisms, mainly bivalves (e.g., mussels). The ingestion of these seafood cause diarrhoea, abdominal pain, vomiting and nausea from half an hour to several hours after consumption. This is known as Diarrhoeic Shellfish Poisoning (DSP), entailing the most frequent and spread phycotoxin intoxication in Spain. The lipophilic toxins okadaic acid (OA), dinophysistoxins (DTX) 1 and 2, as well as analogues are the agents responsible of the symptomatology described. To protect public health, monitoring programs for OA group biotoxins have been adopted in many countries for detecting the presence of these compounds in shellfish tissues. European Union Regulation establishes the LC-MS/MS as the
Referencemethod for the detection of lipophilic toxins for the purposes of official controls at any stage of the food chain. Based on the widely and recurrent consumer risk, it is of interest to understand the mechanism through which OA and derivatives induce DSP. Whether PP inhibition is the only responsible for symptomatology has been long discussed. Here we first designed an *in vivo* dose-response approach to determine the dynamics of diarrhoea at different OA oral dosages. Evaluation of parameters such as diarrhoea score and physiological changes (e.g., food consumption) verified a dosage-dependent effect of the phycotoxin. We also measured Peptide YY (PYY) variations in digestive tissues, since this compound is involved in absorption processes. As a result, we detected modification of the intestinal peptide, suggesting a possible disturbance of PYY absorption mediated mechanisms.

Keywords: *diarrhoeic, shellfish poisoning, okadaic acid*

N61

LC-MS/MS ANALYSIS OF EMERGING LIPOPHILIC TOXINS IN COMMERCIAL MUSSELS FROM GALICIA (SPAIN)

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The presence of emerging lipophilic marine toxins from harmful phytoplankton called cyclic imines (CIs) is increasing in European waters. This group comprises spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs) and pteriatoxins (PtTXs) and all can be accumulated in the marine food chain. There are yet no official methods or regulatory limits for this toxin group. Galicia (Spain) is the region with the most intense mussel aquaculture in Europe producing more than 250,000 tons in 2018, thus, further investigations into potential consumer risk from CIs is required. In this work, levels of emerging lipophilic marine toxins in commercial mussels *Mytilus galloprovincialis* from 3 Galician Rías (Ares-Sada, Arousa and Pontevedra) is provided. Toxin identification and quantification were performed by a 1290 Infinity ultra-high-performance liquid chromatography system coupled to an Agilent G6460C Triple Quadrupole mass spectrometer. Analysis showed the presence of 13-desmethyl spirolide C (SPX-13) in the 46% of the samples at levels up to 29 µg/kg. The presence of SPX-13 is frequent in Galicia and other European regions and thus this toxin in often included in the monitoring programs. In addition, analysis showed the presence of pinnatoxin-G (PnTX-G) in the 12% of the samples (levels up to 1µg/kg), confirming their presence in Galician commercial mussels for the first time. Despite data on PnTXs occurrence obtained in this study, it could not be concluded this toxin represent a risk for public heath in Galicia, it is necessary to monitor PnTXs to check future risk derived from mussel's consumption.

Keywords: *LC-MS/MS, emerging marine toxins*

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N62

QUANTIFICATION OF THE TOTAL CONTENT OF OKADAIC ACID GROUP TOXINS BY UPLC-MS/MS: SUITABLE TOXICITY EQUIVALENCY FACTOR

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Diarrhoeic Shellfish Poisoning (DSP) in humans is characterised by the occurrence of diarrhoea, nausea, vomiting and abdominal pain following the ingestion of contaminated bivalves. The toxic agents leading to DSP are lipophilic toxins such as okadaic acid (OA), dinophysistoxin-1 and -2 (DTX-1 and DTX-2) and its derivatives. LC–MS/MS is the technology recognized as the Referencefor the detection of this group of toxins as states the Commission Implementing Regulation (EU) 2019/627. Likewise, EU Regulation (EC) Nº 853/2004 establishes a maximum concentration of 160 µg equivalents OA/kg shellfish meat. To express the results, the use of Toxicity Equivalency Factors (TEFs) is required. This value is the ratio between the toxicity of each analogue and that of reference compound which is OA since different analogues have different toxicity. The European Food Safety Authority (EFSA) has set TEFs values as 1 for OA and DTX-1 and 0.6 for DTX-2. These correlations rely on intraperitoneal (i.p.) toxin administration to mice. However, TEFs values based on *in vivo* oral toxicology studies are different, even though ingestion is the main via of poisoning. In the present work, we quantified DSP toxins in commercial mussels by UPLC-MS/MS. Mussels were extracted and quantified using the Standard Operating Procedure (SOP) for Lipophilic marine biotoxins determination. Then, after calculated the individual content of each toxin/analogue, EFSA TEFs and oral values were used to express results as µg equivalents OA/kg. Therefore, to evaluate the total toxicity of a shellfish sample regardless of the DSP present, TEFs based on oral toxicity are suitable to protect human health.

N63

KOMBISPEC - DEVELOPMENT OF A FULLY AUTOMATED SYSTEM FOR THE EXTRACTION AND DETECTION OF MYCOTOXINS FROM GRAIN USING A MICROFLUIDIC PLATFORM

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The KOMBISPEC project focused on the development of a fast, affordable and reliable device that can be integrated in grain process operations. The goal was a fully automated system for the extraction and simultaneous detection of four mycotoxins that are most relevant for on-site testing in the European grain industry: zearalenone (ZON), deoxynivalenol (DON), aflatoxin B1 (AflaB1), and ochratoxin A (OTA).
To achieve the goal, extraction and assay were to be integrated in a single-use laminar flow chip. For mycotoxin detection fluorescent immunoassays (FIAs) were set up. Antibodies against all four mycotoxins were characterized and rated according to their suitability for the assay. The FIA compounds' concentrations were optimised for an indirect and competitive assay design. The assays' limit of detection and quantification were determined. Moreover incubation times were shortened and the effect on signal intensity was analysed. Hence incubation times could be strikingly decreased. Finally, an assay could be reproduced with same detection limits in the flow on the fluidic chip.
Required miniaturisation of extraction was achieved by using dust instead of kernel samples. Dust sampling allows simplified sampling procedures and direct analysis without grinding. Extraction was tested in integrated cups on the chip. After continuous solvent addition, samples were stirred for 1 min, and subsequently filtered. For the integrated system it was important to test the assay towards its tolerance against changes in pH, salt and extraction solvent composition, first. A strategic design of experiments was generated to optimise mycotoxin extraction from grain dust under the defined conditions. As the four toxins are differently enriched in grain dust, have to be tested at different legal limits, and respective FIAs showed different sensitivities dependent on their antibody quality, the proper dilution factors for the whole system were to be found.
In combination, the system would allow fully automated control of the relevant mycotoxins within 10-15 minutes. Required limit of quantification was easily achieved for the ZON assay. Also for AflaB1 and OTA, as well as DON the suitability of the format was proven. However, the main challenge of the project remains an affordable, sensitive measurement instrument to manage the chip and read the assay on-site.

Keywords: *fluorescence immunoassay, grain, lab-on-the-chip, microfluidic cartridge, mycotoxins*

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N64

USING AUTOMATION TO IMPROVE UNCERTAINTY IN AFLATOXINS ANALYSIS

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The effect of homogeneity when sampling for aflatoxins is well documented and appropriate sampling procedures are set by EC 401/2006. Measurement uncertainty has also an important part to play and it becomes even more critical when the concentration of the analyte is close to the decision limit (MRL). Typically a result above the MRL can be acceptable when the recovery corrected concentration taking account the uncertainty can be less than MRL at a 95% confidence level.
The project was focussed on using an automated approach to improve method uncertainty. The laboratory used the well-established immunoaffinity clean up (EASI-EXTRACT® AFLATOXIN) based approach followed by HPLC and detected by fluorescence after post column electrochemical derivitisation (Kobra Cell®). The aim was to assess the method performance using the same extraction conditions (solvent combination, blending, filtering) but by performing an online clean up immediately prior to the injection on the HPLC. This was validated using IMMUNOPREP®ONLINE AFLATOXIN cartridges on RIDA®CREST automated clean-up. The uncertainty was calculated using a range of naturally contaminated matrices, spiked products at their respective MRL as well as at a higher level. The analysis was performed under controlled conditions to minimise any other contributors to the measurement of uncertainty. The uncertainty was calculated at a 95% level of confidence using a factor K=2.
The products tested were corn flour, peanut, oats, almonds, pistachios, curry spice mix, turmeric, raisin, animal feed, infant formula, baby rusks and paprika. The results presented showed a decrease of measurement uncertainty by a factor of sometimes higher than 50% in all products.

Keywords: *aflatoxin, food, mycotoxins, HPLC, uncertainty*

Acknowledgement: *Liz Manning and Martin Sibum from r-Biopharm, Vainius Skukauskas (PhD student, University of Glasgow)*

N65

AUTOMATED ANALYSIS OF OCHRATOXIN A USING RIDA®CREST IN COMPLEX MATRICES - A COLLABORATIVE APPROACH

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Laboratory automation is a complex approach that requires research, development, and optimisation in order to capitalise on technologies that enable improved processes. The purpose of the project was to adapt the online immunoaffinity clean up to a range of food and feed products routinely tested in the laboratory. Initial trials show several issues with the chromatography in spices and coffee varieties. In collaboration with R-Biopharm we developed and validated a new method meeting the method performance requirements set by EC 401/2006 whilst being suitable for delivering accurate results fast, which is important to a high volume testing laboratory. The method was applied to cereals, wine, dry fruits, herbs, spices, coffees, baby food and feed. Ochratoxin A is isolated using a reusable the IMMUNOPREP® ONLINE Ochratoxin cartridges to be incorporated directly online with an HPLC-FLD (online method). Following extraction and filtration, the sample is placed in an autosampler vial. The diluted sample is then injected into the online immunoaffinity cartridge which contains a specific monoclonal antibody. After washing and elution, the sample is quantitatively analysed for the mycotoxin of interest. The instrument is capable of processing two cartridges simultaneously. The online clean up approached changes in the analysis procedure helped with improving uncertainty and increasing throughput. The cartridges stability study showed that the RDS for 15 injections was less than 3% and ruggedness study RSD was less than 20%. The new robust and automated online clean up increased, productivity and reduced lab process time by eliminating steps from the extraction. The average recoveries for online method were compared with the HPLC-FLD and manual clean up with the immuno-affinity column (offline method) and all are within the range of 80-100%. The accuracy for online method was better than for offline and RSD was less than 15%.
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 wide range of commodities.

NANOPARTICLES

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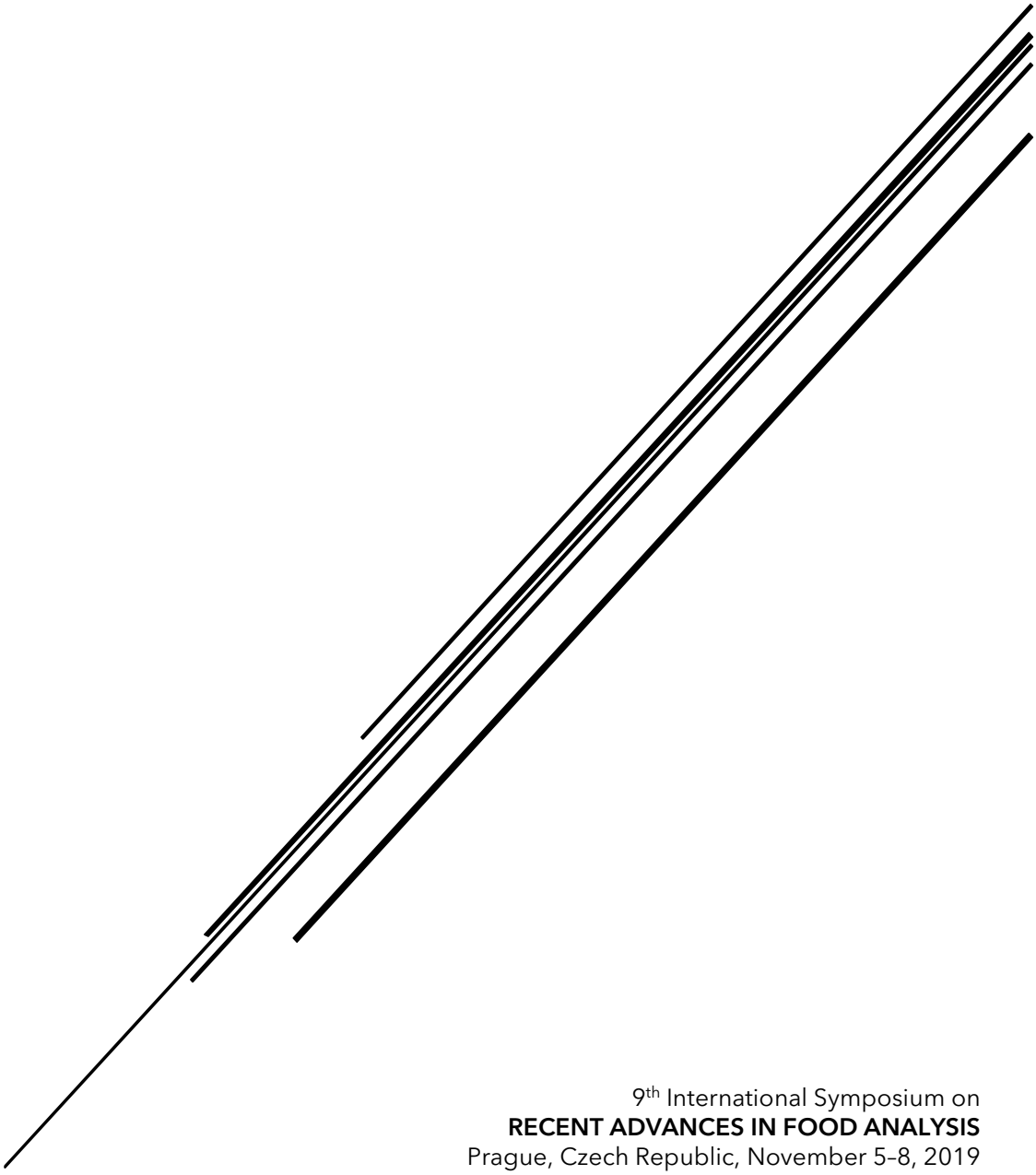

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O1

MESOPOROUS SILICA MATERIALS AS POTENTIAL ANTIOXIDANTS' CARRIERS

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Mesoporous silicas are modern types of materials, which become popular due to their appropriate physicochemical and biological properties. They possess high surface area, high pore volume, easily modifiable surface, physical and thermal stability. Moreover, Food and Drug Administration classified silica as "Generally Recognized as Safe" [1]. This creates the possibility of using them as carriers and adsorbents of many active substances, including antioxidants. Polyphenols have an important role in prevention of diseases of affluence. The use of suitable antioxidant carriers may enhance their antioxidative properties as well as to increase their bioavailability.
The aim of this work was to verify whether mesoporous silica materials can be used as adsorbents of compounds, which exhibit antioxidant properties.
The selected polyphenols, i.e. gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid and rutin, were adsorbed on, previously synthesized, mesoporous silica materials, which were the MCM-41 and SBA-15 types. Efficiency of adsorption process was optimized using as an example gallic acid. Firstly, the adsorption properties for MCM-41 and SBA-15 materials and for MCM-41 and SBA-15 materials modified with amino groups were checked. Afterwards, time of adsorption process assisted by shaking was optimized. Eventually, all the above mentioned antioxidants were adsorbed on the all mesoporous silica materials. Assessment of adsorption process efficiency was performed using two methods: HPLC-Corona CAD and DPPH. The second one is a popular spectrophotometric method designed to evaluate antioxidant properties. All the methods applied were previously optimized and validated. The obtained parameters for both methods, especially recovery (86,3-113%) and precision (0,22-6,37%) were satisfying.
Based on the results it was confirmed that modification of mesoporous silica surface by amino groups increases the adsorption efficiency of all polyphenols. All the polyphenols were adsorbed on MCM-41-NH_2 and SBA-15-NH_2, what was confirmed by FTIR analysis, DPPH and HPLC methods. Rutin was characterized by the highest adsorption at SBA-15-NH_2 (95,3%). The reasonable adsorption for this type of material was also observed for gallic acid (62,8%). On the other hand, 3,4-dihydroxybenzoic acid and 4-hydroxybenzoic acid exhibited lower adsorption comparing to previously mentioned compounds. These two antioxidants adsorbed slightly better on MCM-41-NH_2 type.
The performed research proves the possibility of successful adsorption of selected polyphenols on mesoporous silica materials. It gives hope for using those types of silica materials as a modern carriers of antioxidants. Moreover, silica materials can possibly be applied to improve and enrich extraction process.

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Keywords: *mesoporous silica, polyphenols, DPPH, HPLC*

O2

PHYSICOCHEMICAL CHARACTERISATION OF THE E171 FOOD ADDITIVE

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The application of E171 (titanium dioxide) as a food additive has been an issue of debate in the European Union. A detailed physicochemical characterization of the E171 particles can objectify the discussions and is essential in the context of risk analysis. This work focuses on the physicochemical characterization of 15 pristine E171 materials by transmission electron microscopy (TEM) and single particle inductively coupled plasma mass spectroscopy (sp-ICP-MS) following CEN/TC 352 guidelines. The E171 samples were purchased on the Belgian market or were obtained from European producers.

To measure the minimal external dimension of the constituent particles of E171, sample preparation protocols influencing particle dispersion (pH, probe sonication and centrifugation) were tested and optimized. In optimized conditions, representative TEM micrographs could be recorded, and it was demonstrated that all examined E171 food additives contained a significant amount of nanoparticles. The large majority of constituent particles, confirmed to be TiO_2 by energy dispersive X-ray spectroscopy (EDX), were reliably detected and measured using the ParticleSizer software. In the most dispersed state, the particle size measurements by TEM and sp-ICP-MS agreed well. The measurement uncertainty budgets of particle sizing by TEM and sp-ICP-MS are in the order of 10% and 16 % (Ucx, k=2), respectively, based on validation studies of a series of representative test materials. Electron diffraction demonstrated that both anatase and rutile TiO_2 particles were found in pristine E171. Eleven E171 materials consisted of anatase. Three materials consisted of smaller rutile TiO_2 particles (20-40 nm) coated on mica. One material contained a mixture of anatase and rutile particles.

In future research, the methodology will be implemented in a systematic and larger scale study of E171 food additives and food items containing E171, available on the market.

Keywords: *E171, titanium dioxide, transmission electron microscopy, particle size distribution, single particle ICP-MS*

Acknowledgement: *This work was performed within the framework of the grants "Implementation and validation of an analytical methodology to assess engineered nanomaterials in EC approved food additives (RF16/6306 Nanofood@)" funded by the Belgian Federal Public Service of Health, Food Chain Safety and Environment, and "Physicochemical characterization and exposure analysis of nanomaterials in food additives in the context of risk assessment (GP/EFSA/AFSCO/2017/06 EFSA-Nano)" funded by EFSA*

O3

DEVELOPMENT OF A NOVEL IMMUNOASSAY FOR DIFFERENTIATION OF MYCOBACTERIUM BOVIS AND MYCOBACTERIUM TUBERCULOSIS

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Tuberculosis (TB) is an infectious disease affecting one third of the world's population and is the leading cause of death worldwide from a single infective agent. *Mycobacterium tuberculosis* (MTB) is the main causative organism in humans, whilst *Mycobacterium bovis* is the primary cause of TB in animals. *M. bovis* ravages agricultural communities reliant on livestock production by; (i) creating barriers to trade of animal products; (ii) reducing meat and milk production, and; (iii) acts as a reservoir of infection for zoonotic TB (ZTB) often spread via consumption of contaminated, unpasteurized dairy products.

Although ZTB incidence is low, approximately 1.5-3% of all TB cases, it is likely to be underestimated as both organisms cause similar symptoms, are >99.95% similar at the genomic level, and can only be speciated using molecular techniques which are rarely undertaken in high TB burden countries. Consequently, human TB infections are routinely considered to be caused by MTB. Speciation is essential as *M. bovis* is intrinsically resistant to pyrazinamide, a first line anti-TB drug, thus misdiagnosis of ZTB as HTB may result in treatment failure and contribute to development of antibiotic resistance.

To date, no rapid immunological based diagnostic assay can differentiate at the species level. Development of immunological assays requires species specific binders. Here we present the development of novel binders for highly pathogenic strains of *M. tuberculosis* H37Rv and *M. bovis* AF2122/97. Pathogens were inactivated by gamma irradiation to preserve the structure of their outer surface. Whole cells and ethanol extracted antigens were used as targets for binder production. In addition, key cell surface and secreted antigens identified from the literature were synthesized as targets for binders. A panel of binders, including polyclonal and monoclonal antibodies, and phage display derived peptides, were produced and evaluated by ELISA. Selected binders, when combined in precise combinations, could differentiate between *M. bovis* and *M. tuberculosis*, resulting in species-specific diagnosis of TB in humans. The ELISA data indicates that both *M. bovis* and MTB could be detected at approximate concentrations of 1 x 10^4 CFU/ mL and there was no cross-reactivity with other Mycobacterium spp. tested. A further aim of this research is to translate the ELISA based assay into a low-cost paper-based format for use in high burden, resource poor countries to enable accurate diagnosis and direct treatment of TB disease in regions of the world where TB is most prevalent. Combining the binders with functionalized nanomaterials such as gold nanoparticles (AuNPs) will produce bioconjugates which can be used as an alternative to conventional biochemical sensing due to their unique optical and chemical properties. The result will be a paper based diagnostic device capable of more sensitive, species-specific diagnosis of TB disease and point-of-care testing.

Keywords: *mycobacterium bovis, mycobacterium tuberculosis, immunoassay, nanoparticles*

Acknowledgement: *This project has received funding from the Department for the Economy (DfE)*

O4

BIOGENIC GOLD NANOPARTICLES (AU NPS) AS ACTIVE PEROXIDASE MIMICKING NANOZYMES FOR BIOSENSING APPLICATIONS

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Over the past few years, food safety has become a hot topic of research due to the rapid rise in foodborne diseases. Consumption of food contaminated with pathogens or toxins has caused numerous fatal health incidents worldwide. Biosensing platforms capable of early detection of food contaminants with high specificity and sensitivity are urgently required. Conventional immuno-based detection techniques, such as enzyme-linked immunosorbent assay (ELISA), utilize natural peroxidase enzymes (horseradish peroxidase: HRP), and although they have merit they also have several drawbacks such as susceptibility for inhibition of its peroxidase activity and high production cost. Artificial nanomaterials that mimic peroxidase activity have attracted interest as an alternative as they can overcome the inherent drawbacks of the natural enzymes. Advancements in nanotechnology have provided a potential solution in the search for a suitable peroxidase mimicking artificial enzyme. The unique surface morphology of gold particles (Au NPs) combined with their chemical characteristics has led to the investigation of their high catalytic efficiency as a peroxidase mimicking enzyme, so-called nanozymes. In the present study, we have biologically synthesized gold nanozymes with controlled morphology and surface chemistry. They possess ultra-active intrinsic peroxidase-like activity and can catalyse the oxidation of 3,3′,5,5′ - tetramethylbenzidine (TMB) in presence of H_2O_2. Synthesis of Au NPs using *Prunus nepalensis* fruit extract was performed after standard biochemical characterizations of fruit extract such as total phenolic content, reducing power assay. Characterizations of the biogenic Au NPs were performed using UV-visible spectrophotometer, X-Ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). Analysis using Michaelis–Menten kinetics determined superior affinity of the biogenic Au NPs towards both the chromogen (TMB) and the substrate (H_2O_2) when compared with the natural HRP enzyme. The Michaelis–Menten constant (K_m) for biogenic Au NPs and for HRP was found to be 3.23×10^{-2} mM and $6.083.23\times10^{-2}$ mM, respectively at the same nanozyme/enzyme concentration of 100 pM and under 6% H_2O_2. To investigate its applicability for biosensing analytics, the biogenic Au NPs surface was modified using a direct coupling conjugation method and a monoclonal antibody specific for *Mycobacterium bovis* attached. The aim was to develop an immunoassay for detection of *M. bovis,* a zoonotic pathogen transmitted via contaminated milk and meat products. The biogenic AuNP conjugate showed ultra-active peroxidase activity (only 1.71% suppression was observed), and the immunological activity of the antibody was maintained. Our preliminary results are promising and indicate the potential for development of a cost-effective biosensing platform with high sensitivity and specificity for the detection of *M. bovis* using antibody conjugated Au nanozymes.

O5

PREPARATION AND PROPERTIES OF TIME-TEMPERATURE INDICATOR USING GOLD NANO-PARTICLES

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Gold nanoparticles (AuNPs) were synthesized by using gelatin as both a reducing agent and a stabilizer. The mass ratio of gelatin to $HAuCl_4 \cdot 4H_2O$ (m_G/m_H) on the effect of TTI solution state and AuNPs color signal was examined. When the mass ratio was small, the TTI solution precipitated, and the endpoint absorbance was linearly related to the temperature. The stabilizer effect hindering the agglomeration of the nanoparticles enhanced as the mass ratio increased, which made the solution free of precipitation and the color change endpoint independent of temperature. The kinetic properties and temperature dependence of TTIs were studied, and Arrhenius activation energy (E_A) was obtained. The results suggest that these TTIs can be mainly applied to indicate the quality of products deteriorated by fat oxidation such as meat and fat. Also, the same TTI has different E_A and color endpoint in the liquid and gel states, thus, further research on gel-type TTI has important application value in food packaging.

Acknowledgement: *The authors are grateful to the National Key R&D Program of China (no. 2018YFC1603300) for financial support.*

O6

QUANTIFICATION OF DECABROMODIPHENYL ETHER IN MICROPLASTICS USING DIRECT INSERT PROBE COUPLED WITH MAGNETIC SECTOR HIGH RESOLUTION MASS SPECTROMETER IN FULL SCAN MODE

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POPs and other contaminants found in microplastic ending up in the food chain represent a rising concern. Here we present a method without the need for sample preparation for the quantification of Decabromodiphenyl ether in microplastic. Recommended methods for monitoring compliance with RoHS limits for PBDEs can be divided into two main approaches: screening and high-accuracy chemical analysis. Screening is usually performed via solid sampling techniques that can only quantify elemental Br as a proxy for the total BFR content. More conventional techniques are recommended for high- accuracy determination of BFRs like PBDEs. Specifically, RoHS requires GC-MS analysis to determine the BFR content in polymers. These traditional techniques can have a number of analytical drawbacks aside from being time consuming and expensive. For these reasons a method that combines the convenience of a solid sampling technique with compound specific quantification is highly desirable. Direct Insertion Probe (DIP) is a solid sampling inlet system that introduces the analytes directly into the ion source of a mass spectrometer (in this case a Thermo Scientific™ DFS™ Magnetic Sector High Resolution Mass Spectrometer) by thermal desorption. Having a strong matrix effect, this technique is used for qualitative evaluation, because it is often impractical or impossible to produce reasonably representative solid
Referencematerials at different concentrations in order to build a calibration curve. In this study, we use *ad-hoc* prepared Acrylonitrile Butadiene Styrene (ABS) solid
Referencematerials (RMs) containing different concentrations of BDE209 to develop a 5-point calibra- tion curve that showed linearity (R^2> 0.999) over a concentration range of 0.1 - 2% w/w BDE209. Relative standard deviation between triplicate determinations of BDE209 ranged from 0.32% to 0.42%. The limit of detection (LOD) obtained for BDE209 was 0.112 mg/kg, 4 orders of magnitude lower than the EU's maximum allowed concentration (MAC) in plastic. To our knowledge, this is the first method for compound specific quantification of BDE209 that does not require any sample preparation, reducing the analysis time from roughly 14 hours to 12 minutes with comparable quality of results.

O7

DEVELOPMENT OF SENSOR-BASED DIAGNOSTICS FOR ANIMAL HEALTH APPLICATIONS

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The sensitivity of common diagnostic techniques such as enzyme-linked immunosorbent assays (ELISAs) are often limited by the properties of their chosen reporter enzyme-antibody conjugate. Gold nanoparticles (AuNPs) offer the ability to provide for signal enhancement potentially improving the sensitivity of traditional diagnostic techniques in a variety of ways. Their inherent peroxidase-like activity enables them to act as enzyme mimics catalysing the oxidation of commonly used substrates such as 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of hydrogen peroxide. This nanozyme activity offers substantial benefits over other reporter enzymes (e.g. horseradish peroxidase (HRP) including ease of synthesis and functionalization and also an increased resilience to degradation and denaturation remaining active in more extreme conditions of temperature, pH or high levels of hydrogen peroxide. Gold nanozymes have therefore become increasingly attractive as alternatives to natural protein enzymes in a wide range of fields including diagnostic applications. Such nanoparticles can be used also as carriers of enzyme-antibody reporter conjugates to provide an amplification of signal in many diagnostic methods. This study reports the demonstration of using gold nanoparticles as both an enzyme mimic and a carrier for antibody-enzyme conjugates. Gold nanoparticles (mean diameter 17 nm) were synthesised using the Turkevich method synthesised and functionalised passively (30 mins) using anti-bovine IgG (final concentration of 0.3 mg/ml) as an alternative to standard HRP-conjugated anti-IgG commonly used as a reporter enzyme in ELISAs. An AuNP-HRP-antibody conjugate was also synthesised using a final antibody concentration of 0.0003 mg/ml. The utility of prepared nanoparticle-antibody conjugates were tested using an in-house developed bovine IgG direct ELISA as a model assay system and performance compared to HRP-antibody conjugates. Results suggest that while nanozymes may not rival the signal levels produced by protein reporter enzymes under standard ELISA conditions, when used as carriers of HRP-antibody conjugates they can provide for reporter signal amplification and increased ELISA sensitivity.

Keywords: *nanozymes*

O8

BIOGENIC GOLD NANOPARTICLES (AU NPS) AS ACTIVE PEROXIDASE MIMICKING NANOZYMES FOR BIOSENSING APPLICATIONS

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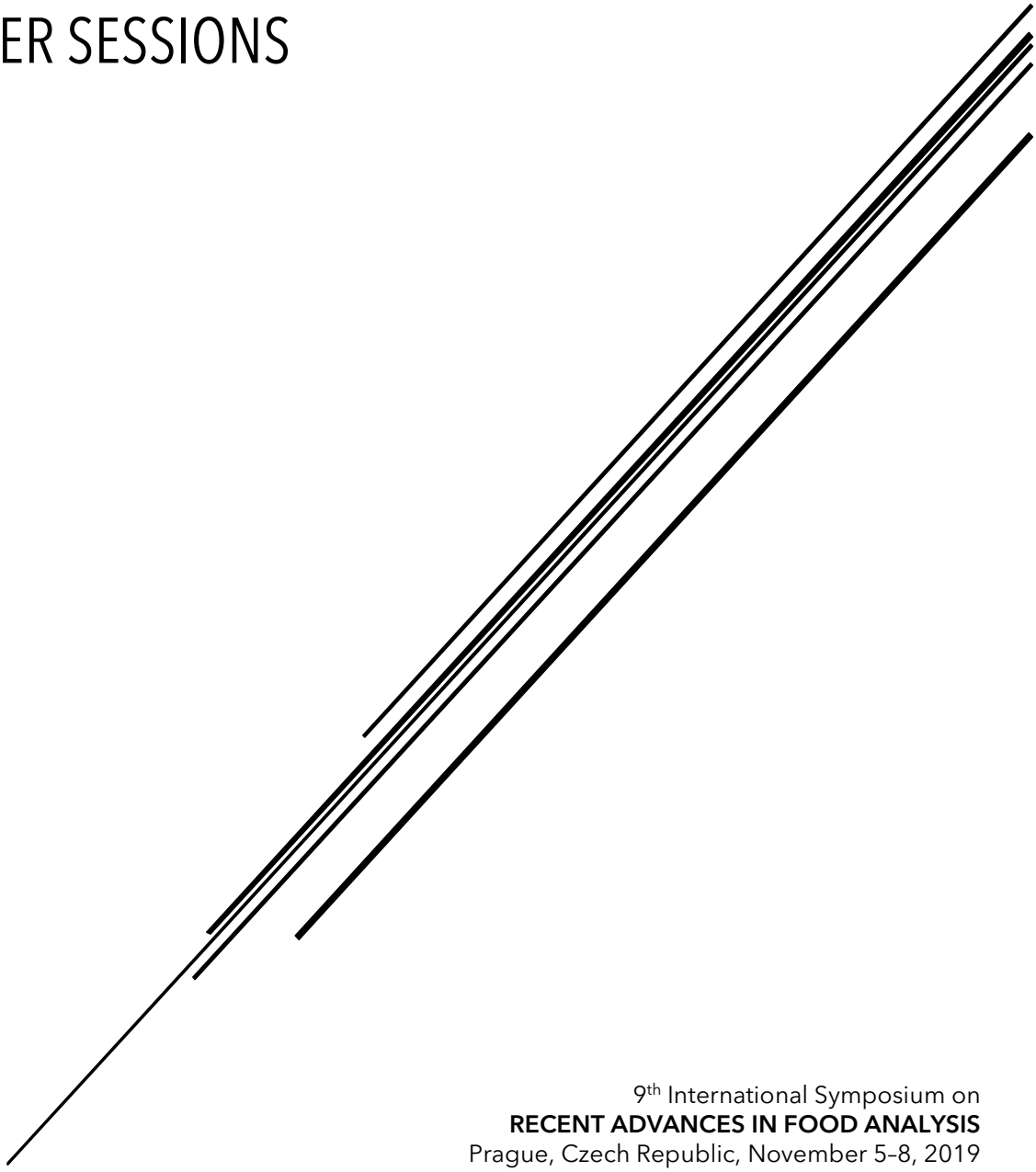
Over the past few years, food safety has become a hot topic of research due to the rapid rise in foodborne diseases. Consumption of food contaminated with pathogens or toxins has caused numerous fatal health incidents worldwide. Biosensing platforms capable of early detection of food contaminants with high specificity and sensitivity are urgently required. Conventional immuno-based detection techniques, such as enzyme-linked immunosorbent assay (ELISA), utilize natural peroxidase enzymes (horseradish peroxidase: HRP), and although they have merit they also have several drawbacks such as susceptibility for inhibition of its peroxidase activity and high production cost. Artificial nanomaterials that mimic peroxidase activity have attracted interest as an alternative as they can overcome the inherent drawbacks of the natural enzymes. Advancements in nanotechnology have provided a potential solution in the search for a suitable peroxidase mimicking artificial enzyme. The unique surface morphology of gold particles (Au NPs) combined with their chemical characteristics has led to the investigation of their high catalytic efficiency as a peroxidase mimicking enzyme, so-called nanozymes. In the present study, we have biologically synthesized gold nanozymes with controlled morphology and surface chemistry. They possess ultra-active intrinsic peroxidase-like activity and can catalyse the oxidation of 3,3′,5,5′ - tetramethylbenzidine (TMB) in presence of H_2O_2. Synthesis of Au NPs using *Prunus nepalensis* fruit extract was performed after standard biochemical characterizations of fruit extract such as total phenolic content, reducing power assay. Characterizations of the biogenic Au NPs were performed using UV-visible spectrophotometer, X-Ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). Analysis using Michaelis–Menten kinetics determined superior affinity of the biogenic Au NPs towards both the chromogen (TMB) and the substrate (H_2O_2) when compared with the natural HRP enzyme. The Michaelis–Menten constant (K_m) for biogenic Au NPs and for HRP was found to be 3.23×10^{-2} mM and $6.083.23\times10^{-2}$ mM, respectively at the same nanozyme/enzyme concentration of 100 pM and under 6% H_2O_2. To investigate its applicability for biosensing analytics, the biogenic Au NPs surface was modified using a direct coupling conjugation method and a monoclonal antibody specific for *Mycobacterium bovis* attached. The aim was to develop an immunoassay for detection of *M. bovis,* a zoonotic pathogen transmitted via contaminated milk and meat products. The biogenic AuNP conjugate showed ultra-active peroxidase activity (only 1.71% suppression was observed), and the immunological activity of the antibody was maintained. Our preliminary results are promising and indicate the potential for development of a cost-effective biosensing platform with high sensitivity and specificity for the detection of *M. bovis* using antibody conjugated Au nanozymes.

NOVEL FOODS & SUPPLEMENTS

POSTER SESSIONS



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P1

THE EFFECT OF MICROWAVE HEATING IN THE STABILIZATION OF RICE BRAN

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Rice bran is a by-product of rice milling industry mainly used for animal feed. This product has the potential to become a major supplementary source of functional nutrients for high-value applications. However, there are serious limitations for its use because it is easily oxidized, releasing substances that are unsuitable for consumption. Endogenous lipases catalyse the hydrolysis of rice bran oil to free fatty acids causing lipid oxidation. The objective of this work is to determine if the microwave stabilization can reduce the oxidation process of the rice bran. In this study the rice bran was submitted to microwave heating (3 treatments of 2 min, at 550W). Two samples of rice bran were used in the experiment: stabilized rice bran (SRB) and non-stabilized rice bran (NSRB), used as control. Moisture content, total acidity and water absorption capacity (WAC) were determined 9 times along the 16 weeks of the experiment in triplicate. Peroxide values, fatty acids profile, and thiobarbituric acid reactive substances (TBARS) were also determined in the first and last week. Scanning electron microscope (SEM) images of the rice bran, before and after stabilization were also obtained. ANOVA with *post-hoc* Tukey test was used to investigate the significance of observed differences. Principal component analysis (PCA) based on correlations was also carried out to find patterns in the dataset. Results showed significative statistical differences ($p<0.05$) between the two samples: the acidity index was higher in the NSRB, and a sudden increase of this value in the first 4 weeks was noticeable. Peroxide values were also higher in NSRB, indicating a fast oxidation. WAC was lower in NSRB, as well as moisture content. Differences in the NSRB and SRB grain structure were visible in the SEM images: the SRB maintained the cell wall integrity, compared to control sample, even at the end of the experiment. It can be concluded that microwave stabilization of rice bran is effective in lowering the acidity index and peroxide values, therefore preventing the rapid oxidation and the formation of undesired substances. The increase of water absorption capacity, showed by the stabilized rice bran, is also a promising result for further applications in meat, bakery, and beverage industries.

Keywords: *microwave, rice bran, lipase*

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P2

GRAPE WATER: RECLAIM AND NUTRACEUTICAL POTENTIAL OF A BY-PRODUCT FROM THE INDUSTRIAL CRYOCENTRATION OF GRAPE (VITIS VINIFERA L.) MUST

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The term "grape water" refers to a by-product from the cryoconcentration of grape (*Vitis vinifera* L.) must, conventionally treated as wastewater. Aim of this study was to evaluate the nutraceutical potential of pasteurized waters, reclaimed from the cryoconcentration of organic Grillo and Moscato grapes musts. Aside from pH, physicochemical parameters, such as conductivity, hardness and fixed point, were in agreement with Italian regulation for drinking waters. The elemental analysis was carried out by means of ICP-MS: both waters showed interesting levels of F^- (3.02-8.02 mg L^{-1}) and SO_4^- (52.85-49.34 mg L^{-1}), a very low Na content, and valuable Mg (5.54-7.78 mg L^{-1}), K (47.12-59.87 mg L^{-1}), Fe (219.09-205.32 µg L^{-1}), and Zn (189.65-127.30 µg L^{-1}) amounts. Through GC-FID, the free fatty acid distribution was determined, with palmitic, oleic and linoleic acids as major components, higher in Moscato than in Grillo samples (27.71% vs. 21.90%, 64.42% vs. 58.22%, and 5.42% vs. 6.07%, respectively). A rich aromatic fingerprint was revealed by means of HS-SPME-GC-(MS/FID) in both the two types of water samples; linalool and a-terpineol were predominant in Moscato water, whereas hexanol, cis-rose oxide, and linalyl acetate in Grillo water. A moderate antioxidant activity, attributed to the presence of specific elements and of polyphenols (total amount <35 mg GAE L^{-1}), was observed in all samples by means of the DPPH free radical-scavenging assay. Statistical analysis (two-tailed Student's t-test) was applied to the whole set of analyses.

Keywords: *winery waste, grape, water, must, cryoconcentration*

Acknowledgement: *Research Project "Valorization of by-products from the wine industry (nr. 1836/5.S), framework PO FESR Sicilia 2014/2020 - Azione 1.1.2*

P3

DETERMINATION OF CANNABIDIOL AND ADDITIONAL CANNABINOID CONTENT IN HEMP TEA

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Cannabis contains more than 500 unique compounds, including over 80 chemical alkaloids known as cannabinoids. Numerous health benefits have been reported that are attributed to their pharmacological characteristics, which allow for use as medical treatment. They can affect physiological processes, such as inflammation, pain perception and seizures, which is a reason for the growing interest in "Medical Cannabis" [1, 2]. While use of cannabis for medicinal purposes is still subject to a lot of debate in Europe, hemp products containing < 0.3 % of the psychoactive compound d9-tetrahydrocannabinol (THC) have always been legal in most countries. With the growing interest in the cannabis plant, the market for cannabidiol (CBD) containing food and cosmetics products is also increasing. Quantification of cannabinoids is essential for the accurate labelling of hemp products, for quality control, as well as to establish legality with regards to the THC content. In this work, High Performance Liquid Chromatography (HPLC) is the method of choice for analysis of cannabinoid content in different CBD rich hemp tea samples. The HPLC-UV method used provides good linearity, low limit of detection, as well as high precision of retention time and peak area for the cannabinoids under investigation.

[1] Perry G. Fine, Mark J. Rosenfeld, Rambam Maimonides Medical Journal, October 2013, Volume 4, Issue 4.
[2] Klein TW, Newton CA, Adv Exp Med Biol. 2007, 601:395-413.

P4

ANALYSIS OF HEAVY METAL CONTAMINANTS IN HEMP AND CANNABIS FLOWER USING ICP-MASS SPECTROMETRY

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The recent changes in legislation in the European Community and other European countries has opened up the cultivation and sale of cannabis and related products in a variety of states and municipalities. With the availability of hemp and cannabis as commercial products such as tea, oil, cannabis drinks and more comes the need for analysis and regulation of potency, pesticides, biological contaminants, and heavy metals, among others. The concentration of heavy metals in plants that are intended for consumption is of concern due to the potentially hazardous effects of these metals related to their toxicity. As they grow, plants can bioaccumulate metals in their tissues that originate from the soil and water in which they are grown. These metals may originate naturally in soils and water as a result of the mineral content of the soil or source of the water, or they may be artificially introduced in the form of fertilizers, pesticides, herbicides, and fungicides commonly applied to increase crop yields. Some of these metals contained in plants have beneficial metabolic uses, such as iron in beans and leafy greens, whereas others, such as lead, can have deleterious effects including toxicity and carcinogenicity. Here, we explore and discuss the applicability of the Shimadzu ICP- mass spectrometer ICPMS-2030 to the detection of the "Big Four" heavy metals (i.e., As, Cd, Hg, and Pb) in digested hemp and cannabis flower samples. In the experimental work Referencesamples were determined using the inductively coupled plasma mass spectrometer ICPMS-2030: Hemp flower tea and two types of cigarette tobaccos [1] [2]. Sample preparation was done in a closed microwave system using about 0.5 g of hemp flower or tobacco in nitric acid. Calibration curves have been prepared immediately prior to analysis to ensure the most accurate quantitation. The Calibration curves for As, Cd, Hg, and Pb show good correlation coefficients r of 0.9998 or better. The recovery rates of the
Referencesamples are within 90 to 110 % and demonstrate the ability of the Shimadzu ICPMS-2030 as a simple, rapid, sensitive and accurate tool for handling acid-digested hemp and tobacco leaves.

[1] Polish Certified Reference Material for Multielement Trace Analysis, oriental Basma Tobacco Leaves (INCT-OBTL-5), Institut Chemi I Techniki Jadrowej. Warszawa Poland.
[2] Polish Certified
ReferenceMaterial for Multielement Trace Analysis, oriental Basma Tobacco Leaves (INCT-PVTL-6), Institut Chemi I Techniki Jadrowej. Warszawa Poland.
[3] Smokeless Tobacco Sub-Group, Proficiency Test Report, The Determination of As, Be, Cd, Cr, Co, Ni, Pb and Se in Reference Material, Sep. 2018, K. Wagner

Keywords: *ICP-MS heavy metals hemp tobacco cannabis*

P5

NEW FRONTIERS OF FOOD MARKET: DART-HRMS TO CHARACTERIZE EDIBLE INSECTS

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By 2050 nine billion people are estimated to live on our planet, so the supply with high quality products is a global challenge [1]. The consumption of insects could become a feasible solution due to their highly nutritious and healthy fat, protein, vitamin, fiber and mineral content, but attention should be paid to their safety and Authenticity [2], [3]. The use of spectrometry in species identification is well known and has been tested also with edible insects [4] In this work we have explored the performances of DART-HRMS (Direct Analysis in Real Time – High Resolution Mass Spectrometry) to differentiate insects' powder of known origin and composition (grasshopper, cricket, mealworm and caterpillars). In particular, analyses were performed to evaluate the characteristic fingerprinting profile of the insects samples and to identify chemical markers able to differentiate insects. All the collected data were used to build a statistical model able to discriminate samples according to composition (species) and to classify insect samples (both single species and multispecies) of unknown origins to identify adulterated powders.
The analyses were performed with a high resolution mass spectrometer with a DART ion source and an Orbitrap mass analyzer. The Samples (250 mg) were extracted in 5ml ethylacetate (lipophilic extract) and 5ml methanol-water mixture (hydrophilic extract). All the mixtures underwent ultrasound bath at room temperature. Finally, 5 µl of the solutions was introduced to instrument using Dip-it tips (IonSense, Saugus, MA, USA). Mass spectra between 75 and 1125 m/z were recorded at a resolution of 70000 FHWD. Finally of 12 spectra samples for each sample were acquired (3 replicated for each combination solvent/ioniziation).
Target analyses allowed the definition of the lipidic and aminoacidic profiles of the samples, whereas non targeted analysis, followed by the chemometric evaluation, achieved promising results in terms of discrimination between different species of insects. Results suggest the possibility to use DART-HRMS as rapid techniques for authenticity evaluation at least as a first screening technique to prevent food frauds.

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[3] EFSA, "Risk profile related to production and consumption of insects as food and feed EFSA Scientific Committee," *EFSA J.*, vol. 13, no. October, 2015.
[4] S. Ulrich *et al.*, "Direct identification of edible insects by MALDI-TOF mass spectrometry," 2017.

Keywords: *edible insect, DART-HRMS, chemometric*

P6

THE LC-UV ANALYSIS OF 16 CANNABINOIDS OF INTEREST IN COMMERCIALLY AVAILABLE CBD OILS

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More than 100 cannabinoids have been isolated from cannabis in addition to the five most commonly tested: THC, THCA, CBD, CBDA, and CBN. While methods have been published that show the separation of these major cannabinoids, many do not take into account the possibility of interference from other cannabinoids that may be present. This is most problematic in concentrates where minor cannabinoids can be enriched to detectable levels that were not observed in the flower. Additionally, some terpenes have been shown to absorb UV light at 228 nm, the wavelength cannabinoids are typically detected, which can result in an additional source of interference. In this study, the LC-UV separation of 16 cannabinoids of interest was performed while monitoring for the potential impact from minor cannabinoids and terpenes on reported potency values. The method is applied to commercially available CBD oils that have recently become suspect due to inaccurate label claims.

P7

SCREENING OF PHYTOCANNABINOIDS AND OTHER BIOACTIVE METABOLITES IN CANNABIS BASED MEDICINAL PRODUCTS AND FOOD SUPPLEMENTS

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Cannabis plants are a key source of unique biologically active compounds - phytocannabinoids. Apart of psychotropic activity of most known phytocannabinoid, delta-9-tetrahydrokanabinol (delta-9-THC), these substances may exhibit a number of positive effects (analgesic, antioxidant, neuroprotective) on a human organism. However, worth to emphasize, that the overall biological effect of cannabis based products is associated by the presence of many other substances (entourage effect), such as terpenes, terpenoids, flavonoids, or phenolic compounds; hundreds of them substances have been identified so far.
Using in-house constructed spectral library (accurate values of possible ions and their isotopic patterns calculated on compound elemental formulas), targeted screening of 227 minor phytocannabinoids and 151 other bioactive substances was performed on 52 samples, including extracts from various cultivars of technical and medicinal cannabis, cannabis-based food supplements, medicinal, and cosmetic products. The instrumental technique of ultra-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS) was in the first step optimized to improve the chromatographic separation of analytes and then applied to the sample analysis.
In analyzed samples, in total 440 phytocannabinoids and 186 other biologically active substances were detected and tentatively identified. While the targeted metabolites profiles in CBD oils made from hemp extract were very rich, only limited number of screened compounds was detected in products containing hemp seeds (hemp oils, hemp oil-containing cosmetics, hemp protein). Hemp teas showed a great similarity with technical cultivars of cannabis, while profiles of medicinal cultivars were richer and more intense compared to technical ones.
Based on the comparison of experimental and theoretical fragmentation HRMS spectra obtained in the analysis of cannabis cultivars a spectral database was created as a tool for confirmation of substances. So far, 66 fragmentation spectra of phytocannabinoids and 12 fragmentation spectra of other biologically active substances have been obtained.

Keywords: *phytocannabinoids, food supplements, UHPLC-HRMS/MS, targeted screening, spectral database*

Acknowledgement: *Financial support from specific university research (MSMT No 21-SVV/2019) This work was supported by METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities. Part of funding was also provided by BIOCIRTECH (TN01000048) project.*

P8

FATS AND OILS AS A NOVEL FOOD

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Novel food is food or a food ingredient that was not significantly used in human nutrition in the European Union (EU) before May 1997. In Republic of Serbia, Regulation, which defines what is novel food, the conditions for it is marketing, declaration and control, was published in 2018. Within the list of novel foods there are many oils and/or extracts, whose characteristics and quality standards are also defined in this Regulation, and they are obtained in various ways from plants, insects, microorganisms,..

National Regulation about novel food defines identification parameters as well as important parameters of quality, whitch is providing conditions for safer and easier control during production,marketing and application of listed food. The specific production method of each of these oils, as well as the specific nature of the raw material, causes each of them to have their own maximum values of quality paremeters,

Which differ from the maximum values defined in the national regulatives of quality and other requirements for edible vegetable oils and fats, margarine and other fat spreads, mayonnaise and related products ("Sl.list SCG" br.23/2006). Most of these foods have defined uses in terms of the maximum amount, indicating that their use in food preparation must be limited and controlled. This Regulation defines the areas of application and quantitatively limits the intake of certain foods. This way, it contributes to the prevention of possible negative consequences of inadequate application of certain food or raw materials, as well as their excessive consumption, with the aim of consumers protection.

Keywords: *fats, oils, novel food*

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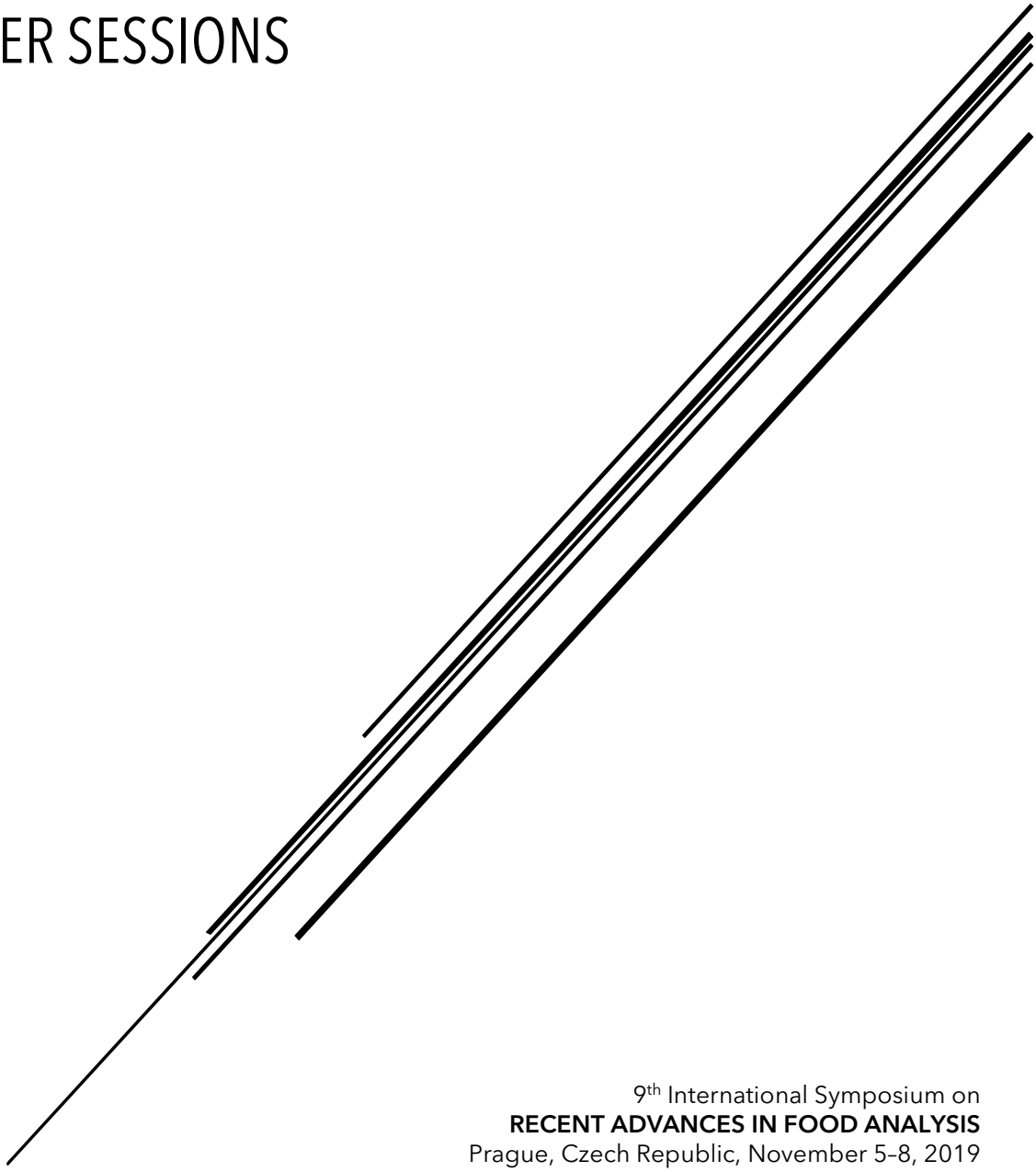

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Q1

POTENTIAL OF METABOLOMICS IN CHEMICAL RISK ANALYSIS APPLICATION TO CHLORDECONE HAZARD CHARACTERISATION

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Introduction: Chlordecone is an organochlorine pesticide (OCP), extensively used during more than 20 years in the French West Indies to protect banana plantations from weevil attack. Its use was banned in 1993. It is still responsible of a widespread environmental contamination and the local population may be exposed through the food chain. Several adverse effects are related to this exposure [1,2]. Although MRLs have been set to secure food production, in certain circumstances consumers may still be exposed above TRV/TDI [3] because of high level consumptions of local and self-productions (root vegetables and products of animal origins) [4]. In order to characterize the possible biological impacts of CLD exposure, the aim of this study was to investigate the effects of chlordecone exposure by highlighting candidate biomarkers during the contamination and depuration periods applying a metabolomics-based approach. Materials and Methods: After a 2 week's acclimatization period, four adult female sheep were exposed to CLD via daily feeding at 0.01 mg/ kg BW/day and during 90 days. This duration was applied to reach the steady-state. The depuration period was monitored during an additional 23 days' period (Bio-DA platform, UR AFPA, University of Lorraine, Agreement n°APAFIS#15366-2018060510344515v8). Sheep serum samples were collected at least every 2 weeks and prepared using a Bligh&Dyer, consisting in a simple liquid-liquid extraction (methanol/water/chloroform). Methanolic fractions, were analyzed using liquid chromatography (RPLC & HILIC) coupled to high-resolution mass spectrometry (LC-(ESI+/-)-HRMS) in full scan mode to generate fingerprints. Data treatment and statistical analysis were performed with the GALAXY open source. Results: Comparative fingerprints analysis using both non supervised statistical analysis (PCA) and Supervised statistical analysis ([Orthogonal] Partial Least Square Discriminant Analysis, ([O]PLS-DA) demonstrated a significant difference between the three different groups of interest (before exposure, during and after). Moreover, temporal dynamics of contamination and depuration could be observed over time. PLS-DA further enabled highlighting a list of candidate biomarkers (n=77) for each studied fraction. Interestingly, the selected candidate biomarkers displayed different trends during the contamination and depuration periods. Some of them did not seem to return to normal levels during the depuration period, which corroborates PLS-DA.

Acknowledgement: *The authors thank Pamela Hartmeyer (URAFPA) for technical support.*

Q2

HAZARDOMICS: APPLICATION OF METABOLOMICS APPROACHES TO THE RISK ASSESSMENT OF BISPHENOL A AND POLYCHLORINATED BIPHENYLS

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Nowadays, there is a growing concern about the effects of chemical exposure on human health and it is of major interest to establish the risks associated with chemicals for the enforcement of health prevention strategies. One of the current priorities of chemical risk assessment is to understand the effects and modes of action of endocrine disruptor compounds (EDCs) such as bisphenol A (BPA) and polychlorinated biphenyls (PCBs). Within this framework, metabolomics has emerged as a novel strategy for the discovery of effect biomarkers that can provide the connection between exposures to EDCs and chronic diseases[1].

HAZARDOmics is a European research project that aims to enhance the knowledge about the risks arising from exposures to low doses of BPA and to a 'cocktail' of PCBs following an innovative metabolomics approach. The pig has been selected as animal model for risk assessment studies because human and pig metabolism show similar responses to disease progression[2]. Exposures to BPA and PCBs were carried out in two different animal experimentations of five weeks each. Four animals were exposed to 850 ng/kg bw/day of BPA (dietary exposure for population groups older than 6 months)[3] for 11 days followed by an exposure to 4 µg/kg bw/day (tolerable daily intake, TDI)[3] of BPA for 11 days. In the same vein, four animals were exposed to 20 ng/kg bw/day (TDI)[4] of a mixture of PCBs (Aroclor 1260) for 22 days. Two control animals were also monitored in both conditions.

The metabolic profiling of blood serum samples is being investigated from a broad analytical perspective in order to obtain the most complete information of metabolic fingerprint and increase the possibility of identifying effect biomarkers. Liquid Chromatography (LC)-High Resolution Mass Spectrometry (HRMS) involving reverse phase-LC and hydrophilic interaction-LC are employed as traditional metabolomics strategies. In addition, Ion Mobility Spectrometry (IMS) integrated in a LC-HRMS workflow is evaluated as a ground-breaking technology for metabolomics, while Capillary Electrophoresis (CE)-HRMS is implemented as complementary technique to LC-HRMS. HAZARDOmics also aims to evaluate, through the implementation of a multi-platform approach, the potential of metabolomics in the service of chemical risk assessment, particularly for hazard characterization purposes.

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2. A. Bassols, *et al.*, *Proteomics Clin. Appl.*, 2014, 8, 175-731.
3. European Food Safety Authority, *EFSA Journal*, 2015, 13, 3978.
4. Agence Française de Sécurité Sanitaire des Aliments, *Avis de l'AFSSA* du 23 octobre 2007 relatif à l'établissement de teneurs maximales pertinentes en polychlorobiphényles qui ne sont pas de type dioxine (PCB " non dioxin-like ", PCB-NDL) dans divers aliments, p. 1-28.

Keywords: *metabolomics, risk assessment, bisphenol A, polychlorinated biphenyls*

Acknowledgement: *This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement HAZARDOmics No 795946. The animal study was approved by the national Ethical Committee (n°6) under project agreement APAFIS#15159-2018051920446340 v2 (ONIRIS agreement E44271).*

Q3

RAW HAZELNUT VOLATILES: CHALLENGES IN DEFINING ODORANT PATTERNS RELATED TO SENSORY DEFECTS BY COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY COUPLED WITH TIME-OF-FLIGHT MASS SPECTROMETRY

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The study of the odor perception of a food, here raw hazelnut, poses severe challenges due to the high chemical dimensionality expressed in these mixtures of volatiles. In this scenario, the separation power and resolution enhancement, the improved sensitivity obtained by the effective band focusing in-space and the generation of structured separation patterns, are key-features that make comprehensive two-dimensional gas chromatography (GC×GC) with thermal modulators a platform of choice to achieve accurate and reliable fingerprinting results. Raw hazelnuts connoted by different sensory defects may show informative 2D patterns of volatiles that could be diagnostic for quality assessment. Volatiles from hazelnuts of different geographical origin and cultivar, selected by flash-profile descriptive analysis for the presence/or not of sensory defects, are sampled by headspace solid phase micro extraction (HS-SPME) with a DVB/CAR/PDMS d_f 50/30 µm 2 cm length fiber and analyzed by GC×GC-TOF MS adopting a polar × medium polarity column set-up. Their 2D patterns are then processed by combined Untargeted and Targeted fingerprinting (UT fingerprinting) and by visual features fingerprinting to highlight compositional differences. Finally, unsupervised and supervised chemometrics is adopted on 2D peaks quantitative descriptors to find reliable and informative peaks and peak-patterns for successful discrimination and classification of samples. The 2D patterns of volatiles from good quality and defected hazelnuts show a great complexity: 120 known compounds on about 350 2D peak-regions detectable, are reliably targeted (i.e.). UT fingerprinting delineates diagnostic patterns that clearly cluster samples with mouldy notes, rich in both saturated and unsaturated aldehydes, short chain fatty acids, linear alcohols and furanones, but fails with rancid samples due to the concurrent presence of additional perceptions like stale and solvent-like odors. To improve the fingerprinting effectiveness and to minimize the impact of confounding variables, a "model peak-pattern" is created after re-alignment and summation of 2D-contour plots from samples showing specific odor defects. The resulting cumulative image is then adopted as diagnostic probe for effective fingerprinting through visual features. Supervised chemometrics effectively extract informative analytes (known and unknown) with high discrimination role.
In conclusion, the univocal identification of chemical patterns related to sensory defects confirms the effectiveness of high-informative fingerprinting by GC×GC-TOF MS and pattern recognition approaches based on template matching for sensory quality assessment of raw hazelnuts. On the other hand, visual features fingerprinting offers a unique option to minimize the effect of confounding variables, leading to more conclusive results.

Keywords: *GCxGC-TOFMS, sensory defects, combined untargeted and targeted (UT) fingerprinting, raw hazelnuts volatiles*

Q4

SEMI-TARGETED ANALYSIS OF FOLATE METABOLITES IN YEAST USING (ULTRA-)HIGH RESOLUTION MASS SPECTROMETRY

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Folates are a group of B_9-vitamins consisting of more than 150 different vitamers. These vitamers function as cofactors for enzymes required in the C1-metabolism and thus play an important role in many metabolic processes. They are required for methylation reactions, nucleotide synthesis, or oxidation and reduction processes. Since folates are involved in cell division and DNA replication, a lack in folates during pregnancy is closely associated with the incidence of neural tube defects in newborns. Shortage of folates is furthermore assumed to be a major risk factor for several chronic diseases such as cardio vascular diseases or dementia. The analysis of folates is of special importance since the German Nutrition Society suspects an undersupply in the whole population. Thus, a daily intake of 300 µg of folic acid is advised for adults. One option of achieving those values would be the consumption of foods rich in folates.
A huge variety of food has been analyzed for its folate content whereby yeast turned out to be a good source for folates. Until now, folate analysis was mainly focussed on the different monoglutamate vitamers. Little research has been done so far on the respective polyglutamates, which are the major vitamer forms in food. Thus, it is important to gain deeper insights into the metabolism of folates including their polyglutamylated forms. Therefore, different semi-targeted methods were applied to unravel the folate metabolome in more detail. Baker's yeast served as model organism due to its high folate content. Samples were analyzed by means of direct injection Fourier transform ion cyclontron resonance mass spectrometry (DI-FT-ICR-MS) and supporting measurements applying ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC/QToF-MS). For obtaining higher sensitivity, sample clean-up via solid phase extraction had to be performed prior to measurements. Due to ultra-high resolution, several signals could be unraveled as tentative folate vitamers in FT-MS spectra. For verification of those compounds, analysis by QToF-MS was performed applying data dependent acquisition. The latter measurements offered the possibility to reduce matrix interferences further as a result of separation on a C18 column before electrospray ionization. Thus, the FT-MS results could not only be confirmed but several more folate polyglutamates could be detected. The applied methods complemented each other quite well since both of them have limitations for some critical vitamers due to measurements in different ionization modes. A combination of both semi-targeted approaches unraveled the folate metabolome in yeast in more detail than reported so far.

Keywords: *yeast, folate metabolome, polyglutamates, ultra-high resolution mass spectrometry*

Q5

ADMINISTRATION OF CLENBUTEROL OR DEXAMETHASONE IN BULLS: A METABOLOMIC APPROACH TO DETECT ANABOLIC TREATMENT

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Surveillance of illegal use of growth promoters is based on the direct detection of drug residues by LC-MS/MS. Screening strategies focusing on indirect biological responses may represent useful tools to overcome limitations of classical analytical methods. Administration of pharmacologically active compounds such as β-agonists and/or corticosteroids can influence the physiology of an organism, thus detection strategies based on the recognition of metabolic changes induced by anabolic practices are promising approaches to strengthen existing targeted monitoring plans.
In the present work, metabolomics analysis was carried out on liver extracts deriving from independent *in vivo* studies in which a group of bulls was used as control (n=16), while the remaining bulls received either dexamethasone (DXM, n=16) at sub-therapeutic dosage or clenbuterol (CBT) alone (n=8) or CBT combined with DXM (n=8) to mimic anabolic practices. LC-HRMS analysis of liver extracts was carried out using either RPLC or HILIC combined with ESI in both negative and positive polarity, thus producing 4 data sets for each treatment.
Principal Component Analysis (PCA) and Partial Least Squares - Discriminant Analysis (PLS-DA) enabled to reveal a set of features/compounds which were used to develop predictive models to classify animals according to their corresponding group (treated or non-treated). The best performances in terms of sensitivity and specificity were observed for the model derived from CBT dataset.

Keywords: *clenbuterol, dexamethasone, anabolic treatment, metabolomic*

Acknowledgement: *Authors thanks Italian Ministry of Health for financing (RC IZS VE 03/2016, CUP B22F17000340001)*

Q6

ANALYSIS OF VOLATILE COMPOUNDS IN PUMPKIN WITH 'TARO-LIKE' AROMA USING SOLID PHASE MICRO-EXTRACTION AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY COMBINED WITH CHEMOMETRICS

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Pumpkin is one of the most popular vegetables around the world. Pumpkin showing 'Taro-like' aroma is an ideal material for the study of pumpkin aromatic trait. However, there is limited information available on its volatile compounds' profiles, in particular, the key aroma compounds causing the typical aroma of pumpkin. Solid phase micro-extraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS) is proved to be an effective way to extract and analyze volatile compounds. In addition, chemometrics has advantages in extracting relevant information and discovering patterns in the large series of data. Therefore, chemometrics methods combined with SPME/GC-MS could be an effective and convenient tool for comprehensive analysis of volatiles in pumpkin with 'Taro-like' aroma.

Three experimental groups, including three replicates, one pumpkin sample with 'Taro-like' aroma (YJ) and two pumpkin samples without the special aroma (EY and 278), were used in this study. All samples (mature fruits about 45 days after pollination) were plucked and then vacuum freeze-dried. Moreover, the content changes of key aromatic compounds at the different development stage of YJ fruit were analyzed. Fruits were sampled in three stages: unpollinated, 25 days after pollination and 55 days after pollination. Gas chromatography/triple quadrupole mass spectrometer (7890B-7000D, Agilent Technologies) was utilized on the analysis of volatile compounds. Then, chemometric method performed by Mass Profile Professional (MPP, Agilent Technologies) was applied to investigate the difference of volatile compounds among three samples.

The volatile components of the mature fruit of YJ were collected and analyzed. A total of 31 volatiles, including 12 aldehydes, 6 ketones, 5 heterocylics, 4 alcohols, 1 esters, 1 lactones, 1 benzenes and 1 alkenes, were obtained. Then, to investigate the differences between YJ and EY, YJ and 278 using MPP. The principal component analysis (PCA) of the volatile components between YJ and EY showed that the contribution rates of PC1 and PC2 were 58.24% and 19.91%. Between YJ and 278, the total accumulative contribution rate of PC1 and PC2 accounted for 78.78%. Thus, the PCA analysis of volatiles can better distinguish the pumpkin resources, indicating the significant difference of volatiles between the pumpkin with 'Taro-like' aroma and pumpkin without the special aroma. Subsequently, to characterize the different volatile compounds. 2-acetyl-1-pyrroline (2-AP) was the unique volatile compound in YJ fruit as compared with the two sets of control samples. Finally, unpollinated fruit and fruit obtained at the 25th day after pollination had a similar amount of 2-AP, while the 2-AP content deceased significantly in fruit at late maturity stag. This study has laid the foundation for future studies on 'Taro-like' aroma trait in pumpkin.

Keywords: *pumpkin, volatile compounds, solid phase micro-extraction, gas chromatography-mass spectrometry, chemometrics*

Q7

CHEMOMETRIC ASSESSMENT OF VOLATILE FRACTION OF PESTO BY SPME ARROW - GC-ORBITRAP MASS SPECTROMETRY

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'Pesto genovese' is a well know tasty and aromatic Italian pasta sauce consisting of crushed basil leaves, cheese (parmesan or pecorino), pine nuts and garlic blended with extra-virgin olive oil. The production of pesto for wide distribution requires the use of additional ingredients and various technologies such as pasteurization and sterilization to extend the product shelf-life ensuring freshness for consumers. The preservation processes usually require high temperatures that can lead to changes in pesto composition affecting its taste and aroma.
In this study headspace solid phase micro-extraction (SPME) with Arrow technology coupled with gas-chromatography (GC) high resolution Orbitrap mass spectrometry was used to determine the volatile profile of various pesto samples that were produced using various technological methods.
The SPME Arrow allowed for sample extraction and concentration in a single step, without the need of time consuming sample preparation and in a fully automated fashion. The improved geometry of the fiber provided a larger volume and a thicker coating phase allowing for fast extraction (15 minutes) of a huge number of volatiles from the major monoterpenes like anethole (RT=13 min) to the less predominant ones as γ-terpinene (RT=9.56 min). Routine high mass resolution of the Orbitrap-GC (60,000 FWHM at *m/z* 200) allowed for consistent sub-1 ppm accurate mass measurments, ensuring high selectivity with no compromise in sensitivity. Unknown compounds identification and sample group assessment was achieved using Compound Discoverer 3.1. In Compound Discoverer, peak deconvolution and spectral library search together with the search index (SI), high resolution filtering (HRF) score ensured confident compound identification. Additionally, positive chemical ionization (PCI) was used to confirm the elemental composition of the parent ions using accurate mass information and adduct formation. Multivariate statistical analysis approach with Compound Discoverer resulted in identification of the principal components linked to the different production processes and that are responsible for the differences observed between the samples.

Keywords: *volatile fraction, pesto, SPME arrow, GC-Orbitrap mass spectrometry*

Q8

A COMPREHENSIVE STRATEGY FOR CONFIDENT DETECTION OF OREGANO ADULTERATION BY GC-ORBITRAP MASS SPECTROMETRY

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Oregano is a herb widely used as ingredient in a variety of food and beverage commodities and is particularly vulnerable to fraud. Adulteration of oregano can be accidental or intentional with the latter being price and demand driven. Common adulterants that can be found in fraudulent oregano samples are leaves originated from olive trees, myrtle, sumac, cistus or hazelnut leaves, among others. As a consequence, the food manufacturers must check regularly for the quality and purity of the oregano outsourced from various suppliers to ensure that the quality of the end product is consistent. Oregano adulteration is commonly investigated using methods such as gas of liquid chromatography coupled to mass spectrometry (GC-MS, LC-MS) that can identify the presence of GC amenable of LC-amenable biomarkers used as adulterants in ground oregano.
In this study a headspace solid phase micro-extraction (SPME) with Arrow technology coupled with gas-chromatography (GC) high resolution Orbitrap mass spectrometry was used to determine the volatile profile of various oregano samples. The SPME Arrow allowed for sample extraction and concentration in a single step, without the need of time consuming sample preparation and in a fully automated fashion. The improved geometry of the fiber provided a larger volume and a thicker coating phase allowing for fast extraction of a large number of volatile compounds present in oregano. High mass resolution of the Orbitrap-GC (60,000 FWHM at *m/z* 200) allowed for consistent sub-1 ppm accurate mass measurements, ensuring high selectivity with no compromise in sensitivity. Unknown compounds identification and sample group assessment was achieved using Compound Discoverer 3.1. In Compound Discoverer, peak deconvolution and spectral library search together with the search index (SI), high resolution filtering (HRF) score and the retention index information ensured confident compound identification. Additionally, positive chemical ionization (PCI) was used to confirm the elemental composition of the parent ions using accurate mass information and adduct formation. Multivariate statistical analysis approach with Compound Discoverer resulted in identification of the principal components responsible for the differences observed between the oregano samples

Keywords: *oregano adulteration, GC-Orbitrap mass spectrometry, SPME arrow*

Q9

EVALUATION OF GRAPE BERRY RIPENING BY NON-TARGETED METABOLOMICS ANALYSIS

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The measurement of grape characteristics which impact on product quality is a basic requirement for vineyard improvement and for optimum production of desired wine styles. It is common industry practice for quality assessment to be achieved by total soluble solids and acidity measurement, visual assessment and also by tasting assessment of fruit and of wines following vinification. However, there is a strong need in the modern wine industry for the holistic analysis of metabolic profiles during berry development and ripening that can be used for grape berry maturity assessment. The main aim of this study was to examine ripening processes of white wine grapes with non-targeted metabolomics. The white table grape cultivar, Grüner Veltliner is Austria's most important and the most planted grape variety, with 36% of grape area. The grapes were harvested over three months (12 time points) and analyzed by LC-TOFMS, to generate a detailed, non-targeted picture of the changing metabolic profiles during late berry development. The dataset obtained was further evaluated via multivariate data analysis and key metabolites with a significant difference in their abundance profile at the optimal ripeness stage were revealed. Furthermore, comprehensive structural characterization of these key metabolites was performed using LC-QTOFMS to provide high-resolution MS2-level information amenable for library-based identity confirmation. Finally, drift tube ion mobility-mass spectrometry (DTIM-MS) was used to generate accurate collision cross sections (^{DT}CCS) for remaining unknown compounds deemed to be of interest following statistical evaluations, allowing a maximum level of structural information for key, but still unidentified metabolites to be reported.

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Q10

POTENTIAL OF TRAPPED ION MOBILITY COMBINED WITH LC-HRMS IN FOOD AUTHENTICITY STUDIES: IDENTIFICATION AND CHARACTERIZATION OF SECOIRIDOIDS ISOMERS FOUND IN GREEK EXTRA VIRGIN OLIVE OIL

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Over the last decade, great effort has been put in the field of food authenticity in order to achieve both quality assessment and further characterization of the products. Olive oil, in particular, has recently been found on the frontline of scientific research due to its particular health-promoting effects. A new legislative framework established in 2012 by the European Union, highlights the health benefits related to bioactive compounds found in olive oil. European Union (EU) establishment, directly associated with European Food Safety Authority (EFSA) preceded pronouncement about the benefits of Extra Virgin Olive Oils (EVOOs) consumption, have set the standards for thorough investigation of bioactive compounds.
One of the most important class of bioactive compounds found in olive oil, is that of secoiridoids, mainly due to their high concertation. Secoiridoids are part of the olive oil phenolic fraction that is also comprised of phenolic acids, phenyl ethyl alcohols, hydroxy-isochromans, flavonoids and lignans. The challenging part in secoiridoids study remains the identification and separation of isomeric compounds already reported but yet to be characterized. Due to high structure similarity, separation of isomeric compounds is not always an easy task to achieve. In many cases identical chromatographic and spectral profile is received, with regards to HRMS-based workflows applied so far. In order to overcome these limitations and achieve reliable identification, an additional dimension of separation is needed.
In the present study, a novel methodology based on Trapped Ion Mobility Spectrometry (TIMS) was applied. TIMS technology was combined with LC-HRMS (timsTOF, Bruker Daltonics) for optimal performance. The use of ion mobility enhanced the identification providing detailed examination of selected compounds utilizing precise collision cross section (CCS) data. Thereafter, further investigation through data dependent acquisition (DDA) was conducted, enabling structure elucidation. EVOOs of different variety and geographical origin were analysed in order to study differentiations among the samples. Data acquired and processed using MetaboScape® 4.0 and the T-ReX 4D algorithm, (Bruker Daltonics Software) aiming at global profiling of compounds. Noteworthy results were retrieved, indicating different profile of secoiridoids isomers among the samples. Moreover, significant differentiations were highlighted in samples of different variety, indicating isomers as potential authenticity markers. In conclusion, the use of LC-TIMS-HRMS along with annotation strategy within MetaboScape (based on retention time, CCS, accurate mass, isotopic patterns and MS/MS spectra) has proved to be a powerful analytical tool, highly-applicable to food authenticity studies, with remarkable capabilities and breakthrough achievements.

Keywords: *extra virgin olive oil, isomers, secoiridoids, LC-TIMS-HRMS, food authenticity*

Q11

RAPID MALDI-TOF-MS-BASED PROTEOMICS APPROACH FOR RELIABLE DETECTION OF PDO FETA CHEESE ADULTERATION

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"Feta" is a Protected Designation of Origin (PDO) Greek cheese, ripened in brine, produced exclusively from pasteurized sheep's milk or sheep's and goat's milk (up to 30%). The worldwide recognition of Feta is attributed to its special sensory characteristics, rendering it as one of the Greece's most important exports. However, in the dairy industry, cow's milk is fraudulently admixed with sheep's milk during the manufacture of ovine cheeses due to the lower yield of ewes combined with the lower price of cow's milk. Due to its high economic impact, fraud control is therefore vital, especially for PDO high-grade cheeses made entirely from sheep's/goat's milk.
A range of analytical methods to detect frauds have been developed, modified, and continually reassessed to be one step ahead of manufacturers who pursue these illegal activities. To assess the authenticity of dairy products and defend consumers' health, a European ReferenceMethodology (ERM) was established to detect cow proteins in dairy products, based on gel isoelectric focusing (IEF) technique. Nevertheless, this method is time-consuming and suffers from interpretation difficulties due to overlapping of species-specific bands.
In the present work, a fast and sensitive matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)-based methodology has been developed for the detection of feta cheese adulteration. Exploiting the total intact protein profile (caseins and whey proteins), an integrated proteomics-based workflow has been elaborated for the detection of the potential feta cheese adulteration with cow's milk. Statistical treatment using advanced chemometric tools, such as unsupervised principal component analysis (PCA) and supervised partial least squares discriminant analysis (PLS-DA) recognition techniques were utilized for the discrimination/classification of the cheese samples. Different adulteration levels were studied and characteristic m/z (species-specific markers) were detected, indicating cow's milk addition during the manufacture of feta cheese. To the best of our knowledge, this is the first study reported in the literature to rapidly reveal feta cheese illegal adulterations using MALDI technology, achieving substantially low LODs and short-time analysis. The results obtained from this study clearly demonstrate that MALDI-TOF MS-based proteomics have the potential to be used as a reliable screening tool for the assessment of dairy products authenticity.

Keywords: *MALDI-TOF-MS, feta cheese, dairy products adulteration, PDO products, protein markers*

Q12

METABOLOMIC PROFILING OF SUPERFOOD SEEDS IN BAKERY PRODUCTS USING A BENCHTOP GC TIME-OF-FLIGHT MASS SPECTROMETER

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Around 20 years ago "*superfood*" started to be used as a marketing tool for selling specific foods, dietary supplements and food additives. Although there is no exact definition of superfoods, one can be classified as food considered exceptionally good for one's health and for boosting the immune system. In the past few years chia seeds became one of the most popular superfoods. As a result of the rapidly increasing demand for superfoods, the market is flooded with counterfeits. In bakery products more expensive chia seeds are often replaced by cheaper flaxseed. The aim of this study was to evaluate the metabolomic profile of chia, flax and sesame seeds, looking for chemical markers, which allow confirming the presence of such seeds in food products. For sample preparation a well established derivatization protocol was applied, including a methoxymation step, followed by a silylation. The samples were analyzed using a LECO Benchtop GC-Time-of-Flight Mass Mass Spectrometer. A new TOF design has significantly improved the detection capability of the system, allowing for the safe identification and quantification of analytes, even at ultra-trace level. Different strategies for data evaluation were compared. Rosmarinic acid is best suited to distinguish between chia and other seeds. The marker was identified by the usage of the Golm Metabolome Database. By using this marker, alterations to bakery products can be easily uncovered.

Keywords: *GC-TOF MS, profiling, TOF, superfood, chia*

Q13

STABLE AND LOW-COST FOOD METABOLOMICS USING GC-FID

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Introduction: Metabolites in biological samples are widely involved in the flavor and aroma of food products. Accordingly, metabolome analysis methods, which provide a comprehensive analysis of metabolites, are being actively applied to research and development of food products, for such applications as analyzing taste components and determining the stability of the quality of fermented foods.
The mass spectrometers (MSs) are widely used for metabolomics due to their separation performance. However, they require high initial costs and sometimes have a problem with data stability in metabolomics. In GC-MS metabolomics, the data would be unstable as time passed after TMS derivatization, that might be caused by MS tuning, pollution, and the stability of derivatized compounds. Therefore, data acquisition with long time span may sometimes need some modification to the data, such as correction with quality control sample. From those backgrounds, metabolomics using gas chromatograph (GC) was investigated as a low-cost and stable method. GC with flame ionization detector (GC-FID) is a conventional analytical instrument that is useful for various fields. However, GC-FID has not utilized for metabolomics compared to GC-MS or LC-MS. While GC-FID is inferior in peak annotation ability, it has a massive advantage especially data stability in metabolomics.
Methods and Results: Commercial beers were prepared as samples and conventional Bligh-Dyer method were conducted for the sample pretreatment. For method comparison, samples were analyzed using both GC-FID and GC-MS with the same column and condition. Obtained chromatogram shapes are very similar for both. The data stability obtained by GC-FID within 60 hours was considerably higher than GC-MS. And stored samples in dry condition were analyzed after two weeks. While there were differences in GC-MS data between measurement days, the data in GC-FID were almost identical not only in chromatogram shape but also in peak area.
Under these background data, we tried to classify the subtle beer samples that were the same as product but differentiated with their lots and manufacturing places. Five beer samples were analyzed using GC-FID and GC-MS, and compared the results of Principal Component Analysis (PCA). GC-FID and GC-MS obtained 161 and 178 peaks that were detected in all the samples, respectively. The score plot of the PCA results were similar. It suggests that GC-FID and GC-MS may seize the similar feature of the samples.
While GC-FID may be inferior to GC-MS from the point of view of peak qualification and sensitivity, its lower initial cost is useful and higher stability is helpful for metabolomics especially when it requires a large number of data.

Keywords: *GC, GC-MS, stable data, low cost, metabolomics*

Q14

REAL-TIME CHARACTERIZATION OF WHOLE COFFEE BEANS USING DART QDA AND CHEMOMETRICS

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Despite the increasing worldwide consumption of coffee, smallholder farmers still lack evidence-based strategies to meet market demands for superior coffee quality. Superior quality often leads to price premiums, which can help alleviate income insecurity for small-scale and subsistence farmers who produce ~90% of global supply. Post-harvest processing methods have been shown to provide product differentiation and better access to high-value markets. Coffee fermentation is a critical control point in post-harvest processing. Traditionally, the fermentation step served to functionally remove the mucilage from the coffee fruit, through biomechanical means, to facilitate the drying process in preparation for storage. However, in addition to serving a functional purpose, coffee fermentation can be optimized to achieve a desired sensory profile and/or elevated cup quality.

Here we report the use of Direct Analysis in Real Time (DART) coupled to a single quadrupole mass detector (QDa) for the direct analysis of whole, roasted coffee combined with multivariant analysis (MVA) to discriminate between post-harvest processing treatments. DART produces relatively simple mass spectra typically characterized by M+, $[M+H]^+$ in positive mode and M^- , $[M+H]^-$ in negative mode. Fragment ions are observed for some compounds and the degree of fragmentation can be influenced by the choice of DART gas. To allow sampling from the roasted coffee surface, the DART gun was configured in the 45° angle position perpendicular to the 10-position tablet carrier rail attachment which was modified to allow for analysis of non-uniform roasted coffee samples. Positive polarity ionization was found to give the most abundant spectral features and considered to be the more diagnostic mode. The MS spectral information is used as a 'fingerprint' for characterization of the post-harvest treatment. Mass bins between *m/z* 50-1000 were used to train MVA models and cross-validation was performed to evaluate the classification accuracy. The results demonstrate the potential for this approach as a high-throughput quality control screen of whole roasted coffee for post-harvest processing treatment effects on coffee quality.

Q15

FLAVONOID PROFILING AND ANNOTATION USING A PRODUCT ION-DEPENDENT MSN DATA ACQUISITION METHOD ON A TRIBRID ORBITRAP MASS SPECTROMETER

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The untargeted profiling of flavonoids provides insights into their biological functions and potential health benefits for humans. However, comprehensive identification of flavonoids from real samples remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds. Previous studies relied upon extensive expert knowledge about fragmentation rules, a priori knowledge of the structures of flavonoids, and simple MS2 based analyses that are often not sufficient for complete structural characterization. Here we present a new flavonoid profiling workflow that uses comprehensive fragment ion information from HCD MS-MS and higher order FTMS^n for rapid flavonoid identification and quantitation on a Tribrid Orbitrap mass spectrometer.
As the proof of concept of the workflow, flavonoid extracts from different types of natural products were tested. A C18 column was used for flavonoid separation, and an ID-X™ mass spectrometer was used for collecting HRAM MS and MS^n (up to MS^5) data. The collected data were processed using Mass Frontier™ 8.0 and Compound Discoverer™ 3.0 software. A novel structure ranking algorithm included in the Compound Discoverer 3.0 software was applied to the MS and MS^n data for confident structure elucidation of the unknown flavonoids based on ChemSpider databases and custom flavonoid databases. The MS^n data were critical, especially for the identification of flavonoid glycoconjugates.

Keywords: *flavonoid profiling, LC-MSn workflow, Orbitrap Tribrid, compound annotation, structure elucidation*

Q16

METABOLOMICS-BASED AUTHENTICATION OF WINES ACCORDING TO A GRAPE VARIETY

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In recent years, there has been growing interest availability of analytical methods for wine authentication. While procedures for geographic origin authentication have been already reported, development of those for recognition of a grape variety used for wine production still remains a challenge. Here, we investigate the suitability of the metabolomic fingerprinting of authentic wine samples, using ultra-high-performance liquid chromatography coupled to high-resolution tandem mass spectrometry. Red and white wines (five varieties of each, 200 samples in total) were analyzed within our study. The generated data were processed by principal component analysis and then by partial least squares discriminant analysis. The resulting statistical models were validated and assessed according to their R2 (cum) and Q2 (cum) parameters. Our results indicate that an HRMS based metabolomic fingerprinting of wine is a promising strategy for wine variety authentication, of course, additional data are needed to verify the models.

Keywords: *wine, authentication, U-HPLC-HRMS/MS, metabolomics*

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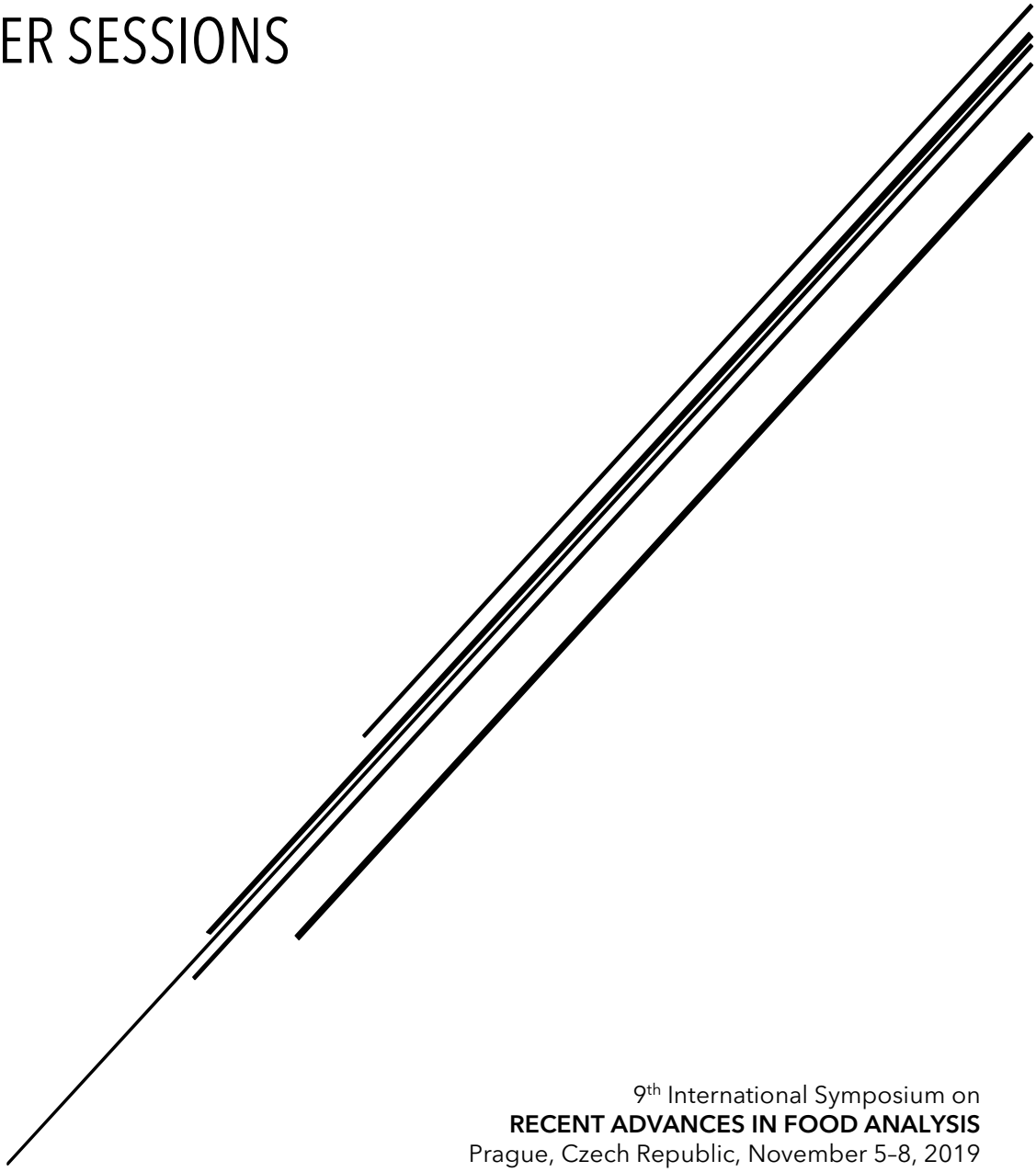
PROCESSING CONTAMINANTS

POSTER SESSIONS



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R1

QUANTIFICATION OF ACRYLAMIDE IN POTATO CRISPS USING LC-MS/MS AND A NEWLY DEVELOPED UHPLC COLUMN

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The Maillard reaction, a reaction between reducing sugars and amino acids, is responsible for the unique colour and flavour of thermally processed food products high in carbohydrates. Such products include crisps, bread and cereals. However, this reaction produces heat-induced by-products including acrylamide, a compound reported as a probable carcinogen by the IARC (International Agency for Research on Cancer). The formation of acrylamide in these foods is dependent on the processing times and temperatures, with higher levels being exhibited in foods cooked for longer, and at higher temperatures.
Commission Regulation (EU) 2017/2158 commenced from April 2018 and establishes best practice, mitigation measures, and benchmark levels (BMLs) for the reduction of acrylamide levels in food products. The BML for acrylamide in potato crisps is 750 µg/kg and is not intended to be used as a maximum limit or for enforcement purposes, but rather used to gauge the success of the mitigation measures.
Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides accurate quantitation of acrylamide levels in potato crisps. The newly developed PerkinElmer Quasar™ UHPLC columns, utilised in this study, feature an ultra-high purity silica base and low residual silanol activity to yield excellent peak shape. Additionally, the optimised ligand bonding process applied provides exceptional surface coverage, yielding high sample loading capacities which helps to improve detection of low-level compounds.

Keywords: *acrylamide, LC-MS/MS, crisps, liquid chromatography*

R2

COMPARING AUTOMATED 3-MCPD ANALYTICS USING A ONE-PIECE WORKFLOW

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3-Monochloropropane-1,2-diol (3-MCPD), 2-monochloropropane-1,3-diol (2-MCPD) and glycidol are known as contaminants in foodstuffs due to manufacturing. MCPD fatty acids may occur during the refinement process of oils and fats at high temperature in the presence of chloride containing salts. However, refinement is an essential chemical and physical process. Only through this kind of treatment with high temperature undesired odorants and flavoring substances as well as possible traces of toxic compounds like pesticides, heavy metals or mycotoxins are eliminated. There is an increasing importance of the analysis of these contaminants due to their carcinogenicity. In January 2018, the European Food Safety Authority (EFSA) declared a reduced value for the tolerable daily uptake of 2 µg/kg body weight for 3-MCPD. But the manual determination is linked to a high expenditure of time.
Axel Semrau® has developed a workstation with which the different methods AOCS Cd29a-13, AOCS Cd29b-13 and AOCS Cd29c-13 as well as Zwagerman can be automated. The poster demonstrates the one-piece-workflow and shows a comparison of the different methods regarding analysis time for a sample, sample throughput, glycidol and MCPD content as well as the use of special modules. The automation of the methods enables faster results and a higher sample throughput per day. The CHRONECT Workstation MCPD not only offers the multi-method approach, but can also be combined with GC-MS systems from any manufacturer due to the powerful data system interface included in the CHRONOS software. Each automation method is tested for analytical performance criteria such as reproducibility, sensitivity, reduction of blank values and elimination of transfers. The elimination of carry over is ensured using the unique robotic tool change approach, which gives the possibility to use different syringes for different automation tasks. Analytical performance is tested for each system using a FAT and SAT protocol developed by Axel Semrau.

R3

IMPACT OF ULTRACENTRIFUGATION ON PREVENTING THE FORMATION OF MONOCHLOROPROPANEDIOL DIESTERS IN VEGETABLE OILS

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This study investigates the benefits of ultracentrifugation on reducing the formation of monochloropropane diol esters (MCPDEs) in vegetable oils. Focusing on sunflower and palm oils, ultracentrifugation experiments were conducted in order to separate the purified oil and the sediment-containing fraction generated. These two fractions were heated and analyzed for their MCPDE content by LC-MS and by the AOCS Official Method (indirect method). The MPCDE levels in sunflower oil showed to be 10 times higher in the sediment-containing fraction compared to the purified bulk oil. However, the mitigating effect of centrifugation was reduced to a factor 3 when the samples were warmed to 90°C beforehand, suggesting that even at mild heating the sediment fraction can partially re-solubilize in the bulk oil.
The effect of residual sediment on the MCPDE formation was also confirmed in the case of palm oil showing 2 to 10 times higher MCPDE levels in the sediment-containing fraction compared to the purified oil.
These results confirm that the mechanical removal of the trace sediments from crude vegetable oils results in reduced MCPDE levels.

Keywords: *monochloropropanediol diesters; mitigation; ultracentrifugation; vegetable oils*

R4

OCCURRENCE OF MONOCHLOROPROPANEDIOL AND GLYCIDYL ESTERS IN INFANT FORMULA AND BABY FOOD PRODUCTS FROM THE DANISH MARKET

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Fatty acid esters of monochloropropanediol (MCPDEs) and glycidol (GEs) are process contaminants that often present in refined vegetable oils. They can also occur in food products containing these oils such as infant formula or baby food. Upon consumption, 2-MCPDEs, 3-MCPDEs and GEs are hydrolyzed by the gastrointestinal tract into their corresponding free forms (2-MCPD, 3-MCPD, and glycidol, respectively). This is of high concern due to their potential toxicological effects on human. Both free 3-MCPD and glycidol are classified as carcinogens by the International Agency for Research on Cancer. In a recent scientific report by EFSA in 2016, infant receiving only formula were at high risks of exposure to 3-MCPDEs and GEs. In 2018, the European Union has published the Regulation No 2018/290 for maximum levels of GEs in vegetable oils and fats, infant formula, and baby foods. Specifically, the concentrations of GEs in infant formula and baby foods from 1 July 2019 onwards must be lower than 50 µg/kg in powder form and 6.0 µg/kg in liquid form. While no legislation is currently available for 3-MCPD in infant formula and baby food, a tolerable daily intake of 2 µg/kg body weight has been established by EFSA.
In this study, an indirect determination of MCPDEs and GEs by GC-MS/MS was developed and validated. The method showed excellent linearity (R^2 > 0.9997), precision, and accuracy. The inter-lab reproducibilities and intra-day repeatabilities were smaller than 9.5%. The recoveries were between 91-106% at two spiking levels of 17 µg/kg and 167 µg/kg. The method LODs ranged from 0.1-3.2 µg/kg and the method LOQs ranged from 0.5-10.7 µg/kg. The validated method was applied to analyze 60 infant formula and baby food products commercially available in the Danish market. The samples comprise of powder and liquid infant formula, baby puree and puree powder, and baby puff and biscuit. MCPDEs and GEs were quantifiable in almost all formulae, puff, biscuit and puree powders but not in fruit/vegetable based purees. The highest concentrations of 3-MCPDEs and 2-MCPDEs were found in a baby biscuit product at 100 µg/kg and 50 µg/kg, respectively. Meanwhile, a baby puff item contained GEs at 150 µg/kg, the highest among all tested samples. Interestingly, MCPDEs and GEs concentrations are well correlated in all but puffs and biscuits. It is possible that de novo formation of GEs have taken place during production of these finger food products. On a legislative perspective, all samples regulated by regulation No 2018/290 in this study show compliance level of GEs.

Keywords: *MCPD esters, glycidyl esters, baby food, infant formula, processing contaminants*

R5

FULLY AUTOMATED DETERMINATION OF 3-MCPD AND GLYCIDOL IN EDIBLE OILS AND FATS BY GC/MS BASED ON THE COMMONLY USED METHODS ISO 18363-1, AOCS CD 29(A&C)-13, AND DGF C-VI 18

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Introduction
3-MCPD, Glycidol and related fatty acid esters are process contaminants that can arise from edible oils refining. Because of their proven or suspected potential human carcinogenetic and genotoxicity, they're nowadays subjected to EU regulations. This work describes a solution for their fully automated determination in edible oils and fats based on the reliable indirect method AOCS Cd 29(a&c)-13, as well as a partial automation of method AOCS Cd 29(b)-13 *Methods*
The complete automated sample prep (including IS addition, derivatization/quenching, evaporation, reconstitution and injection in GC-MS), was performed on a GERSTEL MPS DualHead; key modules are QuickMix (which performs a reliable LLE) and mVAP (wich provides the significant benefit of removing excess derivatization reagent, allowing at the same time to reach required LOQs using a single Quad MS). *Results*
The linearity was checked by a 5 level calibration for both assay A and B, spiking an olive oil with different amounts of Glycidol and 3-MCDP ranging from 0.12 to 1.9 mg/kg. The repeatability was tested as well, with a RSD% < 7% for both analytes
Finally, the matrix effect was taken in account: three different (reference) edible oils were analyzed with excellent results in terms of accuracy. It is also important to underline that the described sample prep allows to quantify, in the same run, even the amount of 2-MCDP *Conclusions*
It's shown that AOCS method can be automated using the GERSTEL MPS and that the results obtained correlate well with
Referencedata. The excellent RSD% achieved for the complete process, including GC/MS analysis, speaks in favor of the presented sample prep station. Moreover, the evaporation step ensures that, for most matrices, the required limits of detection can be reached using a single quad. *Remark*
Although GC/MS technique has been proven to be suitable for "general purpose" analysis, the need to reach lower LOQs (i.e. few µg/kg for baby-food, as required by EU regulation 290/2018), suggests to move towards a GC/MS-MS approach, wich is able to reach such limits of quantitation. Furthermore, the same analytical platform is suitable for PAH quantitation in edible oils and fats according to EU regulation 1881/06 (and subsequent amendments).

Keywords: *MCPD, glycidol, edible oil, AOCS Cd 29c-13, lab automation*

R6

EVALUATION OF ANTHRAQUINONE IN CHALLENGING MATRICES BY GC-QQQ-MS/MS

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Anthraquinone (9,10-dioxoanthracene) is an aromatic organic compound of risk to human health and to the environment. It can be found on food due to its formation and adsorption during combustion processes, which are used to dry vegetables like tea, camomile or coffee. The European Commission recommends its control at low concentrations (5 µg/kg), since these vegetables' consumption is high [1].

The most commonly employed multiresidue analysis methods in the European Union do not allow for anthraquinone's correct quantification and identification. Dry vegetable samples are commonly hydrated with water to create a slurry, which causes the coextraction of natural products that mask anthraquinone's instrumental response and hinder its analysis [2]. When using an isotopically labelled anthraquinone standard –such as deuterated anthraquinone– it is possible to perform a screening analysis of the samples. Nonetheless, this approach does not permit to take legal action if anthraquinone presence is detected, since these analyses do not meet all the required identification criteria.

This study's objectives are (*i*) to evaluate the difficulties associated with the currently used multiresidue methods for food within the European Union; (*ii*) to develop a method that allows the control of anthraquinone at very low concentrations –below the current maximum residue limit (MRL) of 20 µg/kg for tea, coffee, herbal infusions and cocoa [3]–; and (*iii*) to perform a sampling study of potentially contaminated dry vegetables, which is currently ongoing.

For all those reasons, a new method has been developed specifically for anthraquinone analysis, which allows to take legal action in cases where its concentration is higher than 5 µg/kg. This process involves no hydration step prior to its extraction, the use of ethyl acetate as the extractant solvent, and its analysis with gas chromatography coupled to tandem mass spectrometry. Furthermore, a pilot sampling of dry vegetables has been undertaken, where anthraquinone has been detected in 10 % of the analysed samples.

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Keywords: *anthraquinone, gas chromatograpy, mass spectrometry, drying processes, dry vegetables*

R7

2-FUROIC ACID AND FURFURYL ALCOHOL AS POTENTIAL PRECURSORS OF FURAN AND 2-METHYLFURAN IN FOOD

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Furan is a process food contaminant formed during thermal processing which can be found in coffee, canned and jarred foods including baby food. It was classified as possibly carcinogenic for humans by the International Agency for Research on Cancer (group 2B) [1]. Alkylfurans, such as 2-methylfuran (2MFu), can co-occur with furan and are likely to exhibit also a toxicological potential [2]. In the last decade, several mechanistic studies have been conducted, in either model systems or food matrices, with the intention to clarify the chemical pathways involved in the formation of furan and 2MFu [2].
The objective of this work was to study the participation of 2-furoic acid (FA) and furfuryl alcohol (FOL) in the formation of furan and 2MFu in heat-treated foods. Model systems mimicking various thermal conditions (different temperatures/times: 90–190 °C; 2–20 min) were used for this purpose. Two different in-house validated analytical methods based on gas chromatography-head space-mass spectrometry (HS-GC-MS) and liquid chromatography-high resolution mass spectrometry (LC-HRMS) were used for the determination of furan and 2MFu or FA and FOL, respectively. In addition different coffee products (n=27) obtained from Swiss markets were also analyzed with these platforms in order to clarify any correlation between the compounds included on the scope of this study.
Incubations in model system revealed that furan generation from FA increases with temperature and time, therefore confirming decarboxylation (0.15% conversion rate, 30 min incubation at 190 °C). Induction of 2MFu from FA was insignificant. When incubating FOL, no significant formation of furan was observed, however a substantial increase in 2MFu was found as from 170°C (0.01% converstion rate, 30 min incubation at 190°C). Analysis of roasted coffee samples showed a strong correlation between FOL/FA, suggesting an intimate relationship between them. Either FA and FOL originate from the same source, or one decomposes to the other. Correlation between 2MFu/furan was excellent, while no correlation was found between 2MFu/FOL and between furan/FA, suggesting multiple pathways involved in the formation of furan and 2MFu during coffee roasting

[1] Furan: IARC Monographs on the evaluation of carcinogenic risks to humans 63 (1995) 393-407.
[2] Summary report of the standing committee on plants, animals, food and feed held in Brussels on 08 February 2019, European Commission, Health and Food Safety Directorate General: sante.ddg2.g.5(2019)1319051.

Keywords: *furan, 2-methylfuran, thermal degradation, coffee*

R8

A FULLY AUTOMATED METHOD FOR MCPD – AND GE- ESTERS AND THE IMPORTANCE OF GLASS QUALITY OF THE USED AUTOSAMPLER VIALS

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MCPDe and GE are the central point of attention of the food industry. Compounds are unwanted by-products of oil refinery, and predominately occur in palm oil, and great care should be taken in processing the oils. Nonetheless, any oil can contain MCPDe and GE when it is not processed with the utmost care. In the oil matrix, MCPDe and GE occur as esters, but they are analyzed after transesterification and derivatization in order to report total MCPD and GE contents.
In this poster an automated method will be presented based on a method from AOCSCd29c-13 or the so-called Zwagerman method showing excellent recoveries. One of the critical steps in the automation is the choice of autosampler vials. Vials with lower glass quality will have an effect on recovery as the esters tend to absorb at the free silanol groups on the glass walls.
When talking about vial quality, normally it is related to 1st hydrolytic class. Means the percentage of free silanol groups on the surface of the glass. The lower the amount is, the better the glass quality is and as less analytes will stick to it. This poster will show advantages of using vials with the lowest coefficient of mean linear thermal expansion. In theory the highest quality of vials is expected from 33 type vials, as the coefficient of mean linear thermal expansion is the lowest for the basic tubes used for vial manufacturing, as well as the hydrolytic resistance acc. to ISO 719, acid resistance acc. to DIN 12116 and alkali resistance acc. to ISO 695. The expansion coefficient actually describes the activity of the surface of the glass wall, which relates to the amount of free silanol groups present that can react with analytes and bind them to the glass surface.
Especially for MCPDe when they are put on the automation system before they are derivatized, the glass quality is a key factor for success. This is the most critical step, since at this point in the process, polarity of analytes plays a central role. During this step it is key to make sure, that they do not stick on the glass wall in order to reach the necessary detection limits.
An overview about the automation and the used systems, including glass vials, will be given, as well as a method utilizing a GC Triple Quadrupole for detection.

Keywords: *MCPDe, GCMS, automation, glass, quality*

R9

HUPSSE, A FAST EFFICIENT EXTRACTION METHOD FOR PAH IN FOOD

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PAH are a group of organic compounds consisting of two or more conjugated aromatic rings. Due to the genotoxic and carcinogenic properties of PAH, the PAH content in foods should be minimized and continuously monitored. Since 2011, maximum levels for benzo [a] pyrene and the sum of benzo [a] pyrene and three other PAHs (benz [a] anthracenes, benzo [b] fluoranthene, chrysene) have been set by law in the European Union. In addition, twelve other PAH have been identified by EFSA as priority PAH in food.
In order to determine the PAH content in foods, the PAH must firstly be extracted from the food matrix. Previously used extraction methods are either very expensive in terms of apparatus (ASE = Accelerated Solvent Extraction) or very time-consuming (Soxhlet extraction, saponification etc.). HUPsSE, an extraction method developed by SGS Germany GmbH, represents a fast, low-cost, easy-to-use and quickly to establish alternative to previous PAH extraction methods.
For HUPsSE (HUPsSE = Heat-Ultrasonic Pressure-supported-Sovent-Extraction) the sample is weighed into a vial and mixed with a suitable extraction solvent. Subsequently the sealed vial is incubated in the ultrasonic bath at a temperature close to the boiling point of the solvent. Then the sample is centrifuged, the sample extract is separated off and the extraction is repeated several times by adding new solvent.
Laboratory studies of different reference materials (meat, fish, spices) show that HUPsSE achieves with three extraction cycles recoveries comparable to ASE. The repeatability of both extraction methods is also comparable. In contrast to the ASE with HUPsSE a significantly higher number of samples can be processed per hour with minimal expenditure on equipment. As part of a training course, twelve laboratory technicians were trained in HUPsSE within 2 days. Again, a comparison of the obtained results from the participants with the ASE extraction showed a comparable recovery and repeatability. This demonstrates the simple applicability of HUPsSE in the laboratory.

Keywords: *PAH, extraction, ASE*

R10

OPTIMIZATION, VALIDATION AND ACCREDITATION OF GC-MS/MS METHOD FOR MCPDS AND GLYCIDYL ESTERS DETERMINATION IN BABY FOOD AND INFANT FORMULA

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On the basis of the most recent legislative and toxicological indications, an effective and suitable GC-MS/MS method was optimized, validated and accredited for the determination of MCPDs and Glycidyl Esters in baby foods and infant formula. The approach envisages the following:
- Fat Extraction
- SPE (Normal Phase) Purification (MAGs e DAGs if required)
- Glycidol Group Bromination
- Acidic Hydrolysis of esters
- PBA Derivatization
- GC-EI-MS/MS (MRM mode) Analysis

The high level of standardization allows a constant and widespread control of all critical and potentially critical points through the use of:
- Blank Processed Samples (True Positive Verification)
- Non-isotopically labeled standard ester forms (Recovery%)
- Isotopically labeled internal standard free forms (Matrix Effect% and RSD%)
- CPS (Batches Verification)
- Proficiency Test (Absolute Accuracy)

In wet foods, LODs of 1ppb were realized for 2-MCPD esters, 3-MCPD esters, and glycidyl esters reported as 2-MCPD in fat, 3-MCPD in fat, and glycidol in fat respectively.

Keywords: *MCPD, processing contaminants, baby food, mass spectrometry, accreditation*

Acknowledgement: *All authors thank Danone and Mérieux NutriSciences for the opportunity to carry out these activities of Research, Development, Validation, and Accreditation, certainly important for the dissemination of analytical services that are really performing in terms of Food Safety especially for the most sensitive part of the population such as children and babies.*

R11

DETERMINATION OF ACRYLAMIDE IN COFFEE BY LIQUID CHROMATOGRAPHY-TANDEM QUADRUPOLE MASS SPECTROMETRY

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Acrylamide is a well-known carcinogenic contaminant formed at high temperatures during the cooking of starch containing foods. Acrylamide hit the headlines again internationally in 2018, when a judge in California ruled acrylamide fell under the State's Proposition 65 labeling requirements and EU Regulation 2017/2158 was enacted establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food in Europe. The analysis of acrylamide in processed foods has several analytical challenges to consider, including sufficient retention, the complexity of the matrices and the wide range concentrations likely.
A new method, using modified QuEChERS and LC-MS/MS with a high strength silica C18 analytical column, has been developed, to provide a rapid, cost-effective approach for quantifying acrylamide in coffee. Solid phase extraction (SPE) and dispersive solid phase extraction (dSPE) devices were evaluated, to identify simple and efficient cleanup of the samples, to provide selective MRM transitions.
Single laboratory method validation was completed using a selection of store purchased coffee products and a coffee reference material. Acrylamide-d3 was used as an internal standard to correct for any variability through the whole method, including any LC-MS/MS matrix effects. Validation of the method demonstrated excellent performance in terms of linearity, accuracy, precision and repeatability, in accordance with the criteria outlined in Commission Regulation (EU) 2017/2158. Furthermore, results from the analysis of a coffee
Referencematerial demonstrated that the analytical method, using a simple and rapid clean up procedure, was suitable for the determination of acrylamide, in accordance with regulatory requirements.

Keywords: *acrylamide, LC-MS/MS, processed foods*

R12

DETERMINATION OF EIGHT N-NITROSAMINES IN MEAT SAMPLES WITH SPME AND GC-NCD

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N-Nitrosamines are formed by reaction of a nitrosating agent as nitrogen oxide with an organic amino group even if most stable nitrosamines are formed from secondary amines. N-nitrosamines were classified as carcinogens by IARC and US EPA: NDMA (N-Nitrosodimethylamine) and NDEA (N-Nitrosodiethylamine) were classified as 2A, probably carcinogenic to humans, NMEA (N-Nitrosometylethylamine), NDBA (N-Nitrosodibutylamine), NPIP (N-Nitrosopiperidine), NPYR (N-Nitrosopyridine), NMOR (N-Nitrosomorpholine) were classified as 2B, possibly carcinogenic to human.
One of the source for nitrosamines intake is the consumption of sausages and cured meat, where nitrate/nitrite are added as preservatives. Most current methods for nitrosamines detection require extensive sample preparation to isolate these contaminants from the sample matrix. On the other hand, Solid Phase Micro Extraction (SPME) is fast, easy and requires no solvents. Nitrogen Chemiluminescence Detector (NCD) is a nitrogen-specific detector that produces a linear and equimolar response to nitrogen compounds with picogram-level detection limits that could be used for the extremely low levels (ppt) of nitrosamines in food.
A method with the use of SPME in the extraction phase and a NCD detector in the determination phase for the analysis of eight nitrosamines in meat samples was developed. NDMA, NDEA, NMEA, N-Nitrosopropylamine (NDPA), NPYR, NMOR, NPI, NDBA were analyzed. A CAR/PDMS fibre was chosen; a DB 1701 column was used for the chromatographic separation; quantification was based on the use of an internal standard; LOD, LOQ, repeatability, recovery were established.

Acknowledgement: *This work was supported by the Italian Ministry of Health, PGR 2013/101 project*

R13

ROBUST QUANTIFICATION OF ACRYLAMIDE IN FOOD USING GAS CHROMATOGRAPHY-SINGLE QUADRUPOLE MASS SPECTROMETRY

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Acrylamide is a chemical that has been found in some cooked foods (e.g. fried and baked starchy foods such as potato crisps and chips, roasted coffee, breads, peanuts, and cigarette smoke. In foods acrylamide it thought to be formed as a by-product of the Maillard reaction at high temperatures. In baked and fried foods, it may originate from the reaction between asparagine and reducing sugars (fructose, glucose etc.) or reactive carbonyls at temperatures above 120 oC. Acrylamide is highly toxic, can cause neurotoxicity, genetoxicity and reproductive harm, and is a potential human carcinogen. Due to high-water solubility of acrylamide, LC-MS/MS has emerged as the main method for the analysis and favors aqueous extractions from food matrices. However, water will also extract high molecular weight compounds, including proteins, therefore a time-consuming sample clean-up are often also required. Current GC-MS methods mainly involved derivatization via bromination, which is labor intensive. Moreover, breakdown of the brominated acrylamide can occur in the GC injector or column at high temperatures. This work aims to overcome the analytical challenges of current methods applied for acrylamide analysis in food by considering a cost effective, robust approach. A method using silylation of acrylamide with MSTFA (+1% TMCS) as the derivatization reagent for samples extracted with acetonitrile, with detection using single quadrupole GC-MS was developed. Various analytical parameters were considered including selection of ion for increased selectivity (SIM), assessment of chromatographic resolution and peak share, acrylamide linearity, and results repeatability in matrix. Compound linearity was demonstrated over a calibration range of 1 - 1000 ppb (equivalent to 5 – 5000 µg/kg in the prepared sample), with coefficient of determination R2 = 0.9993 and average residual RSD = 4.8%. Good chromatographic resolution with peak asymmetry were observed (tailing factors between 0.94 - 1.03), and peak width (+10%) between 0.063 and 0.066 minutes. Repeatability of results was assessed using spiked ground coffee samples at 1000 µg/kg over n=15 replicate analyses, with acrylamide peak area RSD of 2.9%.

Keywords: *acrylamide, GC-MS, food safety*

R14

THE EFFECT OF NITRITE ADDITION ON N-NITROSAMINE FORMATION IN DIFFERENT PROCESSED MEAT PRODUCTS FROM THE DANISH MARKET

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For decades, nitrite curing has been a widely used method to preserve meat products. Addition of nitrite inhibits the growth of some pathogenic microorganisms as well as lipid oxidation. Furthermore, it contributes to the development of a unique colour, and formation of flavours and aromas. However, during the production and storage of nitrite-cured meat, N-nitrosamines (NAs) can be formed. So far, 20 different NAs have been identified in processed meat products, and among them, many are considered potent carcinogens. NAs include both volatile and non-volatile compounds, and are created by the reaction of nitrite and secondary amines naturally present in meat. Numerous factors, such as the ingoing amount of nitrite or meat quality, can potentially affect the formation of NAs. Therefore, usage of nitrite is regulated in the EU legislation (Directive 2006/52/EC) and generally limited to 150 mg per kg of meat. Due to safety concerns, the Danish authorities maintain national provision generally allowing a maximum of 60 mg of nitrite per kg of meat.

The present study focuses on the estimation of NA levels in processed meat products commonly available on the Danish market. For this purpose, a new efficient, precise and robust analytical method was developed and validated, for the simultaneous determination of both the volatile (NDMA, NMEA, NPYR, NDEA, NPIP, NDPA, NDBA) and non-volatile (NPRO, NTCA, NMTCA) NAs in processed meat products. The method is based on the established methods described in Herrmann et al.[1] and Lehotay et al.[2]. Separation and quantification were carried out by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) using both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). To demonstrate precision, accuracy, specificity, and limit of detection, the method was validated in accordance with ISO 5725-2, using spiked samples of three different processed meat products varying in fat content. Validation results were generally satisfactory with recoveries of 60-110% and RSDs <20. Employing the described method, numerous processed meat samples have been analysed to monitor NA levels and assess the potential hazard to the Danish population, associated with processed meat consumption.

[1] Herrmann S.S.; Duedhal-Olesen L.; Granby K. Occurrence of volatile and non-volatile N-nitrosamines in processed meat products and the role of heat treatment. *Food Control* 2005, *48*, 163-169
[2] Lehotay S.L., Sapozhnikova Y., Han L., Johnston J.J., Analysis of Nitrosamines in cooked bacon by QuEChERS sample preparation and gas chromatography – Tandem Mass Spectrometry with Backflushing. *JAFC* 2015, *63*, 10341-10351

Keywords: *N-nitrosamines, processed meat, cured meat, nitrite, LC-(APCI/ESI)-MS/MS*

R15

SEDIMENT FORMATION IN VEGETABLE OILS AND ITS EFFECT ON THEIR MONOCHLOROPROPANE DIOL ESTER CONTENT

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This work investigates if there is a correlation between the sediment formation in crude vegetable oils and their susceptibility to develop monochloropropane diol esters (MCPDEs). For this purpose, different crude vegetable oils were separated into the sediment-rich and the purified bulk oil. Both fractions were heated and analyzed for their MCPDE content by LC-MS. The measured MCPDE levels and their correlation with the sediment formation will be reported.

Keywords: *monochloropropanediol diesters, sediment formation, vegetable oils*

R16

IMPACT OF TEMPERING AND LASER PRINTING ON THE AMOUNT OF FURAN AND ALKYLFURANS IN CHOCOLATE-BASED PRODUCTS

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New food labelling and packaging innovations are changing the way our foods are brought on the market and consumed today. With a growing demand for less packaging, there are many creative solutions to reduce plastic and label wastes and increase renewability. Natural branding is also oriented toward consumer needs and specifically toward customized labelled foodstuffs such as chocolate. Etching or engraving by laser based techniques become more and more popular. However, laser engraving can affect the final product in term of quality as well as contamination levels. In this work, we focus on the impact of this engraving technique on the formation of furans and its derivatives in chocolate from the raw callets to final transformed products.
Laser printing is an emerging tool recently used for foods labelling [1]. The resulting temperature increase could promote chemical modifications in the treated local area and, could lead to the formation of process contaminants like furans. Furan (C_4H_4O) and its alkyl substituted are found in many foodstuffs undergoing heat treatments [2]. They are formed through multiple pathways, such as Maillard reaction, carbohydrates degradation or lipid oxidation [3]. They have been reported in the range between ppb to ppm level in products subjected to elevated temperature like chocolate, chicory, and coffee [3]. At those levels, these compounds exhibit some toxicological behaviours; the parent furan, for instance, is classified by IARC as possibly carcinogenic to human (group 2B) [4].
To evaluate the impact of processing during both chocolate tempering and laser labelling on those compounds, Head Space Solid Phase MicroExtraction technique (HS-SPME) coupled to Gas Chromatography / Mass Spectrometry (GC/MS) has been used. The conditions of extraction by the HS-SPME have been optimized by Design of Experiments (surface of response methodology). Preliminary results highlight that tempering is responsible of an increase of 24% of furan contamination, while the laser engraving technique lead to 31% of furan increasing indicating that laser labelling is also responsible of furan formation. In total, the furan concentration increases from (3.7±0.3) to (6.1±0.2) µg/kg for the specific case treated in this paper.

(1) Sood, P.; Ference, C.; Narciso, J.; Etxeberria, E.; others. *Proceedings of the Florida State Horticultural Society, Florida, USA, 2008* 2008, *121*, 297–300.
(2) Maga, J. A.; Katz, I. *Critical Reviews in Food Science \& Nutrition* 1979, *11*, 355–400.
(3) Crews, C.; Castle, L. *Trends in Food Science \& Technology* 2007, *18*, 365–372.
(4) IARC. *IARC* 1995, *63*, 393–407.

Keywords: *Furan formation, laser, chocolate, HS-SPME-GC/MS*

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R17

DETERMINATION OF OXY-PAHS AND N-PACS IN FOOD PRODUCTS

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Polycyclic aromatic hydrocarbons (PAH) are well known contaminants in food. Their mutagenic and carcinogenic effects lead to significant human health risk [1].
However the oxidized form of PAHs (oxy-PAH) and the nitrogen containing polycyclic aromatic compounds (N-PAC) have rarely been researched so far. There have been no thorough studies of the occurrence, stability, dissemination, or potential risk.
Therefore coconut oil, as well as fumigated and barbecued products were examined for oxy-PAHs and N-PACs. So we established two sample preparation methods, size exclusion chromatography (SEC) and dispersive SPE. Tandem mass spectrometry (GC-MS/MS) was implemented for the detection and quantification of these analytes by using deuterated PAHs as internal standards.
Our results indicate the presence of oxy-PAHs in low cost coconut oil, as well as smoked and barbecued products. N-PACs were only found in barbecued products. The distribution of the main detected substances in the individual product groups reveal different formation processes or contamination sources. Moreover the amount of oxy-PAHs was not always comparable to the amount of PAHs.

[1] Tsutomu Shimada (2004): *Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and1B1*. In: Cancer Sci, vol. 95, no 1, 1-6.

Keywords: *oxy-PAH, N-PAC, PAH, GC-MS/MS, dispersive SPE*

R18

DEVELOPMENT OF AN AUTOMATED SAMPLE PRE-TREATMENT AND ANALYSIS OF FREE AND ESTER-BOUND 3-MCPD, 2-MCPD AND GLYCIDYL ESTERS IN VARIOUS FOOD MATRICES

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3-Monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) are substances that can be generated in the food processing. Free MCPD is usually formed through roasting and smoking. After formation free-forms can be bound to molecules present in the food.
Another group of substances identified in fats and oils, of the same interest and analyzable in the same method, are glycidyl esters (GE).
An automated sample pre-treatment method coupled to direct analysis of 3-MCPD, 2-MCPD (bound and free) and GE in edible oil, fat and foodstuff was developed and optimized.
This method is also validated in edible oil, fat and foodstuff for 3-MCPD, 2-MCPD (bound and free) and GE.

R19

POLYCYCLIC AROMATIC HYDROCARBONS LEVELS DURING COCOA BEANS PROCESSING

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Polycyclic aromatic hydrocarbons (PAHs) are contaminants formed during incomplete combustion of organic matter and some of them are considered carcinogenic and genotoxic. Contamination of cocoa and its derived products by PAHs may occur due to high temperatures used to roast cocoa beans and the possible presence of smoke during drying. The objective of the present study was to investigate the behavior of 13 PAHs during cocoa processing and production of cocoa liquor, cocoa powder and cocoa butter. Analytical method involved extraction with hexane, liquid-liquid partition with dimethylformamide:water, cleanup with C18 SPE and determination by HPLC-FLD. Two samples of cocoa beans were used for processing, one dried using a dryer with hot air from wood combustion (resulting in a "smoked" sample) and another dried without the presence of smoke. Beans were roasted at 120 °C for 60 minutes. An initial contamination was detected in both samples, the sum of PAHs detected was 1.78 µg/kg for non-smoke cocoa beans and 66.24 µg/kg for smoked cocoa beans. After roasting, a decrease in contamination was observed with levels of 1.47 µg/kg for non-smoke beans and 16.30 µg/kg for the smoked ones. As for the derived products, the following levels were detected. Non-smoke cocoa beans: 2.02 µg/kg (liquor), not detected (shell), 1.46 µg/kg (powder) and 2.09 µg/kg (butter). Smoked beans: 13.56 µg/kg (liquor), 137.37 µg/kg (shell), 4.94 µg/kg (powder) and 31.70 µg/kg (butter). All samples were in accordance with maximum PAHs levels established by European Union regulation, except for smoked cocoa beans shell. A mass balance was done in order to analyze PAHs behavior during processing of roasted beans. For non-smoke beans, after milling 91% of PAHs were detected on the liquor, 9% remained in the mills leftover and no PAHs was present in the shells. After pressing, PAHs were transferred in equal proportions to butter (34%) and powder (37%). The remaining PAHs (20%) were retained by the leftover liquor in the press. For smoked beans, 55% of PAHs were transferred to liquor after milling, 31% remained in the shells and 14% in the leftover in the mill. After pressing, compounds were transferred mainly to the butter (43%), while 11% were transferred to powder and the remaining PAHs (1%) were retained by the leftover liquor in the press. Results indicate that contamination of cocoa beans with smoke possibly occur during hot air drying as a result of direct contact with smoke from wood burn. A tendency for PAHs to migrate to cocoa butter was observed. Drying cocoa beans without contact with smoke could reduce contamination and, therefore, human exposition to potentially carcinogenic and genotoxic compounds.

Keywords: *polycyclic aromatic hydrocarbons, cocoa, HPLC-FLD, cocoa butter, cocoa liquor*

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R20

OPTIMIZING GC-MS AND GC-MS/MS ANALYSIS OF 3-MCPD AND GLYCIDYL ESTERS

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3-MCPD and glycidyl esters in edible oils are contaminants that are formed through refining processes and have been classified as possible human carcinogens. Methods have been developed by ISO, AOCS, and DGF for analyzing these contaminants. While these methods cover extraction and derivatization techniques in detail, very little attention is paid to the GC-MS analysis method. With emerging automated systems, it is important to simplify and speed up the instrument method by optimizing the parameters and switching to split injection.
Our initial optimization of the temperature program led to an 8-minute decrease in analysis time, however additional time can be saved by utilizing free method development software. The employment of split injection resulted in better peak shape and can achieved limits of detection that were comparable to splitless injection. Further evaluation of split injection revealed that performance similar to splitless injection can be achieved regardless of inlet temperature, which results in greater flexibility for different inlet configurations.

Keywords: *MCPD, glycidyl ester, edible oils*

R21

THE ANALYSIS OF ACRYLAMIDE USING AN AQUEOUS COMPATIBLE REVERSED-PHASE COLUMN BY LC-MS/MS DETECTION

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Acrylamide is formed when substances containing asparagine and aldehyde sugars (i.e. glucose, fructose etc.) are roasted, fried or baked at temperatures above 120 °C. The foods with the most acrylamide are roasted coffee and starchy foods such as potato chips, toasted bread, and cereal. Acrylamide is also found in drinking water that has been treated with polyacrylamide as a flocculating agent. Laboratory studies performed on mice show that high level exposure can cause reproductive harm, neurological defects and cancer. Accordingly, many methods have been developed to determine the amount of acrylamide present in foods, tobacco and water. Multiple sample preparation techniques are effective at extracting acrylamide from matrix, but it is important to determine how much time should be invested on sample preparation and what stress the sample will place on your analytical system. QuEChERS-based methods tend to be quicker than SPE-based methods, but typically have more matrix components co-extracted which can lead to worse detection levels and more stress on your detector. SPE-based methods, result in a cleaner sample, but take a lot of time to prepare. LC columns used to quantify acrylamide often suffer from irreproducibility and poor column lifetimes. This results in longer turnaround times, less instrument uptime, and poor data quality. The Allure Acrylamide column addresses these pain points. This silica-based, aqueous compatible, reversed-phase column is part of a reproducible, retentive, and robust solution. The benefits of this column will be discussed showing examples of acrylamide separation from difficult matrices such as coffee and potato products. Particular emphasis on the role varying degrees of sample preparation will be discussed.

Keywords: *acrylamide, LC-MS/MS, sample preparation, coffee, potato crisps*

R22

FURAN AND ALKYLFURANS IN CEREAL BABY FOODS: OPTMIZATION AND VALIDATION OF A HS-SPME GC/MS METHOD

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Furan and its methyl derivatives are process contaminants involved in the food flavouring. They have been first reported in food in 1979 by Maga et al. [1] and furan itself has been classified as possibly carcinogenic to humans two decades later [2]. Since, the food safety authorities have paid more attention to this food borne contaminant and highlight its occurrence in coffee, baby food and snacks [3]. Those data have been used in risk assessments [4,5] and have demonstrated a risk related to its ingestion by babies. Recent studies [6,7] put forward that furan methyl derivatives such as 2-methylfuran, 3-methylfuran, and 2,5-dimethylfuran can have toxic effect as well. In that framework, food safety authorities within EU Member States were asked to provide additional data regarding the occurrence of alkylfurans in foodstuffs.
A HeadSpace Solid Phase MicroExtraction method coupled to Gas Chromatography/Mass Spectrometry (HS-SPME GC/MS) using the isotope dilution for the quantitation has been optimized in naturally contaminated cereal baby foods through a Central Composite Design approach. It highlights that optimal conditions are different but close for every analyte. Therefore, compromised optimal conditions were found to be an extraction temperature of 30 ℃ for 35 minutes.
This method has been validated in spiked cereal baby food at three levels (10, 30 and 60 µg/kg) for three days in triplicate. The validation shows that the method fills the European Commission requirements with a high sensitivity (LOQs < 2 µg/kg) and an intermediate precision between 2 and 13%.

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[7] H. K. Knutsen, J. Alexander, L. Barregård, M. Bignami, B. Brüschweiler, S. Ceccatelli, B. Cottrill, M. Dinovi, L. Edler, B. Grasl-Kraupp, and others, *Risks for public health related to the presence of furan and methylfurans in food*, Wiley Online Library, 2017, vol. 15.

Keywords: *furan, methylfuran, SPME, Central Composite Design, Validation*

Acknowledgement: *The authors would like to thank the Belgium Food Safety Agency (FAVV-AFSCA) for financial support (FAVV-NRL-CHEMIE1-2017: lot 3)*

R23

SIMULTANEOUS DETERMINATION OF 3-MCPD, 2-MCPD AND GLYCIDYL ESTERS TOGETHER WITH THEIR OXIDATION PRODUCTS BY SUPERCRITICAL FLUID CHROMATOGRAPHY – HIGH RESOLUTION MASS SPECTROMETRY (SFC-HRMS)

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Refined vegetable fats and oils (especially palm oil) are a primary dietary source of process contaminants such as esters of monochloropropanediol (MCPD esters) and glycidol. Since they have shown adverse effect on a human health, their occurrence in refined fats/oil and products for preparation of which were used (e.g. bakery products) should be monitored. Number of analytical methods have been developed in the past for this purpose mainly employing reverse phase liquid / gas chromatography coupled with mass spectrometry. However, both of these widely used techniques may suffer of some limitation such as time-consuming sample preparation, primary compound pattern losses etc.
This study was focused on the development of an innovative method applicable for simultaneous determination of 3-MCPD esters, their positional isomers 2-MCPD esters and glycidyl esters. A simple rapid 'dilute-and-shoot' approach (sample preparation not needed) based on a supercritical fluid chromatography (SFC) coupled with high-resolution mass spectrometry (HRMS) was employed. In addition to quantification of target analytes, detection and identification of MCPD esters with oxidized fatty acid chain was enabled by HRMS/MS. The use of ion mobility (IM) for a better separation of positional isomers of 2- and 3-MCPD esters was tested.
The developed method was validated for 9 esters of 3-MCPD, 2 esters of 2-MCPD, 6 glycidyl esters. The limit of quantification for all 3-MCPD esters was 5 µg / kg fat. Recoveries and repeatabilities of measurements were determined at two concentration levels 156 and 1560 g / kg of fat. For the lower one, recoveries were 85–102% and repeatabilities (expressed as RSD) ranged between 2.7-5.3%. For a higher spiking level, recoveries were 94–101% and repeatabilities were in the range 0.6–9.4%. The trueness of the method was verified through analysis of a ´reference´ material (palm oil) obtained from the proficiency test. Validation parameters for the other analytes are under development.

Keywords: *MCPD esters, SFC, MS, glycidyl esters, processing contaminants*

Acknowledgement: *This work was supported by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities, "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 and by the specific university research (MSMT No 21-SVV/2019) - JIGA project.*

R24

APPLICATION OF MODIFIED QUECHERS METHOD FOR THE SIMULTANEOUS DETERMINATION OF THERMAL PROCESSING CONTAMINANTS IN CRAFT BEERS

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Beer, the oldest fermented drink, is world's most widely consumed alcoholic beverage and is the third-most popular drink overall, after water and tea. Industrial beers are usually obtained by fermenting malted barley and wheat. However, apart from commercial beers produced by large worldwide companies, craft beers manufactured in small regional breweries have recently become recognised and willingly consumed by sophisticated gourmets. Craft beers have more unique taste and aroma, resulted from the use of many specific ingredients during its production. Nevertheless, the question is, whether craft beers are safe for consumers?
Among the different contaminants that can be present in beers, thermal processing contaminants are a group that poses a major threat to human health, due to their toxic and carcinogenic effects. This group involves acrylamide (AA), polycyclic aromatic hydrocarbons (PAHs) and 3-monochloropropane-1,2-diol (3-MCPD). All these compounds are formed during e.g roasting, smoking or caramelisation, hence, some beer ingredients such as malt or flavour additives can be contaminated with these compounds.
Therefore, the aim of this study was to assess the occurrence of AA, PAHs and 3-MCPD in selected dark craft beers available on Polish market. The experiment covered development and optimalisation of modified QuEChERS method that was applied for the preparation of beer samples. The protocol included extraction with acetonitrile, dispersive SPE clean-up, preconcentration by dispersive liquid-liquid microextraction (DLLME) and derivatization in the case of 3-MCPD. Final extracts were analysed by GC-IT-MS (PAHs, 3-MCPD) and HPLC-DAD (AA).
Results showed that PAHs were detected in all tested beers. However, its levels were low and the total PAH sum reached only 20 μg kg^{-1}. ΣPAH4 did not exceed 1.3 μg kg^{-1}. Acrylamide was identified in all beers with the highest result at the level of 59 μg kg^{-1}, while free 3-MCPD was found only in several samples approaching the level of 12 μg kg^{-1} in the most contaminated sample beer. Although the obtained results are low, it should be remembered that regular consumption of standard portion of beer (approximately 500 g) in the addition with other source of these contaminants may contribute to negative effects for human health.

Keywords: *craft beer, polycyclic aromatic compounds, acrylamide, 3-monochloropropane-12-diol, QuEChERS*

Acknowledgement: *This research was performed with the financial support from Ministry of Science and Higher Education of Republic of Poland within the statutory R & D activities (DS-3700/KTGiK/2018).*

R25

ASSESSMENT OF THE ACRYLAMIDE LEVEL CONTAMINATION IN FOOD CONCENTRATES

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Diet has always been an important factor in human life and its main aim is to provide appropriate amount of nutrients, vitamins and minerals to human body. Society in 21st century is increasingly becoming a conscious consumer. People pay attention primarily to the energy value of individual products, the content of carbohydrates, proteins and fats. Unfortunately, during technological processing, interactions occur between individual food ingredients, giving completely new contaminants. One of them, acrylamide (2-propenamide), which is toxic to organisms, is formed during such heat treatment at temperatures above 120 °C. It occurs in many thermally processed food products e.g. food concentrates. Food concentrates in general are food products that are characterized by high dry matter content obtained through drying process and the concentration of individual ingredients.

The determination of acrylamide was carried out in three different groups of food concentrates: poultry and beef cubes; light, dark and hunting instant sauces and two types of powdered soups: broth ad mushroom soup. Analyses were carried out using modified QuEChERS method with the final detection by HPLC-DAD.

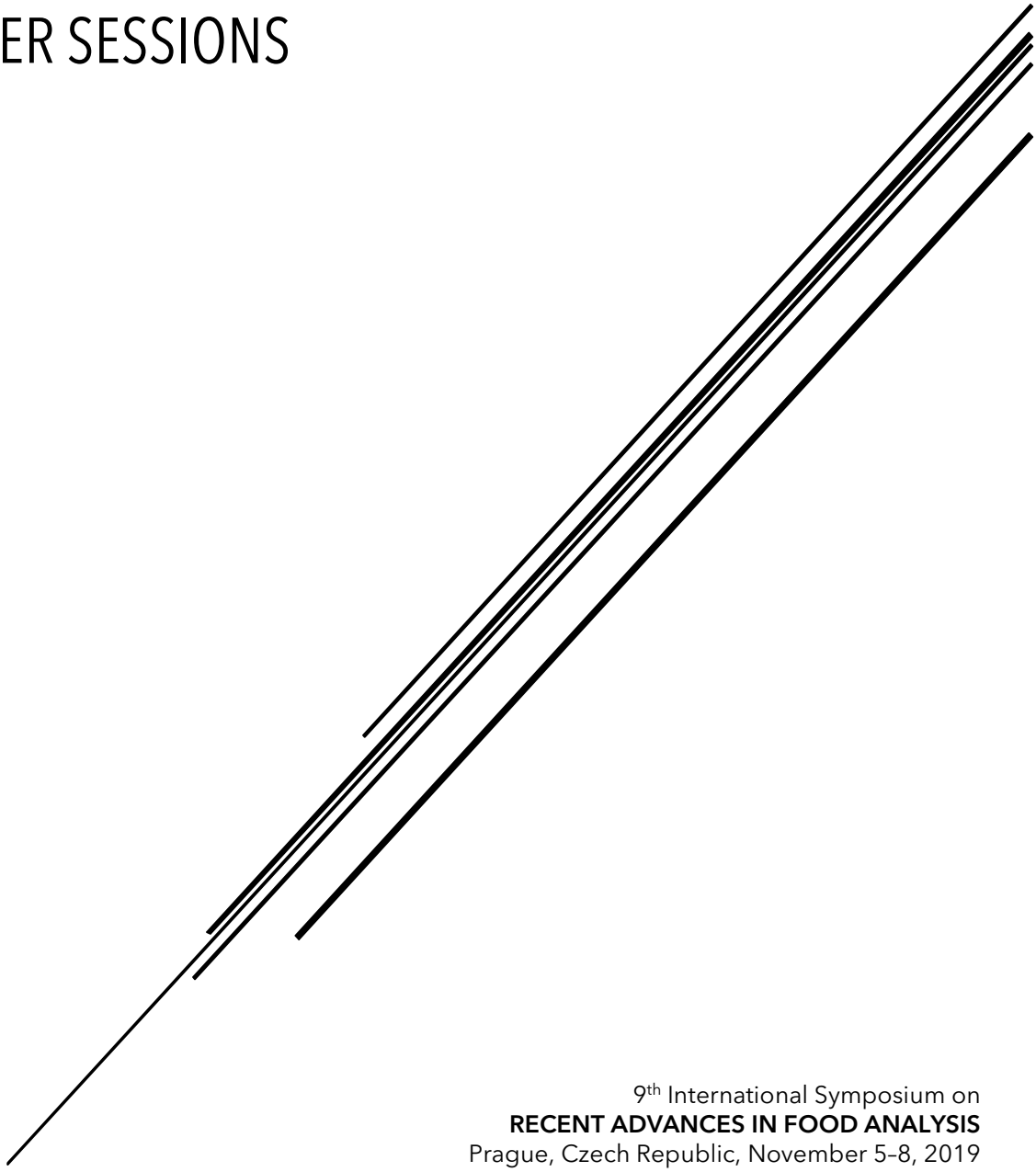
The results showed that acrylamide was not detected only in two samples: one poultry cube and one light sauce. Generally, AA levels ranged from 338 to 2537 μg kg^{-1} for poultry cubes, from 319 to 1923 μg kg^{-1} for beef cubes, from 0 to 3361 μg kg^{-1} for light instant sauces, from 1025 to 5801 μg kg^{-1} for dark sauces, and from 2129 to 2767 μg kg^{-1} for hunting sauces. In broth and mushrooms powdered soups AA content was in the range from 901 to 2129 μg kg^{-1} and from1074 to 2770 μg kg^{-1}, respectively. The highest level of 2-propenamide was detected in one sample of dark sauce - around 5801 μg kg^{-1}. Generally products with darker colour, such as: beef cubes, dark and hunting sauces, were characterized by much higher level of acrylamide in comparison to the products with lighter colour, e.g. chicken cubes, light sauces. This probably results from the addition of caramel, sugars and various types of fats to food concentrates. Therefore, this should be limited to minimize the formation of acrylamide. Assuming that the body weight is about 70 kg and that the acceptable daily intake of acrylamide is 1μg kg-1 body weight, tested food concentrates can be considered safe for adult health. However, a regulation should be created regarding the admissible amounts of this toxic substance in food concentrates.

Keywords: *food concentrates, acrylamide, processing contaminants, QuEChERS*

Acknowledgement: *This research was performed with the financial support from Ministry of Science and Higher Education of Republic of Poland within the statutory R & D activities (DS-3700/KTGiK/2018).*

RESIDUES – PESTICIDES

POSTER SESSIONS



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S1

FIPRONIL IN EGGS: A NEW CERTIFIED REFERENCE MATERIAL ON ITS WAY

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In August 2017 millions of eggs were destroyed and egg products removed from the shelves of supermarkets and stores. According to the RASFF (Rapid Alert System for Food and Feed) alert triggered by the Belgian authorities, high levels of fipronil were detected in chicken eggs (from 0.0031 to 1.2 mg/kg)[1]. The contaminated eggs were discovered in Belgium, the Netherlands, France, a dozen other European countries and beyond.
Fipronil is used as a broad-spectrum insecticide to protect crops as well as in veterinary medicine in flea control products. If a pest infestation at a farm is treated with fipronil, the animals's skin, or feathers in case of chickens, could absorb the insecticide. Traces can then also be found in animal products like eggs.
To ensure safe food for the citizens, European legislation[2,3] establishes maximum residue levels (MRL) of pesticide residues in food stuffs. For control and monitoring, laboratories need reliable analytical methodologies developed and validated in accordance with the standard requirements listed e.g. ISO 17025[4] or other standards. Certified Reference Materials (CRMs) are the preferable option to provide evidence of method performance[5].
Soon after the fipronil crisis, the Joint Research Centre of the European Commission (EC-JRC) launched a survey to collect information about needs of CRMs in that area. As a follow up of the survey and as a response of the needs, the EC-JRC initiated the production of a CRM for determination of fipronil in eggs (ERM-BB125).
The ERM-BB125 is processed at the JRC's dedicated installations from a batch of contaminated eggs originating from an affected farm. Fipronil is mainly present in the material as fipronil sulfone. Tests for homogeneity, stability during transport and long-term stability at different temperatures are successfully conducted in-house. Meanwhile the candidate CRM is characterised for fipronil content, expressed as a sum of fipronil and fipronil sulfone mass fraction, through a certification campaign conducted as inter-laboratory comparison. Analytical methods applied fulfil ISO 17025 requirements.
The different steps followed for the production of the CRM, performed under accreditation scope of ISO 17034, will be presented.

[1] RASFF–the Rapid Alert System for Food and Feed (https://ec.europe.eu/food/safety/rasff_en)
[2] Regulation (EC) No396/2005 of the European Parliament and of the Council of 23.02.2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin amending Council directive 91/414/EEC
[3] Commission regulations (EU) No 1127/2014 of 20.10.2014 amending Annexes II and III to Regulation No 396/2005
[4] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories, International Organization for Standardization
[5] Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed SANTE/11813/2017

S2

QUALITATIVE SCREENING FOR FUNGICIDE DITHIOCARBAMATES USING QUECHERS METHODOLOGY

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Despite of the fact that the first representatives of dithiocarbamate fungicides (DTCs) were introduced more than 50 years ago (thiram in 1942, zineb and nabam in 1943, maneb in 1955, mancozeb in 1962), DTCs are still among the most extensively used organic fungicides in modern agriculture.
For routine pesticide residue laboratories, being in charge with controlling compliance of food with maximum residue levels, analysis of DTCs remains challenging since the physicochemical properties of various representatives belonging to this compound class lead to analytical difficulties. The polymeric DTCs (ethylene-*bis*-DTCs fungicides (e.g. mancozeb) and propylene-*bis*-DTCs fungicides (propineb)) are macromolecular metal-coordinated complexes of variable size and are thus virtually impossible to analyze directly and are practically insoluble in aqueous and organic solutions. Thiram is soluble e.g. in toluene, but easily degrades during analysis. For these reasons, routinely applied multi-residue methods which involve an extraction/partitioning step of individual DTCs into organic solvents are not applicable. Various single residue methods are described in literature for the analysis of DTCs (e.g. by derivatization with dimethyl sulfate), but most routine pesticide laboratories apply a methodology that is based on the chemical cleavage of DTCs by a mixture of tin(II)-chloride and hydrochloric acid and the partitioning of the released carbon disulfide (CS_2) into an organic solvent. Then, the quantitative analysis of CS_2 is either achieved by spectrophotometry or gas chromatography with different detector options (e.g. GC-ECD). From the practical point of view, the disadvantage of this method is that a laborious analysis has to be conducted without having any information if the analytes of interest are present in the sample or not. A qualitative screening approach for DTCs by a routinely applied multi-residue method would help to overcome this drawback by allowing the selection of positive samples. Consequently, workload and costs could significantly be reduced for the quantitative DTC-analysis.
In this study, characteristic degradation products of (a) ethylene-*bis*-DTCs (e.g. mancozeb, maneb, zineb), (b) propylene-*bis*-DTCs (propineb) and (c) N,N-dimethyl-DTCs (e.g. thiram, ziram) were identified and it was tested if these substances could be used as screening indicators for DTCs in QuEChERS-extracts analyzed by routine GC- and/or LC-MS techniques. The screening detection limits were determined according to Document No. SANTE 11945/2015 for high-water content commodities to ensure the reliable and sensitive identification of these indicator substances at a specific level of concentration. QuEChERS-extracts of numerous samples of plant origin were screened. Positive samples were re-analyzed by the chemical cleavage approach involving the release of CS_2 for confirmatory and quantitative purposes. The results of this study are discussed during the presentation.

Keywords: *dithiocarbamate, fungicide, QuEChERS*

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S3

FAST ANALYSIS OF MULTI-CLASS PESTICIDES PANEL IN WINE AND OLIVE OIL EXTRACTS USING A SINGLE RUN LC-TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Increasing food safety concerns and the growing agricultural trade has resulted in stringent pesticide regulations globally. To comply with such regulatory standards, screening methods for large numbers of pesticides is becoming important. Using liquid chromatography coupled Tandem quadrupole mass spectrometry offers highly sensitive, specific and selective detection in complex matrices for analysis of multi-class pesticides in food samples (wine and olive oil).
An Accucore aQ column was utilized for the separation of all analytes within 15minutes. Wine and olive oil were extracted with organic solvent using a simplified QuEChERS method and lipid removal cartridge to help remove excess fat or oil from olive oil and 1uL of sample was injected with a Vanquish Flex HPLC coupled to a TSQ Quantis triple quadrupole mass spectrometer. A multi-residue method was developed for screening (550+) and quantitation of approximately 300 pesticides in one 15-minute run with polarity switching. Ion ratios were used to confirm each analyte (±30%), plus accuracy of retention time to ±0.1min to show robustness of the method which are required for the EU SANTE Guidance 11813_2017. All pesticides analyzed show excellent Limits of Quantitation and Detection between 0.5 to 10ppb, while reproducibility (injection = 8/level) showed excellent precision and linearity with R2=0.9900. Utilization of the lipid removal cartridge showed good %Rec between 10ppb and 50ppb between 70-120% which is within the SANTE Guidance. Unknown samples of wine and olive oil were also screened. Furthermore, the method was developed using software with built-in workflows for streamlining method development and routine analysis.

Keywords: *multi-class pesticides, quantitation, screening, SANTE, QuEChERS*

S4

A MULTIRESIDUE METHOD FOR PESTICIDE ANALYSIS USING AN ORBITRAP TRIBRID MASS SPECTROMETER AND AUTOMATIC BACKGROUND EXCLUSION

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Introduction: Pesticides are routinely applied to crops for preventing, destroying or controlling pest activity. Given the large number of pesticides used and the globalization of the food industry, multiresidue methods offer a great advantage allowing analysis of hundreds of pesticides in a single run. We have implemented a multiresidue method for the analysis of 250 pesticides on an Orbitrap ID-X Tribrid mass spectrometer utilizing an automatic background extraction workflow (AcquireX).
Experimental: Strawberry samples were obtained from a local retail store. Following homogenization, strawberry samples were extracted using a QuEChERS extraction kit. The matrix extracts were spiked with the pesticide standards (250 pesticides) at different concentration levels ranging from 0.05 to 200 ppb. Chromatographic separation was performed on a Vanquish UHPLC system using an Accucore aQ column. Mass spectrometric analysis was performed on an Orbitrap ID-X Tribrid mass spectrometer using AcquireX workflow, for automated generation of background exclusion list, or data dependent acquisition (DDA).
Results: We have evaluated the performance of a multi-residue pesticide method utilizing high mass accuracy and high resolution for semi-quantitation and screening of pesticide residues in a strawberry matrix. Excellent detection limits, reproducibility, linearity and accuracies were obtained. Overall, for 250 pesticides, out of 251 tested, the LODs were at/or below 5 ppb with 215 pesticides having LODs at/or below 1 ppb. LOQs were below 5 ppb for 247 pesticides tested. When the AcquireX workflow was applied for automated background exclusion we observed a significant increase in the number of library matches compared to DDA, especially at the lower concentration levels. For instance, at a spiked concentration of 0.5 ppb the presence of 19 pesticides was confirmed via library search with DDA. When utilizing the AcquireX workflow, at the same concentration level, the presence of 145 pesticides was confirmed. Similar trends were observed at a concentration level of 1 ppb in which we observed 178 library matches with AcquireX versus 65 library matches with DDA.

Keywords: *pesticides, AcquireX, Orbitrap ID-X, multi-residue, quantitation*

S5

VALIDATION OF THE ANALYTICAL METHOD FOR TRIFLUMEZOPYRIM IN AGRICULTURAL PRODUCTS USING QUECHERS AND LC-MS/MS

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A method for the determination of triflumezopyrim in agricultural commodities was developed using the QuEChERS(Quick, Easy, Cheap, Effective, Rugged and Safe) method followed by LC-MS/MS. Samples were extracted with acidified acetonitrile, followed by addition of sodium chloride and anhydrous magnesium sulfate. Dispersive solid phase extraction(d-SPE) cleanup was carried out using $MgSO_4$, C_{18} and GCB. The analytes were quantified and confirmed with LC-MS/MS using ESI(electrospray ionization) in positive ion MRM(multiple reaction monitoring) mode. The matrix-matched calibration curves were constructed using six levels(0.002~0.2 mg/L) and coefficient of determination(r^2) was above 0.99. Recovery results at three concentrations(LOQ, 10xLOQ, and 50xLOQ, n=3) were from 81.2 to 109.6% with relative standard deviations(RSDs) less than of 8.4%. The limit of detection and limit of quantification for triflumezopyrim were below 0.003 and 0.01 mg/kg, respetively. All results satisfied the criteria ranges requested in the Codex guidelines(CAC/GL 40-1933, 2003) and the Food Safety Evaluation Department guidelines(2016). The proposed analytical method was accurate, effective and sensitive for triflumezopyrim determination. This study can be useful as official method for triflumezopyrim residues.

Keywords: *triflumezopyrim, QuEChER,S LC-MS/MS*

S6

DETECTION OF PESTICIDES AND HERBICIDES IN CRAFT BEER BY DART-MS

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The plants that produce the main agricultural ingredients used in the crafting of beer, such as barley and hops, are often treated with pesticides to reach good yields and reduce the losses during storage by protecting the plants from insects and pests. Herbicides are also used to protect the plants from weeds. Glyphosate is a known carcinogenic herbicide that has been recently found in 14 beers, which included beers from major brands like Budweiser, Guinness, Samuel Adams, etc. These pesticides and herbicide agrochemicals can persist in the plants for a long time and could be carried over to the beer from raw materials, malt and hops. As a result, it is important to be able to detect pesticide and herbicide residues in the finished beers.
Here we describe a high throughput analytical method that employs Direct Analysis in Real Time combined with mass spectrometry (DART-MS) for detecting pesticides in beer. Craft beers are spiked with various concentrations of glyphosate and pesticides such as azoxystrobin, flonicamid, metalaxyl, and imidacloprid to simulate finished beer containing pesticides. Beers are sampled using stainless steel pins for automated analysis by DART-MS. Limit of detection is determined for each pesticide. Chemometric models are created and employed to determine beers that contain trace amounts of pesticides. These beers containing pesticides are then searched against our DART-MS library database to identify the specific pesticides. This method can be potentially used to monitor pesticides in finished beer products.

Keywords: *DART-MS, BEER, PESTICIDE*

S7

USE OF SUPERCRITICAL FLUID CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY FOR THE ANALYSIS OF PESTICIDE RESIDUES IN FRUITS AND VEGETABLES

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Supercritical fluid chromatography coupled to electrospray mass spectrometry (SFC-ESI-MS) has proven to be an alternative to the very well-know reverse-phase liquid chromatography. This type of chromatography has experienced an increment in terms of robustness providing many advantages for the analysis of pesticide residues in fruits and vegetables. As SFC use CO_2 as mobile phase, high flow rates can be applied providing short run times with good chromatography resolution. The CO_2 used for the elution return to gas state when it reaches atmospheric pressure after the backpressure. The absence of water in the mobile phase and the low flow that reach the ESI source provide an excellent ionization efficiency. This different chromatographic operation allows the possibility to detect and quantify some compounds usually analyzed by gas chromatography (GC) like pyrethroids. However, some drawbacks related to isobaric interferences and injection volume/solvent limitations have been observed. Also, retention time cannot be predicted because of the many parameters involved in the SFC elution, not just the polarity. A complete evaluation coupling SFC to triple quadrupole (QQQ) and high-resolution mass spectrometry (QTOF) was performed in terms of limit of quantification (LOQ), reproducibility, linearity, and matrix effect. More than 200 pesticides were analyzed by both detectors, most of the pesticides studied were identified at a concentration of 10 µg/Kg. High reproducibility of the areas and retention times were achieved using SFC. The linearity of the method was evaluated employing matrix-matched standards, coefficients of determination equal to or higher than 0.99 were achieved in the corresponding linear ranges and the back-calculated concentration deviations were lower than 20%. Low matrix effect can be observed because of the high sampling efficiency in the source even in complex matrices like spices. As a summary, supercritical fluid chromatography represents a good alternative for reverse-phase liquid chromatography multi-residue methods. Similar accuracy was achieved using SFC, but this different type of chromatography offers many important advantages.

Keywords: *SFC, pesticide residues, supercritical fluid chromatography, mass spectrometry*

S8

PLANT UPTAKE OF PESTICIDES PRESENT IN IRRIGATION RECLAIMED WASTEWATER: A PILOT EXPERIMENT IN RED CABBAGE

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Reclaimed wastewater usage is increasing worldwide, particularly, its use in crop irrigation shows a growing trend. However, the potential risks associated with these waters have not been extensively studied to date. The presence of organic microcontaminants (pesticides, drugs, etc.) is one of such hazards. As an illustrative case, in Spain one third of all recycled wastewater per annum is employed for crop irrigation. For this reason, the assessment of the potential risks is critical.

Pesticides are microcontaminants which encompass organic compounds with vastly differing physicochemical properties, and hence their environmental and plant behaviour can also be disparate. To learn about the behaviour of certain pesticides that could be present in regenerated wastewaters, and how much (if any) reaches plants grown with these waters, a pilot experiment has been undertaken.

A selection of 20 pesticides of varying water solubility and octanol-water partition coefficient has been used to spike irrigation, unpolluted water. The spiked water was used to grow red cabbages in a greenhouse under controlled agronomic conditions from October to December. The experiment was designed in a way that 1 µg/L of each microcontaminant would reach the plants. Water samples were collected weekly from three irrigation points, and red cabbages were harvested once they reached maturity, hearts and leaves separately. Control red cabbages, irrigated using the unspiked water, were also grown during the experiment under the same agronomic conditions. Throughout the experiment, the irrigation flow was adjusted so that at least 1 µg/L of most pesticides could be quantified in the collected water samples.

Red cabbages were extracted using the QuEChERS procedure, and all analyses were performed on a liquid chromatography (LC) system coupled triple quadrupole mass spectrometer in tandem mass spectrometry mode (MS/MS). Water samples were diluted with 10 % acetonitrile and directly injected into the LC-MS/MS instrument.

The analysis of red cabbage hearts demonstrated that 14 out of 20 pesticides were detected at concentration levels below 2 µg/kg. In the case of red cabbage leaves, 13 out of 20 spiked pesticides were detected, but concentration levels were around ten times higher. Eight pesticides were found at markedly higher concentrations in the outer leaves compared to the hearts, which suggests that accumulation of these microcontaminants is taking place. In no case the concentration levels rose above the maximum residue level for the detected pesticide in red cabbage.

Finally, a strong correlation was found when plotting a pesticide's water solubility versus its concentration in red cabbage, which suggests that plant uptake of pesticides is highly dependant on said solubility. As such, water soluble pesticides in reclaimed wastewaters ought to be carefully monitored when intending to reuse these waters for crop irrigation.

Keywords: *reclaimed wastewater, crop irrigation, plant uptake, pesticide*

Acknowledgement: *We would like to thank the Ministry of Economy, Industry and Competitiviness for the grant "Riesgos emergentes de contaminación química y microbiológica en la reutilización de aguas residuales para riego agrícola: estudio integrado (ROUSSEAU)" (grant CTM2017-89767-C3-1-R).*

S9

UNTARGETED DIA ANALYSIS OF QUECHERS EXTRACTED PESTICIDES BY HIGH RESOLUTION ACCURATE MASS LCMS

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To minimize the risk to public health, maximum residue levels (MRLs) have been set for pesticides in food products/groups within the EU and guidance for analytical quality control and method validation procedures for pesticide residues and analysis in food and feed have been published (SANTE/11813/2017). In the SANTE/11813/2017 guidance document, criteria are defined for the identification and confirmation of pesticides using both nominal mass and high-resolution accurate mass (HRAM) detection. A common approach in pesticide monitoring programs is to use triple quadrupole nominal mass detection using a defined list of pesticides in a targeted workflow. However, HRAM detection has the advantage of supporting both targeted workflows for quantitative analysis and untargeted workflows for a retrospective for unknown or unexpected pesticides.
In this work, QuEChERS extracted food commodities were spiked with a panel of over 200 pesticides over a calibration range between 0.002-0.2 mg/kg and injected onto the LCMS-9030 QTOF system (Shimadzu Corporation) with no further sample pretreatment. Samples were separated using a mobile phase water/methanol containing 2mM ammonium formate, 0.002% formic acid (column: Restek Raptor Biphenyl, 100 x 2.1mm 2.7µm). In this analysis, the DIA method was configured with a full scan MS acquisition (m/z 140-900) and 38 DIA MS/MS scanning events isolating at 20Da widths from m/z 140 to m/z 900. All product ion masses were assigned using an in-house application to verify fragment structures with theoretical mass values. DIA collision energy was configured to scan 0-30V to enable detection of unfragmented parent ion in the MS/MS data stream.
Using the DIA method with a list of expected targets, over 200 pesticides in the apple calibration matrix could be detected at the default MRL concentration of 0.01 mg/kg. All data were acquired using external mass calibration with no mass correction applied to any batch analysis. Typically more than 15 points across a peak could be achieved due to the high speed data acquisition. The coefficients of determination (R^2) were >0.996 within a concentration range of 0.002-0.2 mg/kg, using both linear and quadratic curves (compound dependant).
In conclusion, a HRAM DIA-MS/MS method has been applied to the quantitation of a panel of pesticides in agreement with the criteria defined in the SANTE/11813/2017 guidelines. This approach resulted in a robust quantitative method which can be used for both targeted and untargeted workflows.

Keywords: *pesticides, DIA, Q-TOF, QuEChERS, LCMS*

S10

SCREENING VALIDATION ON PESTICIDE RESIDUES IN EGGS USING LC/Q-TOF

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Nowadays pesticide residue laboratories are facing lot of challenges, that puts a significant burden on how they run their lab infrastructure. Analyses should be fast, easy, economical and provide continuously decreasing limits of detection/quantification. In order to monitor the extensive number of approved pesticides on the market, the objective is to analyse as many pesticides as possible using multi-residue methods (MRM). The introduction of high resolution accurate mass instruments, like Time-of-Flight or Orbitrap, has revolutionized the possibilities of MRM, particularly in terms of mass accuracy and "unlimited-scope" of analytes.
The Agilent 6540 LC/Q-TOF (Agilent Technologies) has been successfully used by EURL-AO for the qualitative screening of pesticide residues in food of animal origin over the last years. Validation studies in honey, milk, meat or egg with established sample preparation methods like QuEChERS demonstrated the broad application area as well as the limits of the method.
For this study, we used the Agilent 6545 LC/Q-TOF, which is a mid-range Quadrupole Time-of-Flight instrument, offering higher sensitivity and resolution compared to the legacy 6540 LC/Q-TOF. The instrument was used for a validation study that included 149 selected LC-amenable pesticides spiked into different blank egg samples from the local market. Recovery studies were performed at the levels of 0.001 mg/kg, 0.002 mg/kg, 0.005 mg/kg, 0.01 mg/kg and 0.02 mg/kg.
For sample preparation the Q-EMR-method developed in 2018 by EURL-AO [1] was used. The Q-EMR method was derived from the citrate buffered QuEChERS method by including an optimized clean-up procedure using EMR lipid® [2]. The benefit of Q-EMR was that polar as well as less polar pesticides could be covered by the method. This provided the opportunity to screen for both GC and LC amenable pesticides with only one sample preparation, which saves significant amount of money and time.
Applying the corresponding criteria of the SANTE AQC guidelines [3] it was possible to validate 93% of the pesticides for the screening approach. The screening detection limit (SDL, detection capability - ccß) was 0.001 mg/kg for 58% of the cases. The results are compared with a validation study in egg acquired using a 6540 LC/Q-TOF system.

1. Q-EMR-method by EURL AO (2018). https://circabc.europa.eu/w/browse/01c0939b-bd9f-4962-b034-4ba797522b1a
2. Agilent „QuEChERS Enhanced Matrix Removal – Lipid"-material. https://www.agilent.com/en/products/sample-preparation/sample-preparation-methods/quechers/enhanced-matrix-removal-lipid#promotions
3. SANTE/11813/2017. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.

Keywords: *LC-QToF, multi-residue method, high resolution accurate mass instruments, pesticide residues*

S11

DEVELOPMENT AND VALIDATION OF MULTI-RESIDUE PESTICIDES METHOD FOR ROUTINE ANALYSIS OF FOOD SAMPLES USING UPLC-MS/MS

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In addition to the efficiency benefits (cost, time, and labor), multi-residue pesticide methods can address challenges associated with global trade and regulatory issues in different countries when it comes to pesticide use and misuse, regulatory limits, or pesticide residue definitions. It is the strategy of choice for laboratories performing routine surveillance monitoring.
This work describes the development and validation of a robust, quantitative method for the routine determination of a wide range of LC amenable pesticides. Analytes were extracted according to QuEChERS procedure followed by UPLC separation and detected by tandem quadrupole mass spectrometer. The multi-residue method performance was assessed in accordance with the SANTE/11813/2017 validation guidelines for quantitative methods [3]. The performance of a multi-residue method was evaluated for food commodities belonging to groups 1 (high water content vegetable and fruits) and 5 (high starch and/or protein content and low waters and fat content) defined under SANTE/11813/2017 [1]. For the purposes of validation, a representative selection of analytes were used. These representative analytes were selected based on a) spanning the physiochemical diversity; b) including those defined in the coordinated multiannual control plan 2017/660 [2] and c) a selection of pesticides reported as border rejections in 2019 under the European Rapid Alert System for Food Feed [3].
For all representative analytes in matrix extract, a minimum of two product ions were detected, with S/N ≥3, achieving ion ratios within ±30% of those of the averaged calibration standards. The retention time of the analytes in matrix was found to be within ±0.1 minute of both the solvent and matrix matched standards. Gaussian peaks, with widths of between 3–6 seconds were obtained across the elution profile. All the LOQs were found to be ≤MRL of 0.01 mg/kg and for 93% of representation analytes the estimated LOQ values are equivalent to a matrix concentration of ≤ 0.5x default MRL.

[1] Regulation (EU) 2017/660 concerning a coordinated multiannual control programme of the Union for 2018, 2019 and 2020 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin. (2017) Official Journal of the European Union, L94/12-24.
[2] Regulation (EU) 2017/660 concerning a coordinated multiannual control programme of the Union for 2018, 2019 and 2020 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin. (2017) Official Journal of the European Union, L94/12-24.
[3] Rapid Alert System for Food and Feed portal – Europa EU accessed on 20th June 2019 https://webgate.ec.europa.eu/rasff-window/portal/

Keywords: *pesticides, UPLC-MS/MS, multi-residue method*

S12

DETERMINATION OF PESTICIDE RESIDUES IN EGGS USING GC-Q-ORBITRAP MS

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Targeted GC- and LC-QqQ-MS/MS approaches for multi residue pesticide methods are the currently established procedures as they are well studied, validated and documented. A disadvantage is the limited number of pesticides which can be detected in a single run. Especially with an increasing demand on laboratories to extend their scope of pesticide residue analysis high resolution accurate mass systems have found more and more popularity.
Recently, GC-EI-Q-Orbitrap MS instruments became commercially available - nearly a decade after the development of LC-Orbitrap systems. The instruments perform in full scan mode with a resolving power of 60,000 and achieve a mass accuracy within 2 ppm over a wide concentration range. Furthermore, the information obtained can be used to reanalyse the sample in case of later findings in similar samples.
The main focus of accurate mass instruments was set on developing screening methods. Few works have been done to use such systems for quantification. Therefore, a combined method for screening and quantification with GC-Q-Orbitrap MS was developed for pesticide residue analysis in sample extracts from food of animal origin. For sample preparation Q-EMR-method developed in 2018 by EURL-AO [1] was used. Q-EMR is derived from the citrate buffered QuEChERS method by including an optimized clean-up procedure using EMR lipid® [2]. Q-EMR is an alternative for extraction and clean-up especially for fatty matrices and the application of gas chromatography.
Method validation was carried out on 203 selected GC-amenable pesticides spiked into different blank egg samples from the local market. Recovery studies were performed at the levels 0.001 mg/kg, 0.002 mg/kg, 0.005 mg/kg, 0.01 mg/kg and 0.02 mg/kg.
Screening validation and quantification validation applying full scan mode and measuring 3 ions per analyte led to similar results. Applying the corresponding criteria of the SANTE AQC guidelines [3] it was possible to validate 94% of the pesticides for the screening and 93% for the quantification approach. The screening detection limit (SDL, detection capability - ccß) respectively the limit of quantification (LOQ) was 0.001 mg/kg in 55% of the cases. In 75% of the cases SDL and LOQ were identical. Just 50 analytes had different SDLs and LOQs, whereby half of the pesticides had a lower LOQ than SDL.

1. Q-EMR-method by EURL AO (2018). https://circabc.europa.eu/w/browse/01c0939b-bd9f-4962-b034-4ba797522b1a
2. Agilent „QuEChERS Enhanced Matrix Removal – Lipid". https://www.agilent.com/en/products/sample-preparation/sample-preparation-methods/quechers/enhanced-matrix-removal-lipid#promotions
3. SANTE/11813/2017. Analytical quality control and method validation procedures for pesticide residues analysis in food and feed.
https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf.

Keywords: *GC-Q-Orbitrap MS, pesticide residues, multi residue methods, high resolution accurate mass instruments*

S13

FAST MEASUREMENT OF METHYLATED PHENOXY CARBOXYLIC ACIDS BY LPGC-MS/MS

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We developed a fast method using low-pressure gas chromatography coupled to triple quadrupole mass spectrometry (LPGC-MS/MS) to achieve faster turn-around-times for samples containing phenoxy carboxylic acids. We use a restriction capillary (5 m × 0.18 mm i.d.) at the injector side connected to a wide bore separation column (15 m × 0.53 mm i.d., 1 µm film thickness) which is combined with a GC-MS/MS using electron ionization (EI). This set-up maintains injection conditions, which are similar to conventional GC methods, while the analytical column is operating under low-pressure conditions. The injection and oven procedure is optimized for the separation of 30 methylated phenoxy herbicides, another 12 pesticides are added currently. The extracts undergo several processing stages in the laboratory to ensure the measurability with the GC. The samples are hydrolyzed in an alkaline medium to release any bound herbicide residues (in the form of esters or conjugates). After neutralization the acidic pesticides are extracted by liquid/liquid partitioning with acetone/cyclohexane/ethyl acetate, followed by a GPC clean-up and a derivatization step to form the corresponding methyl esters. The LPGC method shows almost no matrix effect and allows for measurements of the compounds against solvent working standards down to the required limits of quantification over all kind of matrices. Reporting limits between 0.003 mg/kg (haloxyfop) and 0.02 mg/kg (quizalofop) are reached in different matrices, e.g. baby food, tea, spices, and lecithin. We have reduced the run time of our measurement down to about one quarter of the previous conventional GC method; from 40 to 9 minutes. Hence, the LPGC method represents a fast, cost-efficient and low-maintenance measurement without loss of sensitivity, separation efficiency and signal intensity. We see the potential in expanding our LPGC approach to further single und multi pesticide methods.

Keywords: *low-pressure gas chromatography-triple quadrupole mass spectrometry, LPGC-MS/MS, phenoxy carboxylic acids, phenoxy herbicides*

Acknowledgement: *We want to thank our laboratory staffs for patiently answering our questions regarding the workup process of the samples. Special thanks go to Kirsten Stegmann, Petra Staude, Anna Saib, Maike Wilkens, Michaela Caspar and Sandra Smolka. We further want to thank Steven Jopling for proof-reading and contributing to this abstract and poster.*

S14

BEE POISONING IN UKRAINE BY FIPRONIL. DETERMINATION OF INSECTICIDE RESIDUES IN BEES, POLLEN AND HONEY USING THE MODIFIED QUECHERS METHOD

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In recent years bee poisoning is increasingly occurring in the world. This problem is also relevant for Ukraine. The most dangerous for bees are pesticides such as neonicotinoids, fipronil, bifenthrin and lambda-cyhalothrin.
European Union completely ban the outdoor uses of three neonicotinoids (clothianidin, imidacloprid, and thiametoxam). But in Ukraine neonicotinoids are allowed for use in the open fields. Fipronil is limitedly used for seed treatment.
Therefore, our goal was to develop and validation of method for determining the residues of insecticides in bees, pollen and honey.
Bee samples and bee products always contain large amounts of wax, proteins and other substances readily extractable with organic solvents . The separation of co-extracted beeswax from extract samples containing the pesticide residues of interest was the main challenge in developing the clean-up method.
Sample preparation, based on the QuEChERS method combining salting-out liquid-liquid extraction to acetonitrile and a dispersive-SPE clean-up with Z-Sep+, was adjusted to honeybee samples by adding a small amount of hexane to eliminate beeswax. The recovery of analytes ranged from 70% to 120% with RSD ≤20%.
The studies were conducted of multiple samples of dead bees from different regions of Ukraine. More then 50% of poisonings occurred as a result of the use of fipronil for field treatments. These data are confirmed by analysis of plant and soil samples in which fipronil residues were found too.
Conclusion: The modified sample work-up procedure based on the QuEChERS methodology is effective, economical and fast.
The method was applied to determine pesticide levels in real samples from from different regions of Ukraine.
The results obtained confirm that the death of honeybees occurred mainly as a result of poisoning with pesticide residues.

Keywords: *pesticide fipronil bee poison*

S15

MULTI-RESIDUE PESTICIDES ANALYSIS USING SCHEDULED MRM ON SCIEX TRIPLE QUAD™ 3500 IN MANGO AND ONION

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Introduction: The Food Safety and Standards Authority of India (FSSAI) are responsible for ensuring that the food items sold are safe for consumers. Pesticide residue refers to the pesticides that remain on the food crop after being applied. Exposure of the general population to these pesticides most commonly occurs through the consumption of treated food reserves or by their proximity to the farms, lands etc. where they have been applied. Onion and Mango are two of the commodities which are being exported from India to other countries and it is being subjected to routine pesticide analysis. This article describes method for the analysis of 172 pesticides in Onion and mango using Sciex Triple Quad™ 3500.
Method: A Quantitative, screening MRM method was developed on Shimadzu Nexera LC and Sciex 3500 system for 172 pesticides using Scheduled MRM algorithm which gives better data quality by monitoring the targeted analyte around a specified retention time. Gradient mode of HPLC and C18 non-polar stationary phase is used. Mobile phase used was a mixture of Ammonium Formate, formic acid (Aqueous Phase) and methanol, Ammonium Formate and Formic acid (organic phase). Column temperature conditions, injection volume and flow rate were optimized. Sample preparation was done by homogenizing the matrix, followed by dilution with Water, followed by ethyl acetate extraction and cleaning up of the sample by using Na_2SO4 and PSA and then injecting the clear sample into LC-MS for analysis.
Preliminary Data: The method was partially validated using SANTE\11945\2015 guidelines. The pesticide standards were spiked into the matrix to check the recovery which is found in between 70 to 120% for most of the analytes. Matrix matched calibration was used for plotting linearity in the range from 1.0 µg/kg to 100 µg/kg and found the regression coefficient of $r > 0.99$ for most of the compounds by applying weighing factor of $1/x^2$. All of the pesticides were analyzed in ESI positive mode. Two MRM transitions were acquired to confirm and quantify the residues. The qualifier and quantifier ions are selected from the fragmented ions based on sensitivity and selectivity. Automated data processing was performed using Multiquant Ver.3.0.2, thereby reducing the sample turnaround time.
Novel Aspect: Highly sensitive, quantitative method for estimating pesticides at below current regulatory level.

Keywords: *food safety, pesticides analysis, LC/MS/MS, triple quadripole, SANTE guidelines*

S16

BRINGING RELIABLE DETERMINATION OF ANIONIC POLAR PESTICIDES IN FOOD TO THE ROUTINE LABORATORY

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Interest in the direct determination of highly polar, anionic pesticides in foodstuffs has noticeably increased over the last five years, driven by the potential safety concerns of the herbicide, glyphosate. As a result, routine testing labs strive to achieve efficient and reliable methodology to meet the demands of increased surveillance and brand protection. Whilst there have been many methodology advances enabling direct analysis in previous years, many of the solutions require the need for specialised LC equipment or the highest performing MS systems.
Representative commodities were identified, and these organic foodstuffs were purchased from local retail outlets and prepared for the determination of anionic polar pesticides using the Quick Polar Pesticides (QuPPe) extraction procedure. A panel of representative anionic polar pesticides, including aminomethylphosphonic acid (AMPA), glufosinate and glyphosate were targeted using a LC-MS/MS method. Chromatographic separation was achieved on a novel hydrophilic interaction liquid chromatography (HILIC) column, applying an acidified mobile phase gradient. Method performance was evaluated, in the absence of isotopically labelled internal standard, by assessing chromatographic repeatability, linearity, accuracy and sensitivity. Chromatographic performance was evaluated targeting the key challenges in the determination of underivatised anionic pesticides, ie analyte retention, chromatographic separation of isobaric pairs and repeatable retention times. The method's accuracy (% recovery) and precision (%RSD) was determined using spiked food matrix prepared in replicates (n=5), where all recoveries fell within 70 and 120 % and %RSD < 20 %.

Keywords: *polar pesticides, glyphosate, LC-MS/MS, pesticides*

S17

DIRECT DETERMINATION OF PARAQUAT, DIQUAT, AND RELATED CATIONIC POLAR PESTICIDES IN HOMOGENIZED FOOD SAMPLES USING ION CHROMATOGRAPHY AND HIGH-RESOLUTION ACCURATE MASS SPECTROMETRY

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Due to their acute and chronic toxicity, analytical methods are needed to determine six polar pesticides (paraquat, diquat, chloromequat, mepiquat, trimethylsulfonium, and morpholine). Here we demonstrate direct determinations of six cationic polar pesticides in food products using cation-exchange chromatography with serial detection by suppressed conductivity and high resolution accurate mass spectrometry (HRAM MS) in full scan and Parallel Reaction Monitoring (PRM) modes for targeted MS/MS. The pesticides were separated using an electrolytically-generated acid gradient from 1 to 40 mM at 0.4 mL/min and 40 °C. After passing through a desalting electrolytic suppressor, the pesticides were detected by suppressed conductivity and ionized by positive ESI-MS and acetonitrile for detection by MS. The method was applied to extracted, diluted homogenized food samples following the Quick Polar Pesticide (QuPPe) method. The six polar pesticides had good peak shape with peak asymmetries and eluted from the column within 20 min. Mepiquat and chlormequat exhibited good chromatographic resolution with Rs >2. Diquat-paraquat with the carbon isotopic masses within 2 m/z fully coeluted but were easily resolved in PRM mode by HRAM MS. The six pesticides had good accurate mass, between <2 to 2.5 ppm m/z normalized to the true isotopic mass. Good accuracy was found, 80-120% recoveries of spiked in reagents. Sensitivities were single digit µg/L LODs.

Keywords: *cationic polar pesticides, ion chromatography, high resolution accurate mass, paraquat, diquat*

S18

DETECTION OF PESTICIDE RESIDUES IN BASMATI RICE SAMPLES USING SURFACE-ENHANCED RAMAN SPECTROSCOPY (SERS) TECHNIQUES

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Food security faces difficulties across the globe which burden crop and food production. World population is estimated to grow by ~2.3 billion people between 2009 and 2050. Additionally, climate change increases extreme growing conditions (e.g. flood, draught, pests) for food producers, resulting in crop and economic losses. Therefore, the production of safe, nutritious and sustainable food is under immense pressure. Rice is a staple food for more than 60% of the world's population, and by 2050 must be doubled to keep up with the demands of population growth. The overuse of pesticides is a common agricultural practice used by food producers to eliminate pests, reduce crop losses and improve yield. However, less than 0.1% of applied pesticides reach targeted pests, and the majority of residues end up polluting the environment and food supply chain. Residues can adsorb through the skin or via the respiratory or digestive systems and can be extremely toxic, carcinogenic and mutagenic to humans. The analytical techniques for detecting pesticide residues in rice rely on gas chromatography (GC) and liquid chromatography (LC). However, these conventional techniques are complex, expensive and rely on bulky instrumentation. Thus, more efforts are required to develop simple, low-cost, sensitive and portable methods applicable to rice and other complex foods. Surface-Enhanced Raman Spectroscopy (SERS) is a vibrational surface-sensitive technique which enhances Raman scattering through the adsorption of molecules close to the surface of noble metals, and is widely used to improve sensitivity. This preliminary work focuses on the detection of pesticide residues (Carbendazim and Acephate) in Basmati rice samples comparing two SERS techniques; Raman microscope and a handheld Raman device. The SERS techniques developed could successfully detect below the Maximum Residue Levels (MRLs) for Acephate and Carbendazim (10 ppb). Additionally, a swab technique has been developed for the recovery of spiked pesticide residues in Basmati rice. Overall, there is potential for the handheld Raman and swab technique to be applied to on-site analysis, for the detection of pesticide residues in rice and other agricultural crops (i.e. grains, fruit, vegetables) in the future.

Keywords: *pesticides, SERS, rice, handheld, swab*

S19

USING A QUECHERS METHOD FOR GC-HRMS ANALYSIS OF INSECTICIDES AND ACARICIDES IN DEAD HONEYBEES

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Abstract: A QuEChERS sample preparation method with high resolution GC-MS (GC-HRMS) was applied to evaluate 18 pesticides (propoxur, carbaryl, chlorpyrifos-methyl, fipronil, thiamethoxam, bifenthrin, acetamiprid, lambda cyhalothrin, amitraz, permethrin, coumaphos, beta-cylfluthrin, cypermethrin, t-fluvalinat, fenvalerat, esfenvalerat, thiacloprid, deltamethrin) in dead honeybees. Pesticides were found in all samples at 8 - 112 ng/g.

Introduction: Pesticides belong to many different categories such as insecticides, fungicides, herbicides, growth regulators, acaricides etc. Among them, the most dangerous for bees are insecticides and acaricides. In Russia, there are more than 350 insecticides and acaricides licenced for use[1]. Within last years due to global decline in honeybee population the bee health is a matter of public concern. According to mass media, commercial beekeepers in the Russian Federation report greater loss of bee colonies in the summer than in the winter. Summer loses are usually connected with pesticide poisoning. In 2019, more than 20 regions of the Russian Federation registered the death of honeybees in the summer.

Our goal was to analyse whole dead honeybees, which supposedly died from pesticide poisoning, using modified QuEChERS work-up and GC-HRMS [2].

Materials and methods: All standards and chemicals were purchased from Sigma-Aldrich. Supel QuE acetate tube (magnesium sulfate 6g, sodium acetate 1.5g) and Supel QuE PSA/C18 Tube (C-18 50mg, PSA 50mg, magnesium sulfate 150mg) were used for dSPE.

The sample preparation is as follows: 1 g of dried and homogenized honeybees was weighed in a 50 mL centrifuge tube. Then 10 mL of acetonitrile was added and sample was shaken in a Multi Reax Vortexer (Heidolph, Germany) for 15 min. After that Supel QuE acetate tube was added and the sample was shaken for 15 min. again. The mixture was then centrifuged at 4,000 rpm for 10 min. 1 mL of the supernatant was transferred to Supel QuE PSA/C18 Tube and shaken in a Vortexer for 15 min and again centrifuged. Further supernatant was filtered in a 1.2 ml Vμ-Vial (Agilent, USA) through a 0.2 μm pore size PTFE filter. Then extract evaporated to dryness, re-dissolved with 0.1 mL of n-hexane, and injected into Q Exactive GC-HRMS system (Thermo, USA).

Results and discussion: The method was applied for analysis of five honeybee samples from two regions of Russia. Pesticides were found in all samples. In a sample from Orel region, Fipronil was found at approx. 31 ng/g. Samples from Sverdlovsk region showed different patterns: only fipronil was found (112 ng/g) in a sample from Novopishminskoe, whereas in three samples from Zaykovo, both fipronil and t-fluvalinate were found at approx. 23 – 43 ng/g and 8 – 49 ng/g, respectively.

[1] Ministry of Agriculture of the Russian Federation, «State catalog pesticides and agrochemicals, approved for applicaton in the territory Russian Federation,» Moscow, 2018
[2] K. Stenerson, Reporter US, Volume 32.1

Keywords: *QuEChERS, pesticides, honeybees, GC-MS*

S20

DEVELOPMENT AND VALIDATION FOR THIOCYCLAM AND NEREISTOXIN IN FRUITING AND VEGETABLE BY LC-MS/MS

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Thiocyclam, a nereistoxin analogue insecticide, is widely used to control the occidentalis and armigera etc. Thiocyclam is mainly used in the form of a hydrogen oxalate salts. Thiocyclam is rapidly converted into nereistoxin *in vivo* and in the environment after pesticide application, resulting in insecticidal activity. Indeed, the residue definition of thiocyclam is also based on the conversion of thiocyclam to nereistoxin. In this work, a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction method was developed for the simultaneous quantification of thiocyclam and nereistoxin in five commodity (apple, pear, strawberry, paprika and tomato. To minimize matrix effects, the samples were diluted without any additional purification prior to liquid chromatography-tandem mass spectrometry injection. All commodities spiked with two concentration levels of 0.01 and 0.1 mg/kg. The recoveries were within 73.4~102.4% with relative standard deviation (RSD) of < 15% and the limit of quantification (LOQ) of method were ≤ 0.0011 mg/kg in all commodities. Linear calibration functions with correlation coefficients were obtained R2>0.997.

Keywords: *pesticides residue, modified QuEChERS, thiocyclam, nereistoxin*

S21

ANALYSIS OF GLYPHOSATE AND OTHER POLAR PESTICIDES RESIDUES IN STONE AND POME FRUITS USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-QQQ)

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The aim of this study is the determination of polar pesticides, such as Glyphosate, Fosetyl aluminium, Ethephon, and their metabolites using liquid chromatography tandem mass spectrometry (LC-QQQ). Over the decades, Glyphosate has been used worldwide as an active substance in plant protection products. Its herbicide function in agriculture, in horticulture and even in some uncultivated areas makes it suitable for weed control. In addition, its function minimizes the use ploughing machines reducing soil erosion. However, Member States, the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA) have thoroughly evaluated Glyphosate to determine whether it has adverse effects on human and animal health and the environment. The European Union and EFSA has established a Glyphosate Assessment Group (AGG) with the purpose of reporting a scientific evaluation for the Glyphosate approval. In this sense, Regulation (EU) 2019/724 was formally adopted on 10 May 2019 by the European Comission. These pesticides were determined using the EURL-SRM QuPPE method (version 10) with few modifications. The extraction was carried out with acidified methanol and a total of 9 polar pesticides were analysed. Separation was achieved in 18 min employing liquid chromatography in a Xevo TQ-XS equipment. Stone fruits (cherry) and pome fruits (apple) were validated with good results. Good sensitivity and selectivity were obtained at the quantification limit of 0.1 mg kg-1 for Glufosinate-ammonium (sum of glufosinate, its salts, 3-MPPA and NAG), Fosetyl, Glyphosate, Ethephon, Hydroxy Ethephon (HEPA) and AMPA, and for Fosfonic acid was of 1 mg kg-1. Following SANTE/11813/2017 criterias, excellent recoveries between 70-120% with RSD below 20% were achieved, as well as a good linearity with r2≥0.99.

Keywords: *glyphosate, polar pesticides residues, Fruits, LC-QQQ*

Acknowledgement: *This research was funded by the Generalitat de Catalunya. Acknowledges the Departament d'Agricultura, Ramaderia, Pesca i Alimentació.*

S22

DETERMINATION OF 181 PESTICIDES RESIDUES IN FLOWERS, LEAVES AND TREE TRUNKS BY QUECHERS FOLLOWED BY TRIPLE QUAD ANALYSIS, AND RESULTS OF CATALONIAN AGRICULTURE DURING 2014-2019

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Official pesticide analysis laboratories follow current regulations that include Directive 2009/128/CE, Commission Regulation (EU) 396/2005 and the Spanish National Plan for the Official Control of the Food Chain (PNCOCA). The main objective of all these regulations is to monitor the sustainable use of pesticides in the environment. The aim of this study is the analysis of these new commodities to ensure compliance with these regulations. A multi-residue method for determining 181 pesticides in flowers, leaves and tree trunks is described. Pesticide residues are extracted using a modified QuEChERS protocol. Three different clean-up methods have been validated, one for each matrix, using the same chromatographic method for all matrices. The extracts obtained are chromatographed by LC-QQQ for 87 pesticides and separation is achieved in 30 minutes. The same extracts are also chromatographed by GC-QQQ for 94 pesticides in 22 minutes. Sample quantification was by matrix matched standards. The method has good sensitivity and selectivity and the quantification limits obtained are 0.005 or 0.01 mg kg-1. Good accuracy with recoveries between 70-120%, good precision with RSD below 20% and good linearity with r2≥0.99 are also achieved. These results conform to recommended values for SANTE/11813/2017. During routine analyses in 2014-2019, 385 samples of these matrices included in different control projects were analysed: from 206 tree trunk samples, 95% were positive; from 176 leaf samples, 65% were positive, and from 3 flower samples, 66% were positive.

Keywords: *pesticides residues, leaves, QuEChERS, LC-QQQ, GC-QQQ*

Acknowledgement: *This research was funded by the Generalitat de Catalunya. Acknowledges the Departament d'Agricultura, Ramaderia, Pesca i Alimentació.*

S23

DETERMINATION OF PESTICIDES IN EDIBLE OILS BY GC-MS/MS

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The determination of pesticides in edible food oils, such as olive oil, has many challenges. Due to the complexity of the matrix, sample clean-up is crucial to ensure robust methodology and does not lead to significant contamination of and reduce the lifetime of consumables. Traditional approaches to the clean-up of such high fat content samples are a liquid-liquid extraction followed by Gel Permeation Chromatography (GPC) clean-up. This technique, still in use today is undesirable as it is both time consuming and has high solvent consumption. This poster presents an alternative multi-residue extraction method for the determination of pesticides in edible oils by GC-MS/MS.
Edible oils were spiked with known concentrations of pesticides. The extraction of pesticides from the oils and a simple pass through clean-up procedure was optimised. Thus eliminating the need for nonpolar solvents and lengthy GPC or SPE conditioning and washing. All extracts were run on GC-EI-MS/MS, using a splitless injection of 1 µL. In house validation was completed for olive oil in accordance with the SANTE/11813/2017 guidelines. Method validation demonstrated the overall reliable and rugged performance for accurate quantitation, by evaluating the linearity, accuracy, precision and repeatability.
The extraction optimized in this study yielded improved method recovery for representative pesticides when compared to the alternative acetonitrile extractions with a hexane partition step. The loss of analytes during the pass through clean-up step was also evaluated separately, where analyte recoveries were >70% for all analytes, showing negligible loss of the analytes during clean-up.
While the overall method recovery demonstrated improved performance over traditional techniques, additional investigations were conducted to improve the linearity and precision. An alternative type of calibration, using procedural standards, was employed. This mode of calibration compensates for low extraction efficiency and showed excellent improvements in terms of accuracy and precision, where the method's trueness (for edible oil samples spiked prior to extraction and clean-up) ranged from 99.2 to 108.5 % for a selection of challenging organochlorine pesticides. Excellent linearity, over the relevant calibration range of 0.005 to 0.1 mg/kg, was achieved for all pesticides with correlation co-efficients > 0.995 with residuals <20%. The method's accuracy, precision and bias was evaluated at 0.01 mg/kg (n=5), 0.02 mg/kg (n=5) and 0.1 mg/kg (n=5). Following in-house validation of this optimized extraction, clean-up and GC-EI-MS/MS analysis, the method is shown to be simple, quick, solvent friendly, reliable and fit for the routine determination of multi-residue pesticides in edible oils.

Keywords: *pesticides, multi-residue, GC-MS/MS, edible oils*

S24

LARGE SCALE MULTI-RESIDUE METHODS BY USING LC-MS/MS AND GC-MS/MS FOR THE DETERMINATION OF PESTICIDE RESIDUES AND THEIR METABOLITES IN FOOD OF PLANT AND ANIMAL ORIGIN

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Recent advances in mass spectrometry have led to the development of new analytical methods to ensure food safety. Gas and liquid chromatography with mass spectrometry methods are widely applied for the detection of known and unknown chemical contaminants, in food commodities for the protection of human health, international trade and regulatory controls. Monitoring of pesticides residues and their metabolites in different types of food of plant and animal origin necessitates the development and validation of large scale multi-residue methods based on general extraction procedures in combination with hyphenated instrumental analysis techniques such as LC-MS/MS and GC-MS/MS. This work is focused on the development and validation of large scale multi-residue methods for the detection, identification and quantitation of pesticides and their relevant metabolites in various food matrices, using QuEChERS based extraction procedures in combination with LC -MS/MS and GC-MS/MS techniques. Selected LC -MS/MS and GC-MS/MS operating parameters were optimized to achieve the highest possible analytical sensitivity, accuracy and detectability in the complex food matrices. LC-MS/MS and GC-MS/MS methods have been developed and validated for the analysis of more than 400 compounds thus enhancing the capacity and the productivity of the laboratory. More than 6000 pesticides/food commodity combinations have been validated achieving recoveries in the range 70-120% with relative standard deviations less than 20% and method quantification limits of 0.01mg/Kg in most cases; method performance criteria described in the European Union guidelines (SANTE Doc. No 11813/2017) have been fulfilled. Both LC -MS/MS and GC-MS/MS methods have been successfully applied for the monitoring of multiclass pesticide residues and their metabolites in food samples of plant and animal origin; results are included to the Annual Reports on Pesticide Residues of EFSA for the assessment of consumer exposure to pesticides in the EU.

Keywords: *pesticide, residues, food, LC-MS/MS, GC-MS/MS*

S25

DISTRIBUTION STUDY OF CHLORDECONE IN DIFFERENT BOVINE MATRICES SAMPLED IN FRENCH WEST INDIES

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Chlordecone (CLD) is an organochlorine pesticide that was extensively used to fight against the black banana weevil (*Cosmopolites sordidus*) from 1972 to 1993 in the French West Indies. CLD was banned in 1990 by the French Government Order and was used under exemption until 1993. This synthetic organochlorine pesticide is highly toxic, very stable, poorly soluble in water, and can strongly bind to soil and sediment particles where it can remain for years with a low release rate. Considered as a persistent organic pollutant and included in the Stockholm Convention substances in 2009, CLD is known to be carcinogenic in mice and rats. In humans, studies have demonstrated a causal relationship between CLD exposure and increased risk of prostate cancer. CLD is persistent in the soil: more than 8% of Martinique and Guadeloupe soils are highly contaminated. That is the raison why farm animals can be contaminated following involuntary soil ingestion. Indirectly, humans are exposed to CLD when consuming contaminated animal foodstuff.
As shown in 2013 by Bouveret *et al*, contrary to other lipophilic POPs, fat is not the only target tissue for CLD, which is mainly stored in liver.
In order to understand CLD distribution in bovine and to improve knowledge on population exposure, for each of 200 bovines, fat, muscle and liver samples collected from French West Indies were analyzed. This work organized by the French Directorate General for Food (DGAl) from the French Ministry for food, agriculture and fisheries consisted in the evaluation of a potential relationship between levels of CLD in tissues of interest.
As French National Reference Laboratory of Single Residues Method, an official Reference method for the analysis of CLD with isotopic dilution in LCMSMS was set up for CLD monitoring in food products of animal origin. The method was validated in fat, in muscle and liver according to SANCO 11945/2015 guidelines and accuracy profile. Limits of detection and quantification (LOD and LOQ) were determined at 0,001 and 0,003 mg/kg respectively for the three mentioned matrices. The method was validated from 0,003 to 0,120 mg/kg for muscle and liver and from 0,003 to 0,500 mg/kg for fat. Recoveries rates ranged from 100 to 103 % for muscle and liver and from 95 to 100 % for fat. Two hundred triplets (fat, muscle and liver) sampled in 2016 have been analyzed by the NRL and an official laboratory following the same Reference method.
Among 200 triplets corresponding to 600 analysis, 48 % of samples were higher than the LOQ, representing 54 % of fat, 66 % of livers and 49 % of muscles. Significant correlation between CLD concentration the three matrices has been stated.
Results of the present work reinforced the French regulation amended in January and May 2019 regarding CLD's MRL. Moreover, a complementary study carried out on serum from the same animals will also be undertaken in order to find a potential relationship between serum, fat, muscle and liver.

Keywords: *chlordecone, bovine, French West Indies, MRL*

S26

AUTOMATED CLEAN-UP OF QUECHERS EXTRACTS FOR GC-MS AND LC-MS

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Since the first publication in 2003 the QuEChERS extraction method for pesticides in food (Anastassiades 2003) became a worldwide standard. While the extraction step is well standardized (AOAC 2007.1; CEN 15662), the clean-up of the resulting extracts from different matrices is still in active discussions. The large variety of differing dSPE clean-up methods for each individual food matrix creates extra burden, source of error and additional cost in routine pesticide laboratories worldwide. The efficient and reliable application of clean-up sorbents for daily different food commodities is hardly possible in practice. A well proven solution for a unified clean-up for all food matrices has been established with the use of micro-SPE cartridges (µSPE) (Hayward 2016). The here described µSPE extract clean-up works for all food commodities with only one cartridge type each for GC-MS and LC-MS. The choice and mix of sorbent materials have been optimized for hundreds of pesticides with the goal of high recovery and optimum matrix separation.

Keywords: *automated QuEChERS clean-up, QuEChERS, micro-SPE, µSPE*

Acknowledgement: *Bruce D. Morris and Richard B. Schriner, Development of an Automated Column Solid-Phase Extraction Cleanup of QuEChERS Extracts, Using a Zirconia-Based Sorbent, for Pesticide Residue Analyses by LC-MS/MS, J. Agric. Food Chem. 2015, 63, 5107−5119, DOI: 10.1021/jf505539e. Steven J. Lehotay, Lijun Han, Yelena Sapozhnikova, Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography-Tandem Mass Spectrometry, published with open access at Springerlink.com, Chromatographia (2016) DOI 10.1007/s10337-016-3116-y. M. Hayward, J. Ho, Automated Chromatographic Solid-Phase Extraction Using an Autosampler, American Laboratory (2016) posted online Sep 01, 2016.*

S27

A LARGE-SCALE SCREENING AND QUANTITATION OF PESTICIDE RESIDUES IN CEREALS BY USING GC-(EI)-MS/MS

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A large-scale screening and quantitation solution for more than 150 pesticide residues in cereals (rice and wheat flour) by using gas chromatography tandem mass spectrometry GC-(EI)-MS/MS. A buffered QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used for extraction followed by GC-MS/MS analysis including electron impact (EI) ionization. The data acquisition in t-SRM and processing were performed by using Thermo Scientific TraceFinder software. Automated data processing was performed by addressing identification and quantitation in alignment with SANTE guideline. Two or more transitions per analyte were used from Thermo pesticide analyzer compound database (CDB). To harmonize the results, matrix match linearity was prepared in the range of 0.005-0.100 mg/kg for both matrices offered excellent correlation coefficient (R^2=0.99). Recovery was checked at 0.010 and 0.050 mg/kg concentration level. The results obtained through this optimized protocol complies with SANTE guidelines requirements i.e. ion ratio (±30%), retention time (±0.1 min), linearity (>0.99 with residuals ±20), recovery (70-120%) and precision (±20%). The optimized method fulfils the FSSAI as well as European commission (EC) MRLs requirement for pesticide residues in rice and wheat Flour.

S28

ULTRA-LOW LEVEL QUANTIFICATION OF PESTICIDES IN BABY FOODS USING AN ADVANCED TRIPLE QUADRUPOLE GC-MS/MS

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The EU maximum residue level (MRL) for the majority of pesticide-commodity combinations is set at the default level of 10 µg/kg. For a small number of the more toxic pesticides, MRLs for baby foods are set as low as 3 µg/kg calculated as the sum of the parent compound and relevant metabolites. In some of these multi-component MRLs the quantification limits of individual compounds can be 1 µg/kg or lower. In this study, the quantitative performance of the Thermo Scientific™ TSQ™ 9000 Triple Quadrupole GC-MS/MS system was assessed for the analysis of ~200 pesticides in baby food at very low concentrations (as low as 0.025 µg/kg). A complete evaluation of method performance included, sample preparation, overall method suitability measured from pesticides recoveries, selectivity, sensitivity, linearity and long term robustness.
The method performance was tested in accordance to the SANTE/10518/2017 guidance document. All detected compounds, at the three spiking levels in both matrices satisfied all SANTE requirements. More than 97% of the target pesticide residues had recoveries between 70 – 120% at the 1 µg/kg spiking level. Over 90% or the target compounds had a Limit of Identification below 0.5 µg/kg and over 60% below 0.1µg/kg – 100 times lower than the default MRL. All results in compliance with all SANTE criteria for method validation. Compound linearity was assessed by injecting matrix matched standards in the range of 0.025 to 250 µg/kg in duplicate for two composite Baby foods; carrot/potato and apple/pear/banana. Both sets of linearity data showed R2 > 0.990, and % RSDs of <20% for over 96% of component peaks. Robustness of the AEI source was demonstrated by maintenance of SANTE compliance at the default MRL throughout a sequence of ~400 consecutive injections of sample matrix (1 g/mL).

Keywords: *pesticide residues, GC-MS/MS, QuEChERS, TSQ 9000, advanced electron ionization*

S29

THE MULTI-RESIDUE ANALYSIS OF POLAR ANIONIC PESTICIDES USING A ROBUST AND SENSITIVE IC-MS/MS 'SAMPLE-TO RESULT' WORKFLOW

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Laboratories are constantly challenged to analyze more classes of pesticides at lower concentrations in more different commodities, with faster turnaround times and little if any increase in costs. And with the expectation that residues will not go undetected and all results can be verified by associated analytical quality control data compliant with method performance guideline criteria. One of the most challenging group of pesticides in the polar anionic pesticides, such as glyphosate, perchlorate, chlorate and the like, which often occur as residues in food, but are not always included in pesticide monitoring programs. This presentation will highlight the development and validation of an IC-MS/MS based workflow for the robust, sensitive and reliable multi-residue determination of polar anionic pesticides and metabolites at low µg/kg levels in a single chromatographic run. The integrated workflow from sample to results is based on the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, TSQ Altis™ Triple Quadrupole mass spectrometer system and all associated workflow components: IC column & suppressor, suitability check standard solutions, software system and comprehensive user guidelines for fast implementation, enablement of ongoing optimum performance. The workflow uses a modified QuPPe extraction with cartridge solid phase extraction clean-up and has been thoroughly tested and validated . Results for wheat, leek and baby food matrices are compliant with SANTE guidelines, and EU MRLs. Quantification limits are 10ug/kg or lower with % RSDs typically <10 %. Recoveries with and without internal standards and using matrix-matched calibration, and matrix extracted calibrations (procedural standards) will be presented.

Keywords: *polar pesticides, glyphosate, IC-MS/MS, glufosinate, ethephon*

S30

SAMPLE PREPARATION FOR MULTI-RESIDUE ANALYSIS IN DIFFICULT MATRICES WITH AN OPTIMIZED PUSH-THROUGH-SPE

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Analytical methods have to be fast and easy and to be able to recover a large number of analytes. The analysis of pesticide residues in spices, herbals, tea or hops is considered difficult due to the complexity of the matrix.
With the "Q19" method[1] using combined mini SPE (PSA, NH2, MgSO4 from Agilent and UCT) we developed a processing approach, which is more suitable for complicated matrices than the current § 64 methods of the LFBG, QuEChERS and DFG S19[2,3] and which is not as time and solvent consuming as modified QuEChERS[4] . But there is only a limited variety of commercially available SPE- cartridges.
To expand the spectrum of analytes we wanted to optimize the SPE sample preparation by varying the composition of the SPE cartridges. For this purpose we tested and combined different commercially available materials und we checked if and which analytes got lost during the sample preparation[5].
As a result we were able to develop a SPE combining different materials, which covers a wide range of analytes with good purification results of difficult matrices.

[1] A. Romanotto, J. Langner, K. Speer. Q19 in der Routine: Automatisierte Probenaufarbeitung für das schnelle Pestizidscreening in schwierigen pflanzlichen Matrices, LABO
01-02/2018, www.labo.de
[2] 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
[3] 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
[4] Multi-residue Pesticide Analysis in Green Tea by a Modified QuEChERS Extraction and Ion Trap GC/MSn Analysis
[5] J. Langner, A. Romanotto, Optimized Sample Preparation for Multi-residue PesticideAnalysis in Difficult Matrices, poster presentation; EPRW 2018

Keywords: *SPE, multi-residue analysis, sample preparation*

S31

ANALYSING 360 PESTICIDES IN LESS THAN 10 MINUTES USING FAST GC-MS/MS

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Analysing 360 pesticides in less than 10 minutes using fast GC-MS/MS
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Contamination of food products with pesticides has been of concern for many years due to the risk of acute or delayed adverse health and environmental effects. The global use of pesticides is increasing, as is exposure to them; and imports of raw foodstuffs from unknown sources is rising too. Consequently, the number of samples in analytical instrumentation as well as pesticide monitoring has escalated significantly in the last decade. To handle this high sample load, a quick, easy and cheap cleanup procedure called QuEChERS was established some time ago. Unfortunately, samples prepared using this method contain large matrix signals, which popularised the use of highly selective tandem MS. Along with matrix interference, the analysis time is a crucial point when handling a high sample load. The usage of narrow bore capillary columns has proven to be a powerful tool for drastically reducing analysis time while maintaining chromatographic resolution in different GCMS applications. Combining the speed of fast gas chromatography (GC) and the selectivity of tandem mass spectrometry (MS) increases laboratory efficiency and reduces working costs. Fast MRM (Multiple Reaction Monitoring) switching modes with no interfering crosstalks are therefore needed. The potential of this approach is demonstrated by analysing 360 pesticides in QuEChERS apple extract in less than 10 minutes by using a Shimadzu GCMS-TQ8050 NX ultra-high sensitivity Triple Quadrupole system.

S32

A MULTIRESIDUE METHOD FOR QUANTITATION AND SCREENING OF PESTICIDE RESIDUES IN BABY FOOD USING LC-MS/MS

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A large number of pesticide residues is used worldwide in food products for preventing, destroying or controlling pest activity. Therefore, for consumer protection, regulatory agencies have established maximum residue levels (MRLs). For baby food products MRLs are even lower[1,2] compared to other food commodities, and therefore sensitive analytical methods that allow simultaneous analysis of a large number of pesticides in challenging matrices are required. We have developed a 15-min multiresidue method for quantitation and screening of pesticide in baby food using a triple quadrupole mass spectrometer, coupled to a HPLC in a single run with polarity switching.

We have developed a multiresidue LC-MS/MS method for the analysis of pesticides in baby food using a triple quadrupole mass spectrometer. The 15-min method allows for pesticide quantitation and screening at low concentration levels (ppb) which are required for baby food.[1,2] Pesticide confirmation was performed based on one or two ion ratios. Optimum SRM transitions (quantifier and qualifier) were determined for each compound by optimizing RF lens values and collision energies for each of the 230 pesticides in neat standards. Preliminary data indicates that the performance of the method in terms of RT reproducibility, detection limits, CVs, RSDs and accuracy is excellent. Limit of quantitation was between 0.05 ppb to 10 ppb for 219 out of 230 pesticides with 84% of all pesticides tested having an LOQ at or below 1 ppb. Obtained % RSD and %CV values were within 10% for 88% of all pesticides tested. Future work will focus on method validation according to the SANTE guidelines. We will discuss in details the data, results and method validation.

1 Commission Directive 2006/141/EC
2 Commission Directive 2006/125/EC

Keywords: *pesticide residues, baby food, LC-MS/MS, SANTE Guidelines*

S33

A NEW AUTOMATED ON-LINE QUECHERS-HPLC DIRECT INJECTION-CLEAN-UP FOR FATTY MATRICES

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The QuEChERS methodology has become the leading method for pesticide determination in food and feed labs throughout the world. Nevertheless, in many cases difficult to analyse matrices, in particular fatty matrices, were analysed without using QuEChERS. For fatty matrices additional steps are required in order to apply the QuEChERS methodology (e.g. freezing out). Consequently, many labs are searching for a working and automated alternative that is easy to use. The new QuEChERS automation concept is based on the second clean-up step applying a non-dispersive approach in specifically adapted cartridges containing a proprietary sorbent. The cartridges are specifically developed for fatty matrices and do not require additional steps or filters.
Furthermore, as a novelty the eluted extract is on-line injected onto the LC-MS/MS. Because of the high matrix-retention capacities of the cartridge in combination with the non-dispersive approach, a minimised ion suppression with high recoveries and low standard deviations is observed.
The new methodology is successfully applied to avocado and other fatty matrices and gave excellent results.
On the poster, the automation concept of the FREESTYLE SPE-HPLC-Direct Injection system designed by LCTech GmbH is presented, as well as the results (recoveries and standard deviations) for the matrix mentioned above.
Subsequent analysis for more than 200 pesticides is performed using a core-shell silica phase with octadecyl modification and polar endcapping. Core-shell particle technology leads to fast and efficient HPLC-MS/MS results due to low bleeding characteristics of NUCLEOSHELL® Bluebird RP 18 columns designed by MACHEREY-NAGEL GmbH & Co. KG. A chromatographic run needs less than 10 minutes and allows analysing a large number of samples in food and feed labs. Because of the benefits of core-shell technology, the chromatographic separation can be done with low backpressure on conventional LC systems.
The matrix-retention capacity of the cartridge is presented by comparing the UV-VIS spectra and the dry weight of crude sample extracts and cleaned QuEChERS extracts. Furthermore, the minimised ion suppression is illustrated by a better signal to noise ratio for cleaned QuEChERS extracts and leads as a result to better limits of detection and quantification.
The automated sample preparation coupled with an efficient HPLC analysis meet the requirements of a modern high-throughput contract laboratory.

Keywords: *fatty matrices, QuEChERS, non-dispersive, LC-MS/MS, pesticides*

S34

ASSESSMENT OF A POTENTIAL OF SELECTED MICROBIAL CULTURES TO DEGRADE RESIDUES OF ´MODERN´ PESTICIDES

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The input of pesticides into soil due to a food crop protection may represent a serious problem, not only because of impact on natural microbial consortia but also due to a possibility of transfer of residues into food chain and drinking water. Although various physico-chemical processes contribute to successive reduction of soil residues, persistence of some compounds is rather high. The presented study was focused on investigation of potential of several bacterial species (*Pseudomonas spp*., *Bacillus spp*., *Peanibacillus spp*.) to degrade residues of commonly used pesticides such as acetamiprid, boscalid, thiacloprid, etc. The bacteria (isolated from soil) were cultivated in liquid medium spiked with respective pesticide (10 and 100 mg/L) up to 25 days. Validated UHPLC-MS/MS method was employed to monitor the fate of pesticides across cultivation period. Microorganism species proved to possess a degradation potential will be used for future experiments under real-life conditions at contaminated localities and collected research data will be used for planning bioremediation experiments aimed at protection human environment.

Keywords: *pesticide, degradation, UHPLC-MS/MS, bacteria*

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S35

IDENTIFICATION OF NICOTINE SOURCES IN INDIAN TEA

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Investigations of the German Federal Institute for Risk assessment (BfR) in 2009 demonstrated that various plant-based foods including tea exceed the residue limit of 0.01 ppm for nicotine according to regulation (EC) No 396/2005 [1]. Even the European Food Safety Authority (EFSA) introduced a temporary limit of 0.6 ppm for nicotine in 2011, because the nicotine sources have been unknown [2]. The only plant with a significant nicotine content is tobacco (1 % in dry mass). Because India is simultaneously one of the five biggest producers of tea and tobacco in the world [3], we suspected a correlation between the tobacco cultivation and the nicotine contamination in Indian tea.
Therefore specific markers were established for identification of tobacco and tobacco smoke to proof the source of nicotine [4]. Various plant materials from the tea-growing area Darjeeling were examined with HPLC-MS/MS on nicotine, 3-vinylpyridin, cotinine, nornicotine and anabasine with a low LOQ.
The generated results indicate a relation of the contamination and cultivation as mentioned above. Nonetheless a clear correlation has to be confirmed in further studies.

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Keywords: *nicotine, HPLC-MS/MS, tea*

S36

RESULTS OF THREE YEARS HRMS TARGETED SCREENING OF MONITORING SAMPLES WITH FOCUS ON PESTICIDES NOT INCLUDED IN THE QUANTITATIVE DANISH PESTICIDE CONTROL

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As part of the national and EU multiannual control programme (MACP) the Danish Food Administration (DFA) annually collects and analysis approximately 2000 samples for pesticide residues. The pesticide scope for the quantitative methods employed primarily include pesticides authorized for use in Denmark as well as pesticides included in the MACP. As a supplement to this quantitative control, approximate 100 selected samples are further analysed by targeted screening at the Technical University of Denmark. The techniques used are HRMS LC-QTOF-MS and GC-TOF-MS (2016 and 2017) or GC-Orbitrap (2018). The targeted screening scope include more than 200 pesticides for which screening detection limits have been established and are not included in the scope of the quantitative control programme.
The results obtained by the targeted screening analysis performed over three years (317 samples) are presented. The samples include primarily fruit and vegetables but also rice and few samples of e.g. honey and wine. The sampling had been performed in accordance with the sampling directive 2002/63/EC and were taken as targeted or non-targeted sampling with focus on samples from third countries. Pesticides that are found several times by the screening are considered for inclusion in the quantitative control programme.
The number of findings of pesticides included in the screenings scope are few and generally only occurring sporadically. The compounds ametoctradin*, ametryn, amisulbrom, butachlor, etoxazole*, pentachloranilin, penthiopyrad, pyridalyl and spirodiclofen* were each detected in one of the 317 samples. Fluopyram*, methoprene, metrafenone*, and tetrahydrophthalimid (metabolite of captan*/folpet*) were detected in 2-15 of the 317 samples. The pesticides marked by an asterisk (*) are also included in scope list for the EU MACP (Regulation (EU) 2019/533 of 28 March 2019). Etoxazole, fluopyram and metrafenone were included in the Danish quantitative control programme during 2017 or 2018.
The overall few findings of the pesticides included in the screening scope compared to the quantitative national programme and the MACP indicate that scope for the latter two are adequate for control of MRL compliance and provide a good foundation for dietary intake estimations. Thus, the effect or benefit of expanding the scope of the quantitative control with the pesticides in the screening scope would be limited.

Keywords: *pesticide residues, monitoring, targeted screening, HRMS*

S37

QUANTITATIVE ANALYSIS OF FLURALANER IN EGGS, EGG-PRODUCTS, CHICKEN MUSCLE AND -FAT WITH THE AGILENT 6470 TQ

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Quantitative analysis of Fluralaner <10 µg/kg
Fluralaner, An isoxazoline ectoparasiticide, originally used to protect our pets from:
Arthropods (Dermanyssus galinae)
Nemotodes
Ecto- and endoparasites

Counter sample
30 unknown egg samples were analyzed at MSD and Eurofins LZV
93% conform
Criterium: 20% deviation

The methods are validated and accredited
Criteria
Reporting limit 0.01mg/kg
Repeatability <20%
Reproducability <20%
Recovery 80% - 120%

S38

MULTI-RESIDUAL METHOD FOR THE ANALYSIS OF PESTICIDE RESIDUES IN THE VEGETABLE ORIGIN PRODUCTS AND MONITORING PROGRAMME IN REPUBLIC OF MOLDOVA

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Pesticides are potentially toxic to humans and can have both acute and chronic health effects, depending on the quantity and ways in which are person exposed. Republic of Moldova is mainly agricultural country, so the pesticides play a significant role in food production.
Central Phytosanitary Laboratory, Chemical Laboratory, Determination of Pesticide Residues is responding for the implementation of the state monitoring programme for residues of pesticides which are used in the conventional agricultural production in Republic of Moldova (GD 567/2014).
Since 2015 the purpose of the Laboratory has been the determination of residues of the pesticides which are intensively used for plant protection.
The Laboratory developed a multi-residual method for the analysis of 100 pesticides in fruits, vegetables and cereals. Sample preparation methods were based on the citrate-buffer QuEChERS approach (EN 15662:2008) and liquid-solid phase extraction. GC-MS/MS and LC-MS/MS were used for separation and detection of pesticides. The sistem was operated in multiple reaction mode (MRM). The samples were analysed using matrix-matched standard calibration curves which were created by spiking the pesticides mix in range 0.005 mg/kg - 0.5mg/kg. Reporting limit was in range 0.005 - 0.01 mg/kg. The method was validated using negativ sample spiked at two different concentration (0.005 and 0.05 mg/kg) and evaluating the analytical parameters according to SANTE 11813/2017.
The multi-residual method for the analysis of pesticides was used for the implementation of the state monitoring program. That allows controlling the utilization frequency of the plant protection products and the distribution of their residues in various agricultural crops, at the same time this control permit to minimize the risk for to human health, in the way that non-conform products appear on the market.

Keywords: *pesticide residues, QuEChERS, GC-MS/MS, LC-MS/MS, monitoring programme*

Acknowledgement: *The authors acknowledge the support of the Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic*

S39

SELECTIVE SPE DISKS AND CARTRIDGES CLEAN-UP METHODS BASED ON MOLECULARLY IMPRINTED POLYMERS FOR GLYPHOSATE&AMPA ANALYSIS WITHOUT DERIVATIZATION IN FOOD AND WATER

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Glyphosate is part of herbicides family referred as phospho-herbicides and is the most commonly used herbicide worldwide. 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 0.05mg/Kg in meat or milk and 30mg/Kg in cereals.
As very polar molecules, the analysis of glyphosate, AMPA and glufosinate (a closely structured herbicide) is very challenging. Indeed, they are difficult to extract with organic solvents and common solid phase extraction (SPE) sorbents.
In spite of these difficulties, a new SPE sorbent based on Molecularly Imprinted Polymers (MIP) could be developed for these analytes. A MIP is a synthetic material with artificially generated three-dimensional network which shows affinity for a target molecule. This new and powerful SPE clean-up method was evaluated for the analysis of glyphosate, AMPA and glufosinate on a broad range of diverse matrices such as cereals, tea, wine, honey or juice. Then concentrations could be determined with a LC-MS/MS detection without any derivatization.
With very similar clean-up methods for all these matrices, recovery yields higher than 80% were obtained. This method is simple, fast and cost-saving for easy obtained results on very complex matrices.
In addition to cartridges format described above, a format SPE disks of MIP glyphosate was also manufactured. We have developed a complete range of SPE disks for environmental purposes with HLB, DVB, SCX, SAX, C18 and C8 as sorbents. A mixture of MIP glyphosate with HLB was used for the disks to extract glyphosate as well as a broad range of pesticides. These SPE disks are very useful to extract contaminants in water with a very high flow rate (10min for 1L). Some results are shown for glyphosate, AMPA and glufosinate in water.

Keywords: *glyphosate, SPE Disks, food analysis, solid phase extraction, without derivatization*

S40

OPTIMIZING A 190+ PESTICIDES MULTI-RESIDUE SCREENING WORKFLOW FOR THE PREPARATION AND ANALYSIS OF PRODUCE BY LC-MS/MS

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Pesticides are ubiquitously used to help increase crop yields; however, they can pose health risks for the general public and pollinators. Faster multi-residue screening workflows, which combine easier sample preparation techniques that yield higher recoveries with lower instrument detection limits in fruits and vegetables, are often sought. Accomplishing these goals increases sample throughput, and reduces costs for laboratories and their clients. To demonstrate the feasibility of developing improved methods, organic celery and other representative matrices were spiked with pesticides down to 10 ppb. Samples were extracted using QuEChERS salts (AOAC 2007.01 and original unbuffered), and cleaned up with complementary dSPE containing $MgSO_4$ along with appropriate amounts of C18, PSA and GCB sorbents for each matrix. Each sample was diluted 10x with water prior to analysis. Separations were performed with a sterically protected superficially porous C18 (Raptor ARC-18) column (100 mm x 2.1 mm, 2.7 µm) analyzed by a UHPLC-MS/MS in selected reaction monitoring mode. Optimized LC-MS/MS conditions, pesticide separations, and recovery (accuracy and precision) results from organic celery, spinach, orange, avocado, brown rice flour and honey will be presented.

Keywords: *pesticides, multi-residue, QuEChERS, dSPE, LC-MS/MS*

S41

A COMPREHENSIVE REVIEW OF OUR GLYPHOSATE DATA IN COMMON FOODS BY ELISA AND LATERAL FLOW IMMUNOASSAY

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Glyphosate, or N-(phosphonomethyl) glycine is, the world's most widely used broad-spectrum herbicide and crop desiccant. As of 2016, 18.9 billion pounds have been used globally, accounting for 25% of the global herbicide market. Glyphosate is an organophosporous compound (phosphonate) that was first discovered by Monsanto and introduced into the herbicide market in 1974 under the trade name "Roundup". It is also frequently applied to crops as a pre-harvest drying agent to speed up harvest operations. Due to its widespread usage, it tends to be ubiquitous in the environment and our food supply.
Glyphosate works by blocking a metabolic pathway involved in the synthesis of three aromatic amino acids (tyrosine, tryptophan, phenylalanine) which are essential for the plant's growth.
Contradictory findings on carcinogenic risks have thrust glyphosate into the center of dispute between EU and U.S. politicians, regulators and researchers. In March 2015, the World Health Organization international Agency for Research on Cancer classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological, animal and in vitro studies. In November 2015, European Food Safety Authority published a report concluding that "glyphosate was unlikely to be genotoxic or pose a carcinogenic threat to humans". While glyphosate and formulations have been approved by regulatory bodies worldwide, concerns about their negative effects on humans and the environment persist.
In the past, we have reported on glyphosate in honey, beer, wine and soy products. Here we present our complete and up to date data sets for glyphosate in common foods as measured by ELISA and lateral flow dipstick.

Keywords: *glyphosate, herbicide, food contaminant, Roundup, pesticide*

S42

OPTIMIZING SAMPLE PREPARATION FOR THE ANALYSIS OF OVER 200 MULTI-RESIDUE PESTICIDES IN PRODUCE BY GC-MS/MS

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Optimization of sample preparation is an important step in mitigating matrix effects in the pesticides multi-residue workflow. Using the QuEChERS approach followed by dispersive solid phase extraction (dSPE) allows for customization of solutions based on matrices. In order to efficiently select the combination that yields the highest analyte response and provides sufficient clean-up, we first tested 40 representative pesticides with different QuEChERS salts and dSPE clean-up materials. The results were evaluated based on; the responses of all tested pesticides, responses of commodity relevant pesticides, and the overall cleanliness of the samples. To demonstrate the feasibility of developing optimized methods, organic celery and other representative matrices were spiked with over 200 pesticides at two levels, 100 ppb and 10 ppb. Non-spiked commodities were also analyzed for the presence of incurred pesticides. Separations were performed using a Rxi-5MS column (30 m x 0.25 mm x 0.25 µm) and analyzed by GC-MS/MS in selected reaction monitoring mode. Optimized sample preparation conditions, pesticide separations, and recovery results from organic celery, spinach, and other produce will be presented.

Keywords: *pesticides, GC-MS/MS, fruit, vegetable*

S43

MULTICONTAMINANT ANALYSIS IN TURMERIC POWDER BY LC-MS/MS AND GC-MS/MS

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Testing for pesticide residues in spices is a difficult procedure, although it should be a routine task and is a requirement in some countries. The Environmental Protection Agency (EPA) in the United States of America has set Maximum Residue Limits (MRLs) for a variety of pesticides in root and tuberous vegetables, including turmeric. Canada has set MRLs for 42 pesticides in turmeric root, and the European Union and the Codex Alimentarius Commission has also set MRLs for a number of pesticides. Turmeric *(Curcuma longa L.)* is an economically important food and medicinal spice plant that grows primarily in tropical and sub-tropical regions, including in India, China, Taiwan, Sri Lanka, Peru, Australia and Thailand. It is a complex matrix for pesticide residue analysis due to its high content of secondary metabolites, mainly polyphenols (curcuminoids) and essential oils, which have physico-chemical properties similar to a range of pesticides currently employed in agriculture and can cause interferences in the detection and extraction of the target analytes.
In this work, the sample preparation protocol was optimized for the determination of selected representative pesticide residues, aflatoxins and persistent organic pollutants, all of which are currently regulated in turmeric under international standards including the EU and MERCOSUR Pharmacopoeias, the EU food regulations and the Codex. Sample extraction and clean-up was optimized following the SweEt, QuEChERS-EN15662, Dutch Mini Luke and IAEA sample preparation procedures, and combinations of them. The co-extractives of the different protocols were studied by GC-Ion Mobility spectroscopy in order to evaluate the profile of the obtained extracts.
The method finally selected was an adaptation of the IAEA modified QuEChERS sample preparation technique based on an ethyl acetate extraction followed by dispersive solid-phase extraction. The clean-up step was performed using primary–secondary amine, RP-C18 and $MgSO_4$, with the amounts of the salts and adsorbents optimized for turmeric. The whole procedure was validated for 75 pesticides at 10, 20 and 50 µg kg^{-1} in turmeric powder, with analysis by LC and GC-MS/MS. The performance of the method was tested in FEPL at Seibersdorf, Austria and GACT labs in Uruguay.
The key method performance parameters investigated were specificity, linearity, trueness, within and inter-laboratory repeatability and reproducibility, limit of quantitation and matrix effects. Recoveries for the studied pesticides ranged from 60 to 110 %, and the RSDs were lower than 20 % for the majority of the evaluated pesticides. More than 20 commercial samples have been analyzed and chlorpyrifos ethyl was present in all samples, even in two labelled as organic.

Keywords: *turmeric, multicontaminant*

S44

THE DETECTION OF FIPRONIL AND FIPRONIL SULFONE IN EGGS

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Fipronil is a pesticide in the phenylpyrazole class and is used for a wide array of products, including some home flea and tick preventatives/treatments for household pets. The use of fipronil near animals for human consumption or laying hens is not permitted in Europe, as fipronil is fat soluble and could contaminate meat and chicken eggs. However, millions of eggs were destroyed last year due to illegal use of fipronil in Europe near laying hens, which resulted in the contamination of millions of eggs with the insecticide. Fipronil and its metabolite of similar toxicity, fipronil sulfone, inhibit the action of GABA in the central nervous system. Fipronil is more effective at blocking the GABA action in insects than in mammals, but fipronil sulfone is less selective. Once ingested fipronil can cause hypertension, paralysis, and death in insects and can cause indigestion, sweating, nausea, dizziness, agitation, vomiting, and seizures in humans. Because of the illegal use of fipronil around laying hens, it is crucial to develop a rapid, reliable, and sensitive method for detection of fipronil and its metabolite in eggs. In this study, we optimized methods for extraction of fipronil from eggs using QuEChERS. We also evaluated multiple HPLC stationary phases and developed an optimized method calibrated from 0.1 to 10 ppb.

Keywords: *fipronil*

S45

ANALYSIS OF PESTICIDE RESIDUES IN FATTY MATRICES WITH QUECHERS AND HLPC-MS/MS

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The analysis of pesticide residues in food and feed is part of routine food control. Therefore, the QuEChERS methodology is used for many sample matrices as part of sample preparation. It provides many advantages allowing rapid and cheap cleanup of strongly matrix-contaminated samples. It consists of an extraction and a cleanup step. Each compound of QuEChERS cleanup-mix shows different effects on matrices reduction. The Diamino phase (PSA) removes, e.g., sugars and organic acids. Magnesium sulfate removes water, C_{18} ec removes nonpolar interferences and so on. Subsequently, a sensitive QuEChERS method with an efficient cleanup for fatty matrices like avocado, butter and so on was developed. The sample raw extract was purified with a cleanup-mix with customized composition. In this work, the recovery rates of pesticides and the effects on matrix-reduction for different cleanup-mix composition are shown and are discussed. Finally, an optimal proposal for the analysis of pesticide residues with the QuEChERS methodology in fatty samples will be presented.
For the subsequent analysis, a pesticide multi-method was developed. The liquid-chromatographic determination was carried out on a NUCLEOSHELL® Bluebird RP 18, which is optimal for the determination of pesticides due to low tendency of column bleeding.

Keywords: *pesticides, QuEChERS methodology, fatty matrices*

S46

ANALYSIS OF PESTICIDE RESIDUES IN CANNABIS WITH QUECHERS AND HLPC-MS/MS

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Cannabis markets are growing because of the legalization of using cannabis as medicinal marijuana or for recreational sale. Actual, there is a worldwide discussion about cannabis. In several countries, an increasing number of jurisdictions legalized the acquisition of cannabis. The need for quality control methods for the analysis of pesticide residues increases to ensure product safety. These methods have to be quick, easy, cheap, effective, rugged and safe being useful for pesticide laboratories.
The QuEChERS-methodology is a well-known technique for extraction of contaminants like pesticide residues in food and feed that refills these method properties. This methodology provides advantages allowing rapid and cheap cleanup of strongly matrix-contaminated samples. Each compound of QuEChERS cleanup-mix shows different effects on matrices reduction. For cannabis, the use of C_{18} ec and Carbon phase helps removes nonpolar interferences and pigments, sterols, and so on.
In this work, the recovery rates of pesticides and the effects on matrix-reduction for different cleanup-mix composition are shown and are discussed. Finally, an optimal proposal for the analysis of pesticide residues with the QuEChERS methodology in cannabis will be presented.
The chromatographic separation of pesticides is performed by using core-shell particles that are well known for fast and high-efficient separations combined with a reasonably low backpressure. In this case, a subsequent analytic was developed on a NUCLEOSHELL® Bluebird RP 18 column.

Keywords: *pesticides, cannabis, QuEChERS methodology*

S47

A ROBUST AND SENSITIVE METHOD FOR THE DIRECT ANALYSIS OF POLAR PESTICIDES IN FOOD AND ENVIRONMENTAL SAMPLES WITHOUT DERIVATISATION

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The prevalence of multi-residue LC-MS/MS analyses for the quantification of pesticides in food and environmental samples has been steadily increasing for many years, and they are now considered to be a minimum requirement of most laboratories working in these fields. Modern tandem quadrupoles are capable of detecting such regulated compounds at very low levels with minimal sample preparation, such as QuEChERS, thereby enabling labs to process large numbers of samples for many analytes with a fast turnaround. However, some very polar compounds which are not amenable to the extraction procedure, chromatographic method or are poor ionisers require additional single-residue methods which involve time-consuming preparation and separation and often involve derivatisation to improve detection. The extraction and chromatography of these compounds is well described in the EURL-QUPPE method, but the separation is not robust in practice, so system and method maintenance are intensive. Several different HPLC or HILIC based methods have failed to address the issues of reproducibility and sensitivity, so FMOC derivatisation prior to analysis is often still employed for glyphosate, AMPA and glufosinate. Although possible to automate, this procedure is still time consuming or expensive, and is not applicable to the other polar pesticides of interest. Ion chromatography has been shown to be beneficial for separation, but the need for a suppressor is detrimental to MS analysis and the problem of changing inlet systems on a heavily used mass spectrometer makes it impractical in a busy lab performing primarily reverse-phase LC. So, the final method, presented here, makes use of an IC column in a method-switching RP system with MS amenable mobile phases at around pH 9. Such conditions configure glyphosate ideally for MS detection with good retention and separation of the other analytes and matrix interferences. The method meets the DG-SANTE requirements of reproducibility (<20%) and recovery (80-110%), and the LOD of the method is below 0.01 mg/kg. Excellent long-term stability and robustness were achieved throughout the validation of this method for food samples extracted by the QUPPE procedure Since glyphosate is well retained in this new method, the potential to further develop it for direct large-volume injection was investigated in collaboration with SCIEX. By modifying the gradient conditions and optimising the injection parameters, a second method specific to environmental water samples has been developed. Although the LVI is more susceptible to changes in pH (for example, due to evaporation of mobile phase) robustness has been shown to be similarly good, and allows detection of the same suite of analytes with a LOD of <0.02 ng/l

Keywords: *glyphosate, polar, pesticides, non-derivatized*

S48

BREACHING THE 10-SECOND BARRIER OF TOTAL ANALYSIS TIME FOR COMPLEX MATRICES VIA AUTOMATED COATED BLADE SPRAY

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In the development of modern analytical workflows, parameters such as sample turnaround time, cost of analysis, and ease of use must be prioritized. Automation enables reductions in total analysis time, human intervention, and cost per sample. In this report, a suitable automated coated blade spray (CBS) workflow is proposed for the screening and quantitation of multiple substances (i.e. drugs of abuse and pesticides) in complex matrices. In an attempt to reduce the total sample-analysis time, several parameters were investigated, including tandem mass-spectrometry (MS) dwell time, CBS spray time, and extraction time. SPME method parameters are explored, such as reduction of extraction time for increased signal-to-noise. Model compounds with a moderately wide range of molecular weights (150-500 Da), polarities, and structural diversity were selected in order to monitor analytical figures of merit during method optimization. The resultant automated CBS method proved capable of analyzing the model compounds in human urine in under 10 seconds total analysis time with excellent accuracy (95-120 %) and precision (RSD < 12 %). As an application, an automated method for the screening and quantitation of more than 150 pesticides from apple juice was demonstrated on both triple quadrupole and orbitrap instruments in under 15-second total sample analysis time.

Keywords: *automation, screening, coated blade spray*

Acknowledgement: *We are grateful to the Natural Science and Engineering Research Council (NSERC) of Canada for their financial support through an Industrial Research Chair program. We would also like to thank our collaborators at Thermo Fisher Scientific, particularly Bradley Hart and Mari Prieto-Conaway, for their technical expertise, as well as for lending us the triple quadrupole mass spectrometer that was used in this work (TSQ Quantiva). Thank you to Varoon Singh for synthesis of the particles used in this work and to Milaan Thirukumaran for assistance in preliminary data processing. Finally, we would like to extend our utmost gratitude to members of the University of Waterloo's Technical Services Department. Specifically, we would like to thank Harman Vander Heide, Andrew Dube, Hiruy Hale, Peter Kessel, and Krunomir Dvorski for their help in with the autosampler modifications and for their invaluable machining and electrical advice.*

S49

CRITICAL ASSESSMENT OF MATRIX EFFECTS WHEN ANALYZING PESTICIDE RESIDUES IN COMPLEX MATRICES

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Pesticides are widely used chemical compounds designed to eliminate or minimize the risks of food production damage. However, because of their potential toxicity for humans, the presence of their residues in food commodities is one of a high concerns. Combination of QuEChERS sample preparation method followed by GC-MS/MS analysis is one of the widely used methods in pesticide residues analysis. However, in case of complex matrices, difficulties with quantification and even with screening analysis can be encountered because of matrix effects in liner and front part of separation column.
The aim of this study was to characterize matrix effects occurring when determining pesticide residues in complex matrices such as chilli peppers, ginger or cannabis and critically assess their impact on the results of pesticide residues analysis. For quantification, matrix matched calibration standards containing the mixture of 198 GC and LC amenable pesticide residues at concentration level 0.01 $\mu g \cdot ml^{-1}$ (´baby food´ limit) were employed.
The validation study was performed on samples of ginger fortified by the mixture of 198 GC and LC amenable pesticide residues at two different concentration levels (0.02 $mg \cdot kg^{-1}$ and 0.2 $mg \cdot kg^{-1}$), each in six replicates. Obtained data were evaluated and performance characteristics (recoveries, repeatabilities and limits of quantification) were compared with the criteria stated in SANTE/11813/2018 document. More than 70 % of target compounds met this criteria (recovery 70 – 120 % and repeatability < 20 %).

Keywords: *matrix effects, GC-MS/MS, validation, pesticide residues, QuEChERS*

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) and by the METROFOOD-CZ research infrastructure project (MEYS Grant No: LM2018100) including access to its facilities.*

S50

PESTICIDE RESIDUES IN APPLES AT THE CZECH MARKET: ANY DIFFERENCE BETWEEN DOMESTIC AND IMPORTED FRUIT IN CONTAMINATION PATTERN?

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Apples are one of the most important fruits grown in temperate climatic zones and in the Czech Republic they have a highest share in the total fruit production. During the pre-harvest period and storage, this fruit may be invaded by various pests which may leave residues in the fruit for consumption. Czech producers minimize these residues by, among other things, selecting pesticides that rapidly decompose after application, meaning that the residues are at such low levels that they generally are close, or even below detection limits (LODs) of routine control methods. However, locally-produced high-quality apples compete with imported ones that often contain a rich 'cocktail' of residues, the levels of which often exceed the maximum residue limits (MRLs) set by the EU. To obtain up-to-date information about the presence of pesticide residues in apples on the Czech market, we compared pesticide residue pattern found in domestic apples and imported ones. Both LC-MS/MS and GC-MS/MS methods were used to test for pesticide residues in apples of various varieties harvested in several regions of the Czech Republic (40,58% of total amount of the samples) and from other countries (44,93% from Poland, 10% from Italy, 3% from Chile, 1% from Slovakia). 69 apple samples were analysed. Pesticide residues were found in 67 of them (97.10% of tested set). In total, 44 different pesticides were quantified. Maximum Residue Limits were not exceeded in any of the tested samples. While captan, pyraclostrobin, thiacloprid, boscalid and pyrimethanil were most frequently detected in Czech apples, captan, flonicamid, boscalid, fludioxonil and acetamiprid were most frequently detected in foreign ones. Up to 12 different pesticides were determined in a single sample.

Keywords: *apples, pesticide residues, LC-MS/MS, GC-MS/MS, country of origin - Czech and foreign producers*

Acknowledgement: *Project "Research of metabolomic methods for laboratory authentication of apples geographicity", supported by QK - Applied Research Program of the Ministry of Agriculture 2017-2025, Czech Republic (QK1910104) and Project "Pesticide residues in apples at Czech market: assessment of consumers´ exposure to risky contaminants", supported from a specific university research (MSMT No 21-SVV/2019), which we are gratefully acknowledged.*

S51

ANALYSIS OF PESTICIDES IN PAPRIKA - DEVELOPMENT OF AN SPE CLEANUP METHOD

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Paprika is a spice made from the drying of sweet peppers and used for flavor and color in many types of cuisine. Pesticides applied to the peppers during cultivation can carry through the drying process, ending up in the dried paprika spice. Paprika is a commonly used spice, thus, in the interest of food safety, testing for the presence of pesticide residues is of great interest. For pesticide analysis, the "Quick, Easy, Cheap, Effective, Rugged and Safe" (QuEChERS) approach has become a popular method for extraction of various commodities, including spices. However, the background resulting from dried spices can be problematic. Conventional QuEChERS cleanup may not be thorough enough for these types of samples. In this work, an SPE cleanup using a new dual-layer, multi-sorbent cartridge was developed for cleanup of paprika extracts in the analysis of pesticide residues by LC/MS/MS and GC/MS/MS. The cartridge differs from conventional dual-layer products containing carbon and PSA or aminopropyl silica in that it is much smaller, requiring less solvent for processing. It also contains blends of sorbents optimized to reduce oil and pigment background, while producing better pesticide recoveries than larger cartridges containing graphitized carbon black. The steps undertaken to develop the cleanup method for paprika extracts are described, and method accuracy and reproducibility are reported using replicates spiked at 50 ng/g with a variety of pesticides.

Keywords: *pesticides, dry food, paprika, sample preparation*

S52

ONE YEAR PILOT MONITORING OF PESTICIDE RESIDUES IN STRAWBERRIES FROM SERBIAN MARKET

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An effective method has been developed and validated according to DG SANTE/11813/2017, for the determination of residues of 196 pesticides in strawberries. The proposed sample preparation method is based on acetonitrile (QuEChERS preparation technique for pigmented sample, EN 15662 method) extraction, and dispersive solid phase extraction (d-SPE) clean-up. Gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography analysis coupled to tandem mass spectrometry (LC-MS/MS), were used for the determination. An Agilent 7890A GC System and an Agilent 7000A Series Triple Quadrupole GC/MS together with Agilent 1200 HPLC System with agilent 6490B Triple Quad LC/MS system were used for determination od pesticide residue. The whole method includes 307 molecules (219 by GC-MS/MS, 129 by LC-MS/MS while 41 were determining by the both methods). The LOQ (limit of quantification) was set at 0.01 mg/kg. The analyses comprised 76 strawberries samples collecting during 2019. The most frequent detected pesticides were pyrimethanil, azoxystrobin, fluopyram, acetamiprid, chlorpyrifos, boscalid and metolachlor. With no pesticide residues detection there were 25% of analysed samples. In conclusion, to achieve a high level of public health protection, stricter controls should be enforced towards pesticide residues contamination of food, especially since the detection point to the application of pesticides that are not registered for use in strawberries protection in Serbia. Some detections of pyriproxyfen, bitertanol, propikonazole, fenpripidin and fluroxypyr methyl were above the MRLs. All the other pesticide detections were below the MRLs.

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S53

LC-MS/MS DETERMINATION OF PESTICIDE RESIDUES IN BROCCOLI

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Recently we are aware of growing public concern and scientific investigations related to the presence and control of pesticide residues in plant products to assess the potential health hazards more thoroughly. On the other hand, there is a trend which encourages people to consume unprocessed food. An active lifestyle and a balanced diet have a decisive impact on protecting human health. Fresh fruits and vegetables hold a prominent place in this diet as they are a rich source of fiber and several vitamins and minerals important for health. According to the WHO, consumption of fruits and vegetables in Europe constituted over 30% of consumer diet. In unprocessed food, including raw fruits and vegetables, a considerable number of healthy bioactive compounds can be accompanied by contaminants occurring in atmospheric air, soil, and water. Unfortunately, almost all of the agricultural commodities currently cultivated and distributed are exposed to contaminants. Considering that pesticides have potentially harmful effects on the environment and pose health risks for consumers, many countries have established MRLs in agricultural products, according to the European Community Regulation No. 396/2005. Republic of Serbia also has National Regulation established through Official Gazette RS No 22/2018. In order to control the food quality, a reliable, rapid, cost-effective, and high-throughput method is necessary for the detection of pesticide residues in agricultural products. A method for the simultaneous determination of multiple pesticide residues in broccoli was developed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Broccoli samples were extracted using a QuEChERS procedure. Pesticides in the broccoli extracts were determined by LC-MS/MS using MRM mode. All the validation parameters were performed according to DG-SANTE/11813/2017 guidelines. Limit of quantification (LOQs) were 0.01 mg/kg. Recoveries of 118 pesticides ranged from 63.2 to 123.4% (RSD≤20%). Pesticide residues in 23 broccoli samples were investigated by this method, and residues of 9 pesticides were detected in 11 samples. The most detected pesticides were spiroxamine, pyrimethanil, pyraclostrobin and tebuconazole. The multiple detections were present in 39.13% of the analyzed broccoli samples. Of all analyzed samples 12 were with no pesticide residue detections, or the detections were under the limit of quantification. There were no broccoli samples exceeding the maximum residue levels set by the Regulation EC/396/2005 and Off. gaz. RS 22/2018. The detections pointed out that all detected pesticides are not registered in the Republic of Serbia for broccoli protection (spiroxamine, pyrimethanil, pyraclostrobin, methiocarb, propiconazole, imidacloprid, azoxystrobin, metconazole and tebuconazole). Given suggests that farmers should comply with the principles of GAP and only use products that are registered for the use in the Republic of Serbia.

S54

ONION (ALLIUM CEPA) AND GARLIC (ALLIUM SATIVUM): LC-MS/MS AND GC-MS/MS PESTICIDE RESIDUES DETERMINATION

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This abstract describes the use of QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation technique used in the extraction and cleanup of 307 pesticide residues in Onion (*Allium cepa*) and garlic (*Allium sativum*) samples. The pesticides are all amenable to LC-MS/MS (liquid chromatography with tandem mass spectrometry) and GC-MS/MS (gas chromatography with tandem mass spectrometry) analysis. For the LC analysis, an Agilent 1200 HPLC system with a binary pump and Agilent 6410B Triple-Quad LC/MS system were used. For the GC an Agilent 7890A gas chromatograph and 7000A mass spectrometer was used. The chromatographic separation was done on a fused silica HP-5 MSI Ultra inert 30 m capillary column with 0.25 mm internal diameter and 0.25 µm film thicknesses. For the method development and data evaluation, massHinter Software was used. The analyses comprised 10 onion and 12 garlic samples. The most frequent detected pesticide in garlic were boscalid and difenoconazole, while there were no pesticide detections in onion samples. One garlic sample contained chlorpropham in the concentration of 0.035 mg/kg, which is above the MRL established for this pesticide. The Regulation (EC) No 396/2005 set up the chlorpropham maximum residue limit to 0.01 mg/kg. All the other pesticide detections were below the MRLs.

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S55

MEPIQUAT IN ORGANIC ALFALFA PELLETS

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Mepiquat is a well-known plant growth regulator (PGR) widely used in cereal and oil-seed crops. Mepiquat residue levels are then frequently found in their products. However, this can be either due to a field application or the heat-induced formation in cereal-based and other processed food as evidenced by recent studies. In our work, mepiquat was found out in alfalfa pellets, produced from both conventional and organically grown alfalfa, with a 100 % appearance at levels ranging from 0.0088 mg/kg to 0.3974 mg/kg.
To confirm mepiquat formation in dried alfalfa, a laboratory trial on drying of untreated alfalfa was conducted. Mepiquat in fresh, lyophilized and thermally treated alfalfa samples was analysed after methanolic extraction by high performance chromatography coupled to tandem mass spectrometry. Mepiquat was detected after 8 hours of drying at temperature 100 °C and after 24 hours the content increased to 0.0244 mg/kg. Mepiquat results for fresh alfalfa and alfalfa dried by lyophilization were negative. Heat-induced mepiquat formation in alfalfa might be explained by one of the published mechanisms, particularly by decarboxylation of pipecolate betaine, which is naturally present in various alfalfa varieties at levels of hundreds of mg/kg.
The above-mentioned finding should be taken into account when evaluating processed feed and food products, particularly those of organic origin. Mepiquat maximum residue limit (MRL) according to Reg. (EC) No 396/2005 range in several orders of magnitude up to 40.0 mg/kg. However, the decision limit applied to organic production in various EU countries including the Czech Republic is 0.01 mg/kg. Considering the fact that it is not possible to unambiguously distinguish the source of mepiquat contamination of alfalfa pellets and other processed commodities; interpretations of measured mepiquat levels may easily lead to incorrect conclusions when assessing MRL or organic production rules compliance.

Keywords: *organic processed feed, mepiquat, alfalfa pellets, pipecolate betaine*

S56

A MULTIRESIDUE METHOD FOR PESTICIDE ANALYSIS USING AN ORBITRAP TRIBRID MASS SPECTROMETER AND AUTOMATIC BACKGROUND EXCLUSION

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Pesticides are routinely applied to crops for preventing, destroying or controlling pest activity. Given the large number of pesticides used and the globalization of the food industry, multiresidue methods offer a great advantage allowing analysis of hundreds of pesticides in a single run. We have implemented a multiresidue method for the analysis of 250 pesticides on an Orbitrap ID-X Tribrid mass spectrometer utilizing an automatic background extraction workflow (AcquireX).
Experimental: Strawberry samples were obtained from a local retail store. Following homogenization, strawberry samples were extracted using a QuEChERS extraction kit. The matrix extracts were spiked with the pesticide standards (250 pesticides) at different concentration levels ranging from 0.05 to 200 ppb. Chromatographic separation was performed on a Vanquish UHPLC system using an Accucore aQ column. Mass spectrometric analysis was performed on an Orbitrap ID-X Tribrid mass spectrometer using AcquireX workflow, for automated generation of background exclusion list, or data dependent acquisition (DDA).
Results: We have evaluated the performance of a multi-residue pesticide method utilizing high mass accuracy and high resolution for semi-quantitation and screening of pesticide residues in a strawberry matrix. Excellent detection limits, reproducibility, linearity and accuracies were obtained. Overall, for 250 pesticides, out of 251 tested, the LODs were at/or below 5 ppb with 215 pesticides having LODs at/or below 1 ppb. LOQs were below 5 ppb for 247 pesticides tested. When the AcquireX workflow was applied for automated background exclusion we observed a significant increase in the number of library matches compared to DDA, especially at the lower concentration levels. For instance, at a spiked concentration of 0.5 ppb the presence of 19 pesticides was confirmed via library search with DDA. When utilizing the AcquireX workflow, at the same concentration level, the presence of 145 pesticides was confirmed. Similar trends were observed at a concentration level of 1 ppb in which we observed 178 library matches with AcquireX versus 65 library matches with DDA.

Keywords: *pesticide analysis, Tribrid Mass Spectrometer, Orbitrap, multiresidue*

RESIDUES - VETERINARY DRUGS

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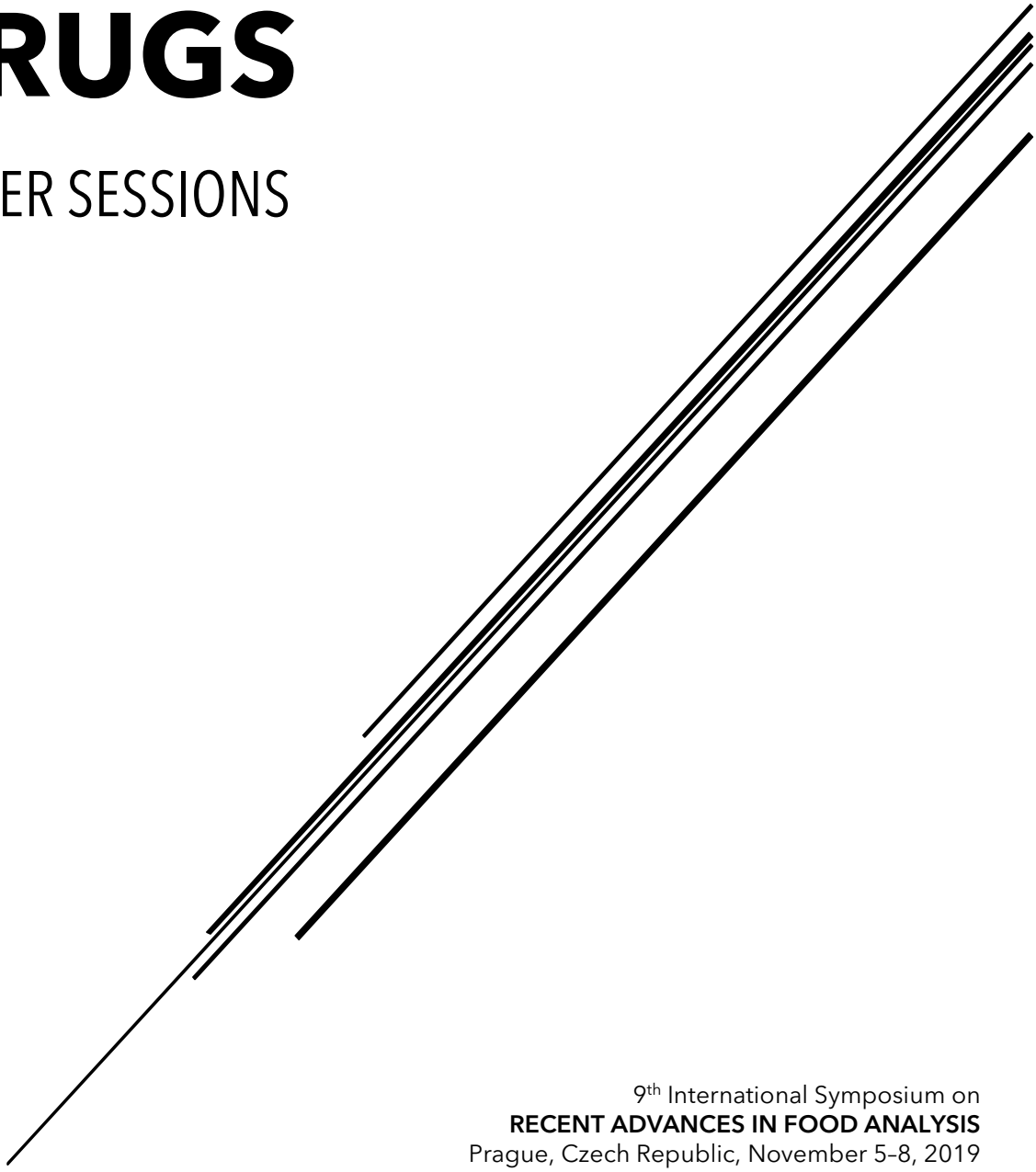

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T1

INFLUENCE OF FOOD MATRIX ON THE FATE OF MALACHITE AND LEUCOMALACHITE GREEN DURING COOKING IN BROOK TROUT AND SHRIMP

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Traditionally used in agriculture, such as dairy farming, veterinary drugs have been used more and more in aquaculture whose contribution to the world's supply of seafood is almost equal to wild capture. Antibiotics are perhaps the largest group of veterinary drugs used, applied for both prophylactic and disease treatment purposes, with other drugs applied for their anti-fungal and anti-parasitic properties. One popular anti-fungal, malachite green, remains frequently used in the aquaculture industry as it is a cheap and highly effective treatment. However, it is currently banned for use in aquaculture, as it is considered a possible carcinogen. Current analytical methods have focused on the parent compound and its main metabolite, leucomalachite green. With some studies showing a reduction in levels following cooking, non-targeted analysis using high resolution mass spectrometry can offer more advantages over targeted analysis, as it can allow for extraction and quantification not only of the parent compounds, but also extraction of other previously unknown metabolites and thermal degradation compounds. Such compounds are important to identify as they may be more toxic or they can be used as markers of contamination. In this study, non-targeted analysis using QuEChERS was used for the extraction of malachite and leucomalachite green from brook trout and Pacific white shrimp. Quantification and identification of other metabolites and degradation compounds was achieved using LC-QTOF-MS. As leucomalachite green is more lipophilic, the influence of fat content on the degradation observed in muscle tissues was investigated. Trout muscle was submitted to four cooking treatments: canning, microwaving, baking and boiling for various time periods. Shrimp muscle was boiled for 10 minutes. All cooking procedures reduced malachite green and leucomalachite green levels up to 50%, with microwaving a more efficient treatment for leucomalachite green reduction. Fat was found to have an influence on the degradation of the compounds, with leucomalachite green being less prone to degradation in trout compared to shrimp. Degradation products unique to each treatment and each matrix will be further discussed.

Keywords: *non-target, thermal degradation, veterinary*

Acknowledgement: *NSERC, FRQNT*

T2

DETERMINATION OF NITROFURAN METABOLITES IN SEAFOOD USING UHPLC-MS/MS AND A NEWLY DEVELOPED UHPLC AQ COLUMN

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Nitrofurans are a class of broad-spectrum antibiotics that are widely used to kill or slow down the growth of bacteria in aquaculture industry. According to previous studies, nitrofurans transform rapidly to metabolites which can bind to protein tissues quickly. Their binding products to tissues are very stable and used as indicator of nitrofuran residues in various food, animal and aquatic products.
The use of nitrofurans and their metabolites have been restricted by different countries and organizations such as European Union, United States and China, due to their harmful side effects to human health. In addition, nitrofurans are defined as class A prohibited drugs in many countries and the Minimum Required Performance Limit (MRPL) of 1.0 µg/kg has been set for food, animal and aquaculture products.
LC-MS/MS methods have been used for the detection of nitrofurans metabolites in many studies because they have high sensitivity, selectivity and specificity. However, nitrofuran metabolites have low ionization efficiency with electrospray ion sources used in LC-MS/MS systems. Moreover, lower molecular weight of nitrofuran metabolites results in higher background noise in different food matrices. The use of a derivatization step for nitrofuran metabolites, before extraction and LC-MS/MS analysis, is crucial to enhance ionization efficiency, reduce background noise and meet low regulatory limits of 1.0 µg/kg in different food samples.
This study demonstrates the good sensitivity of the PerkinElmer QSight® LC-MS/MS system for the identification and quantification of nitrofuran metabolites in seafood. This method meets the requirements of low regulated limits of quantification for routine screening and quantitation analysis. The newly developed PerkinElmer Quasar™ UHPLC AQ column, utilized in this study, features an ultra-high purity silica base for excellent peak shape, and polar end capping to enhance retention of more polar compounds. Additionally, the optimized ligand bonding process applied provides exceptional surface coverage, yielding high sample loading capacities which helps to improve detection of low-level compounds.

Keywords: *LC-MS/MS, residues, UHPLC, nitrofurans, nitrofuran metabolites*

T3

EVALUATION OF SOLID PHASE MICROEXTRACTION (SPME), SOLVENT EXTRACTION, AND QUECHERS FOR THE QUANTITATIVE ANALYSIS OF MULTIRESIDUE VETERINARY DRUGS IN CHICKEN AND BEEF TISSUES

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Experimental results of liquid chromatography-electrospray ionization- mass spectrometry (LC-ESI-/MS) analysis are greatly influenced by the choice of sample preparation procedure carried out prior to instrumental analysis. In this study, a fully automated and high-throughput method using direct immersion solid phase microextraction (DI-SPME) was developed and validated for quantitative analysis of more than 100 veterinary drugs representing more than 12 different classes of drugs in chicken and beef tissue. In addition, this work encompassed a comparison of the developed and validated SPME method to two well-documented sample preparation procedures, namely solvent extraction (SE) and QuEChERS, for quantitative analysis of multiresidue drugs in chicken and beef tissue. The comparison took into account factors such as matrix effects, linearity, limits of quantitation, accuracy, repeatability, sample throughput, and environmental footprint. In terms of matrix effects, SPME showed considerably less matrix effects, with only two compounds out of the more than 100 analytes under study showing significant matrix effects in comparison to 30% of analytes in SE, and 42% in QuEChERS in beef tissue. Similar results were obtained in chicken tissue. Excellent accuracy and precision results were achieved with all methods in chicken matrix with more than 91% of analytes falling within the 70−120% range of their true concentrations and RSD ≤25% at both the 0.75X and 1.5X concentration level, where X is the maximum residue level (MRL) of the analyte. In beef tissue 97% and 99% of the analytes fell in the abovementioned ranges using the SPME protocol, compared to 86% and 92% in QuEChERS, 90 and 96% in SE at 0.75X and 1.5X respectively. In terms of linearity, 99% of the analytes in SPME, 87% in QuEChERS, and 80% in SE achieved linear correlation coefficients > 0.99 % in beef tissue, and 99% of the analytes in SPME, 91% in QuEChERS, and 87% in SE in chicken tissue. In terms of LOQs, the three methods were able to meet regulatory levels for all analytes, with the exception of cephapirin in chicken tissue with the SPME protocol, and amoxicillin in beef tissue with all methods while florfenicol amine could not be quantified by SE and QuEChERS in beef tissue. Other main advantages afforded by SPME included the minimal use of organic solvents compared to SE and QuEChERS, and higher sample preparation throughput.

Keywords: *solid phase microextraction, QuEChERS, multiresidue analysis, veterinary drugs, automated sample preparation*

Acknowledgement: *We are grateful to Perkin Elmer for their financial support. We would also like to thank Thermo Fisher Scientific for lending us the triple quadrupole mass spectrometer that was used in this work (TSQ Quantiva).*

T4

LOOKING FOR INNOVATIVE ANALYTICAL TOOLS FOR THE RAPID IN VIVO DETECTION OF ANTIBIOTIC RESIDUES IN LIVESTOCK

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According to WHO antibiotic resistance is one of the biggest threats to global health, food security, and development today. The current increase on antimicrobial resistance is linked to a misuse of antibiotics and antimicrobial substances. Livestock sector consumes 70% of the antibiotics sold worldwide, thus the implementation of control systems at animal production levels is necessary to reduce and prevent their presence in the environment and food chain. Several post mortem biological based control methods exist, but their adaptation to live animals would help in dealing with the problem and substantially avoid economic losses. Within this project, a commercial biological test based on *Geobacillus stearothermophilus* growth (Explorer 2.0, Zeulab S.L.) and standardised for muscle Maximum Residue Limits (MRLs), was tested for alternative matrices to tissues, in order to observe the response of the microorganism to matrices such as urine and blood serum. For that purpose, a study was carried out on paired samples of 40 piglets, divided in 4 sub lots of 10 individuals untreated and treated with oxytetracycline, sulfamethoxypyridazine and amoxicillin. Samples of muscle, blood serum and urine were analysed in parallel by Explorer 2.0 and LCMSMS for confirmation and quantification of the corresponding antibiotic. Among the matrices studied, blood showed a similar test performance when compared to muscle, whereas urine presented higher concentrations and greater variability. These preliminary results pointed to blood as best candidate to adapt the biological test to be used *in vivo,* but further adjustments need to be done in order to correlate MRLs and cut off levels of the biological test.

Keywords: *antibiotics, in vivo, biological test, LCMSMS*

Acknowledgement: *This Project has been co-financed at a 65% by the European fund of Regional Development (FEDER) through the Program Interreg V-A España-Francia-Andorra (POCTEFA 2014-2020).*

T5

CONTROL OF ANTIMICROBIALS IN FEED BY A LC-MS/MS METHOD: ASSESSMENT OF CROSS-CONTAMINATION RATE IN PIG, POULTRY AND RABBIT FEED AT THE FARM LEVEL

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Animal feedingstuffs in the EU are subject to European regulations in order to protect animals from deleterious health effects but also to protect humans from consuming contaminated animal derived products. Cross-contamination of feedingstuffs by different contaminants may occur during manufacturing, transportation and storage and our objective was to assess the occurrence of cross-contamination by antibiotics during these different processes. Indeed, even if all antibiotics have been forbidden since 2006 as feed additives for growth promotion, they can still be used in a curative way as medicated feed. An LC-MS/MS method for the determination of 11 antibiotics in feed for pigs, poultry and rabbits was developed and validated according to the requirements of decision 2002/657/EC. Antibiotics were selected according to the knowledge of medicinal premixes used for medicated feed in France and which were likely to contaminate the manufacturing chains. They were belonging to the tetracycline, sulfonamide, macrolide, penicillin and pleuromutilin families. In France, tetracyclines and sulfonamides are the most commonly used antibiotics in medicated feeds. The range of validation was set according to the requirements of the Good Manufacturing and Distribution Practices for Medicated Feeds, which states that the methods dedicated to the monitoring of cross-contamination rate should be able to detect up to 0.5% of the concentration of the medicated feed. Procedure for extraction and cleanup as well as chromatographic and spectrometric conditions were established. The method was validated between at least 0,125 and 1 mg/kg for the 11 antibiotics. At this step, colistin and aminoglycoside antibiotics were excluded from the analytical methods due to their specific chemical and chromatographic properties.
The validated procedure was used to analyse 100 pig, poultry and rabbit feed samples which were taken directly from the farms in order to assess the rate of cross-contamination and determine which antibiotics were most frequently found. These results will be presented later, as will the correlation between contamination rates and tonnage of antibiotics sold as medicated premixes. Because of these results, we are considering a research project about the impact of feed cross-contaminations on animal and human health in terms of residues in animal products and antimicrobial resistance.

Keywords: *feedingstuffs, antibiotics, cross contamination, LC-MS/MS*

T6

MACROLIDES, LINCOSAMIDES AND FLUOROQUINOLONES: NEW LATERAL FLOW TESTS FOR INDIVIDUAL AND MULTIPLEX DETECTION IN MILK AND DAIRY PRODUCTS WITH ENHANCED AND ADJUSTED SENSITIVITY

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Modern requirements of food safety are characterized by the increase in the number of controlled toxic contaminants and the adaptation of maximum residue levels to the features of various food stuffs. In this regard, the development of new rapid tests for the control of toxicants needs approaches allowing for highly sensitive detection, controlled thresholds for distinguishing between positive and negative samples, and integration of several test systems into one without deterioration of analytical parameters. This study focuses on enhancing detection limits of lateral flow tests (LFTs, immunochromatographic tests strips) to control the contamination of milk and dairy products with veterinary drugs belonging to the classes of macrolides, lincosamides and fluoroquinolones.
The development of LFTs included multifactorial optimization of the reagents' composition, their concentrations and location on a test strip. Thus, an antibody:nanocarrier and an antigen:(protein carrier) ratios were found to vary detection limits of LFTs up to two orders of magnitude. Assay formats differing in the order of immune complexes formation were compared. The replacing of the specific antibody – nanocarrier conjugate by the combination of free specific antibodies and the anti-species antibodies – nanocarrier conjugate was considered. In this case, all interactions of antibodies with the antigen contained in the sample lead to a decrease in label binding which shifts the detection limit to lower concentrations. Nanoparticles of different chemical composition, size, and shape were compared to select the most optimal labels for LFTs with colorimetric and fluorimetric detection.
Several approaches were used to implement a multiplex testing. The first one was group-specific detection of structurally similar compounds by the same class-specific antibodies. Structural basis of the immune recognition was studied using a QSAR technique on the example of fluoroquinolones. The selected combination of the reactants (with ciprofloxacin as an immunogenic hapten) allows detecting up to 20 fluoroquinolones. Another approach was to form separate binding zones on the test strip for different analytes. Several combinations were tested for the analyses of two or three compounds or chemical classes of veterinary drugs. For example, the simultaneous detection of both macrolide tylosin and lincosamide lincomycin was carried out using a common test strip with non-changed instrumental / visual detection limits of 0.1 and 0.01 ng/mL / 6 and 1 ng/mL, respectively.
The developed LFTs were applied to detect veterinary drugs in milk and dairy products. The recovery of analytes` detection was not less than 80%. Duration of the assay was 15 min without any preliminary treatment. The accordance of the reached detection limits to national and European regulations was demonstrated.
The study was financially supported by the Russian Foundation for Basic Research (grants 18-53-18013 and 18-58-00038).

Keywords: *immunochromatography, test strips, multiplex assay, antibiotics, nanoparticles*

Acknowledgement: *The study was financially supported by the Russian Foundation for Basic Research (grants 18-53-18013 and 18-58-00038)*

T7

VALIDATION OF A NEW MULTIRESIDUE ANALYTICAL METHOD AND MONITORING OF 84 VETERINARY DRUGS IN FISHERY PRODUCT USING HPLC-MS/MS

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Veterinary drugs are a group of chemicals that are widely used in livestock and cultured fish for the protection of them from disease and the growth promotion. The presence of veterinary drug residues in fishery product could be potential risks for human. The suitable analytical methods are required to be capable of detecting these residues at regulated levels. We validated a simple multiresidue analytical method improved to revise the Korea food code in which HPLC-MS/MS was used. Also, the method was applied to monitor the residual amount of 84 veterinary drugs in 70 fishery products purchased from local markets in Korea. Fishery product samples were eel, mudloach, flat fish, rockfish, salmon and shrimp. Amoxicillin, ciprofloxacin, oxolinic acid, oxytetracycline and sulfonamides were detected. The detected levels were from 0.01 to 0.04 mg/kg showing that residual amounts were lower than MRLs. These monitoring results suggest the method would be suitable for analyzing the residual amount of veterinary drug and the use of veterinary drugs in fishery product in Korea might be under control.

Keywords: *fishery product, veterinary drug, HPLC MS/MS*

T8

PROFICIENCY-TESTING SCHEME FOR VETERINARY DRUGS RESIDUES IN HONEY

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BIPEA organizes regular proficiency-testing schemes (PTS) in many analytical domains, including the analysis of veterinary drugs residues in honey. As an example, in December 2018, one test was conducted using an organic honey spiked with 10 veterinary drugs, at levels between 0.5 and 50 µg/kg, with 8 participating laboratories. The techniques used by the laboratories were GC-MS-MS, LC-MS-MS and GC-MS 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. 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.
These tests allow participating laboratories to draw up a general inventory of their analytical skills, and are 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.

Keywords: *proficiency-testing schemes, veterinary drugs residues, honey, quality control, laboratory performance*

T9

ANALYTICAL METHOD DEVELOPMENT FOR NITROFURAN METABOLITES IN VARIOUS ANIMAL MATRICES: BASED ON THE RESULTS OF A DECADES PROFICIENCY TESTINGS

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As European Union Reference Laboratory (EU-RL), the Anses-Laboratory of Fougeres has the duty to organise proficiency tests (PT) to the attention of the European Union National Reference Laboratories (EU-NRLs). The participation to proficiency testing schemes allows these laboratories to assess their competence and to prove the reliability of their results. The aim of one of the series of PT organised over the past ten years was to evaluate the proficiency of the participants to identify and quantify nitrofuran residues in various matrices (pig muscle, shrimp, honey) and to take any decision regarding these samples according to the criteria of the European Decision 2002/657/EC. In European Union (EU), five nitrofurans (furazolidone, nitrofurazone, nitrofurantoine, furaltadone and nifursol) have been banned for use in food-producing animals (Council Regulation (EEC) n° 2901/93, Commission Regulation (EC) n° 1442/95 and Council Regulation (EC) n°1756/2002). So analytical methods have to be implemented in order to control the misuse of these compounds, the evolution of these analytical methods will be presented. Since 2003, a MRPL (minimum required performance limit) was set at 1 µg/kg in EU for all 5 nitrofuran metabolites in poultry meat and aquaculture products (Decision 2003/181/EC). That implies that methods of analysis used to control nitrofuran residues could detect and confirm the metabolite compounds at least down to a level of 1 µg/kg.
Detection of the presence of nitrofuran residues in the EU are based on detection of the presence of a part of the parent molecule, the selective side-chain residues: AOZ for furazolidone, AMOZ for furaltadone, AHD for nitrofurantoin, SEM for nitrofurazone and DNSH for nifursol. These side-chain compounds are released by means of an acidic hydrolysis and their detection is operated in mass spectrometry after their derivatization with 2-nitrobenzaldehyde leading to the nitrophenyl derivatives needed to obtain a satisfactory detection limit with a high degree of certainty on the identity of the analyte. Moreover, the EFSA CONTAM-panel in charge of Chemical Contaminants released an opinion* on safety issues related to nitrofurans in food and confirmed a ReferenceP oint for Action (RPA) similar to the MRPL set at 1.0 µg/kg. More recently, the Commission has strengthened the control of these metabolites at 0.5 µg/kg through the enforcement of RPA, in accordance with a shortly upcoming Decision.
* EFSA Panel on Contaminants in the Food Chain (CONTAM), "Scientific opinion on nitrofurans and their metabolites in food" – EFSA Journal 2015; 13(6):4140

Keywords: *nitrofurans, proficiency testing, LC-MS/MS, residues*

T10

UHPLC-MS/MS MONITORING OF SELECTIVE ANDROGEN RECEPTOR MODULATORS IN URINE

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Selective androgen receptor modulators (SARMs) represent a new generation of non-steroidal compounds designed to separate anabolic effects on muscle and bone from androgenic activity in other target tissues. These structurally diverse molecules have become widely recognised as significant drugs of abuse in human and animal sports, and also as emerging candidates for misuse in livestock-based food systems. As a direct response to the real and possible implications of illicit SARM use and the threats this poses to competition animals and athletes and to food safety, we have developed and validated a semi-quantitative UHPLC-MS/MS-based assay to detect multiple compounds belonging to different SARM families within urine from a range of species (i.e. human, equine, canine, bovine). The current assay was based on a previously published method [1] and re-validated with modification to incorporate an enzymatic hydrolysis step. Briefly, urine samples (200 µL) were subjected to enzymatic hydrolysis (β-glucuronidase/arylsulfatase) and SARM residues extracted with TBME without clean-up and concentrated under nitrogen. Chromatographic separation was performed employing a 14 min gradient on a Luna Omega Polar C18 column (Phenomenex) at 45 °C, using water and methanol, both containing 0.1% acetic acid (*v/v*), as mobile phases at a flow rate of 0.4 mL min^{-1}. The developed assay was 'in-house' validated in all species of interest in terms of selectivity, specificity, detection capability (CCβ 1 ng mL^{-1} excluding andarine (2 ng mL^{-1}) and BMS-564929 (5 ng mL^{-1})), sensitivity (≥95%), limit of detection (LOD 0.002-1.5 ng mL^{-1}), absolute recovery (54-97%), as well as applicability, ruggedness and matrix effects according to respective EU legislation. The newly presented screening assay is shown to be suitable for intended use, being rapid and offering high-throughput testing, with validation findings fulfilling criteria stipulated within relevant doping and food control legislation. Analysis of incurred and surveyed samples (*ca.* 200) from target species demonstrates applicability for use within routine analyses (e.g. residue control programmes) helping both ensure fair play in animal and human performance sports and that food of animal origin is free from risks of residue contamination from this new class of compounds.

[1] E. Ventura, A. Gadaj, G. Monteith, A. Ripoche, J. Healy, F. Botrè, S. S. Sterk, T. Buckley, M. H. Mooney, Development and validation of a semi-quantitative ultra-high performance liquid chromatography-tandem mass spectrometry method for screening of selective androgen receptor modulators in urine, J. Chromatogr. A 1600 (2019) 183-196.

Keywords: *selective androgen receptor modulators (SARMs), UHPLC-MS/MS, urine, doping control, food safety*

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T11

VALIDATION OF A MULTI-RESIDUE ULTRA-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC TANDEM MASS SPECTROMETRY CONFIRMATORY METHOD FOR THE DETERMINATION OF 41 COCCIDIOSTATS IN EGG

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A confirmatory multi-residue method based on electrospray ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) was developed for the simultaneous determination of 41 coccidiostat residues in fresh and lyophilised egg. The samples were extracted using a mixture of acetonitrile:water (8:2), and were cleaned using solid-phase extraction with two different SPE cartridges to eliminate matrix. The chromatographic separation was carried out on a C_{18} Luna Omega Polar column with a mobile phase consisting of (A) 2 mM ammonium formate and (B) methanol, both containing 0.01% formic acid. The MS detection was effected simultaneously in fast polarity switching mode, using a mass spectrometer operated in multiple-reaction monitoring (MRM) mode with electrospray ionisation. The developed method was successfully validated according to Commission Decision 2002/657/EC on the basis of the alternative validation approach. The validation parameters, e.g. linearity, precision, recovery, specificity, decision limit (CC_α), detection capability (CC_β) and robustness, were determined. The recoveries were between 89.6 and 118.8 %, and the within-laboratory reproducibility was in a range between 6.5 and 24.6 %. The proposed method proved to be highly effective in detecting the presence of the targeted analytes, providing a high degree of precision and accuracy.

Keywords: *egg, validation, coccidiostats, CD 2002/657/EC*

T12

VALIDATION OF A MULTI-METHOD FOR ANTIBIOTIC RESIDUES IN RED AND WHITE MEAT WITH AN ALTERNATIVE APPROACH

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Many broad-spectrum antibiotics with different activities against gram-positive and gram-negative bacteria are widely used in the treatment of infections in animals. Maximum residue limits (MRLs) for antibiotics in different food matrices were laid down by European Regulation (EC) No 37/2010 to guarantee a high degree of food safety. In order to ensure the control of antibiotics in food of animal origin against this background, the usage of multi-methods for the determination of antibiotic residues is of growing importance for the laboratories.
The presented validation study comprises the determination of substances of the antibiotic groups quinolones, cephalosporins, penicillins, tetracyclines, macrolides, lincosamides, sulfonamides, pleuromutilins and the substances trimethoprim and dapsone in red and white meat of different species.
The validation of the method was performed on the basis of an alternative factor-comprehensive in-house approach in accordance with Commission Decision 2002/657/EC to obtain information about robustness and reliability among other validation parameters. Different factors and different samples were checked in the laboratory. Using this in-house validation concept, the validation of the multi-method was successfully performed for different kinds of red and white meat from pig, cattle, chicken and turkey. In comparison to the classical approach, the presented alternative study was executed with less experiments. In the course of the validation, the relevant validation parameters, e.g. the critical concentrations CC-alpha and CC-beta, the repeatability, the within-laboratory reproducibility and the recovery rate, were calculated and discussed.
The presented method includes an extraction step, clean-up and measurement by LC-MS/MS to identify and confirm the above-described antibiotic groups in red and white meat. The validation covered a concentration range from 0.25 to 2.0 x MRL for MRL substances, and from 5 to 40 µg/kg for substances without MRL.

Keywords: *antibiotic residues, multi-method, red and white meat, LC-MS/MS, special validation concept*

Acknowledgement: *Special thanks to Ms. Monika Jüsgen for technical assistance.*

T13

QUANTITATION OF MULTI RESIDUES ANTIBIOTICS IN MILK USING THE SCIEX TRIPLE QUAD™ LC-MS/MS SYSTEM

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*Introduction:*The presence of antibiotics in milk is one of the concern due to the potential of increasing bacterial resistance and to hypersensitivity for some individuals. Milk is one of the most consumed foods in India. MRL and MRPL have been established around the world by regulatory agencies and monitors the food supply to ensure that antibiotic residue concentrations do not exceed these permitted levels. The accurate detection of low levels of antibiotic residues in milk is of great importance for the dairy industry. This emphasizes the need to expand the list of antibiotic residues detect at lower levels with greater precision, reduced analysis turnaround time while maintaining the cost of analysis. The liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for Metronidazole, Ronidazole, Tylosin, Trimethprim, and Tilmicosin in one single method for milk analysis as per the regulatory guidelines described in EU/SANCO/12495
Method: A simple LLE (Liquid Liquid extraction) method was performed to extract the antibiotics (Metronidazole, Ronidazole, Tylosin, Trimethprim, and Tilmicosin in Milk samples. Milk sample was diluted with water and extracted using 0.1% formic acid in Acetonitrile. Extracted solvent was evaporated, reconstituted, filtered and injected on LC MS/MS. Data acquisition was performed in using Sciex ExionLC™ AD connected with SCIEX Triple Quad™ 3500 mass spectrometer. Data was acquired using a gradient mode of HPLC and the separation of the analytes were performed on a C18 column. Mobile phase was used as a 0.1% Formic acid in water and 0.1% formic acid in Acetonitrile. Liquid chromatography conditions were optimized to get good separation and reproducibility and Linearity.
Preliminary Data: Method was developed for the quantitation of veterinary drugs, namely metronidazole, ronidazole, tylosin, trimethoprim and tilimicosin were developed and validated in Milk samples. The method was partially validated using EU/SANCO/12495 Guidelines. The Antibiotics standard mix at different concentrations were spiked into the milk samples to evaluate the recovery, linearity, precision and accuracy which was found to be within limits set by the EU. Linearity was prepared in the range from 0.5ppb to 10ppb for metronidazole and ronidazole and from 12.5ppb to 250ppb for tylosin, trimethoprim and tilmicosin. The regression coefficient of $r > 0.99$ was observed using a weighing factor of $1/x^2$. Matrix matched calibration thus plotted was used for quantification. Recovery was found to be 80 to 120% as per the EU guidelines. Antibiotics were run in an ESI positive mode and two MRM transitions were monitored as a Quantifier and Qualifier to get the Ion ratio for the confirmation.
Novel Aspect: Method for highly sensitive detection and quantitation of multi residues antibiotics in milk can be utilized for the routine analysis.

Keywords: *antibiotics, milk, MRL, LC-MS/MS*

T14

SCREENING AND QUANTIFICATION OF UP TO 189 VETERINARY DRUGS IN DIFFERENT ANIMAL SOURCE FOODS USING A COMPREHENSIVE SAMPLE PREPARATION AND AN ON-GOING VALIDATION APPROACH

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In the context of an official food control laboratory, the analysis of veterinary drugs is still very challenging. This family includes a large set of active pharmaceuticals compounds characterized by various physiochemical properties, which can be found in different types of animal source food. It is therefore difficult to have an efficient single analysis method able to cover up such a large number of compounds in different matrices. This is particularly true for food control laboratories in which a high-throughput screening with precise quantification is required to ensure a high level of consumer protection.
Given this, our lab developed a comprehensive analytical method using UHPLC-QTOF systems for the screening and quantification of veterinary drugs in three animal source foods, i.e. egg, milk and meat of different species. The features of this method rely on a single QuEChERS-like sample preparation for the three matrices and a validation based on an "on-going" approach from the guidance document SANTE/11813/2017.
For all tested matrices, the current method allowed the screening and quantification of up to 189 veterinary drugs with a one simple but effective sample preparation. Satisfactory expanded uncertainties (below 35%) were obtained with very good sensitivity (i.e. LOQ between 0.5-5 µg/kg for almost all selected veterinary drugs). Besides, the use of on-going validation approach provided a real added value in simplifying and reducing data treatment for such large set of compounds. It also allowed a continuous monitoring of the quality of the method while promoting data routinely generated.

Keywords: *veterinary drugs, on-going validation, UHPLC-QTOF, screening and quantification, comprehensive analytical method*

Acknowledgement: *We would like to thank Dr. Michael Scherer from AB Sciex for his precious help and fruitful discussions.*

T15

TESTACOS : PIONEERING SOLUTION OF SELF-MONITORING TO MINIMISE THE PRESENCE OF ANTIBIOTIC RESIDUES IN ANIMALS

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According to the World Health Organization, Antimicrobial Resistance (AMR) is one of the main threats to health that humanity will have to confront over the next decades. The emergence of microbial resistance to antibiotics implies that these latter lose their efficacy: they stop being effective in treating common illnesses. AMR currently causes 25,000 annual deaths in Europe, with concomitant losses of 1.5 billion euros from medical expenses along with diminished productivity. It is estimated that AMR is responsible for more than 700,000 annual deaths worldwide. In 2050 it will become the most frequent cause of mortality, surpassing cancer.
The veterinary sector is one of the main users of antibiotics. At the European level, several programs have been initiated with the purpose of improving medicines management in this aim of reducing their use over the next years., On the other hand, EU legislation has established a series of control measures designed to avoid that antibiotics applied by vets become part of the food chain. In that sense, the legislation establishes that all operators in the food and animal feed sector are directly responsible for consumer safety of the foodstuffs they produce or sell.
TESTACOS project is coordinated by the University of Zaragoza, with the participation of the University of La Rioja, the Bilbao Laboratory of Public Health, the University of Perpignan (France), the French National Institute of Agronomic Research (INRA), and ZEULAB (Spain), a SME specialized in developing new analytical tools. Working together, these organizations will elaborate new tools to control antibiotic residues of both sulphonamides and quinolones in live animals as well as in meat commercialized for retail sale, with the purpose of assisting the meat production sector to minimize the presence of antibiotic residues in meat.
In order to reach that general objective, this 3-years project (2018-2020) works on the following activities:
- Creating a "biobank" of benchmark Reference samples: a pioneer sample bank in terms of availability of meat samples naturally contaminated with standard antibiotics.
- Developing autocontrol systems that detect the presence of antibiotics in live animals. (Please see our second abstract "Immunosensor for quinolone detection in meat based on an electrochemically active derivative of difloxacin ».
- Designing a broad-spectrum integrated analytical system placed at the disposal of all actors along the entire food chain (from the farm to the table), offering easy auto control of antibiotic residues in live animals and in commercialized meat.
- Carrying out a broad study of the incidence of residues of antibiotics in meat from different species in order to evaluate the current situation in the Interreg region. Proposing new methods that may enable agrifood sector to adequately control and manage the problem.

Keywords: *antibiotics, biobank, meat, interreg-poctefa, screening tools*

Acknowledgement: *This work cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020)*

T16

VALIDATION OF A TARGETED SCREENING METHOD FOR MONITORING AND CONTROL OF VETERINARY DRUG RESIDUES IN MEAT BY LC/HRMS: QUALITATIVE, SEMI-QUANTITATIVE OR QUANTITATIVE?

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For monitoring and control of veterinary drug residues in foodstuffs of animal origin, the development and implementation of multi-class multi-residue analytical methods is of great importance to get as much information as possible from a sample in a single analysis. In this framework, we developed a method for screening of more than 150 veterinary drug residues belonging to four therapeutic classes (antibacterials, antiparasitics, sedatives, and non-steroidal anti-inflammatory drugs) in meat by LC-high resolution mass spectrometry (LC-HRMS) with a Q-Exactive system. An untargeted data acquisition was carried out using Full MS-variable data independent acquisition (FS-vDIA) mode to record non selective information such as Full MS and MS/MS spectra. A targeted post-acquisition process was undertaken for identification of compounds belonging to our compound database containing such characteristics as retention time, parent ion exact mass, fragment ions exact mass. Criteria of identification were based on the presence of precursor ion with an accuracy mass deviation of less than 3 ppm, in a retention time window of 30 sec, and with presence of at least one fragment ion with an accuracy mass deviation of less than 5 ppm, and additionally isotopic pattern score over 70 %. Validation of the multi-class multi-residue method was applied to assess the performance characteristics for a screening method, in accordance with the "new" Commission Decision (EC) No 2002/657. Taking into account the large range of MRLs of the EU regulated compounds and in order to optimize the workload, it was chosen to characterize the method at three levels (10, 50 and 100 µg/kg) independently of their individual MRL level. The implemented validation plan allowed assessing CCβ (critical concentration), trueness, precision, selectivity, and specificity. From these characteristics, it has been shown that the screening method, while being qualitative, can also be classified as quantitative method for 25 % of the compounds as values for trueness, repeatability and intermediate reproducibility are in accordance with the tolerance of Commission Decision (EC) No 2002/657 for the three levels tested and can be classified as semi-quantitative method for 45% compounds.

T17

IMMUNOSENSOR FOR QUINOLONE DETECTION IN MEAT BASED ON AN ELECTROCHEMICALLY ACTIVE DERIVATIVE OF DIFLOXACIN

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The use of veterinary drugs has played an important role in the field of animal husbandry and agro-industry to prevent and treat diseases and as growth promoting agents, but they have the potential to generate residues in animal derived products (meat, milk, eggs and honey) and cause a health hazard to the consumer. Contamination of food in low-levels may not generate a serious problem on public health. However, extensive use of drugs as well their irregular use may increase the risk of occurrence of microbial drug resistance. Quinolones are among the most widely used antibiotics in veterinary medicine for treatment and prevention of diseases. Therefore, quantification and determination of even low levels of these residuals is crucial for food safety.
Enzyme-linked immunosorbent assays (ELISA) have become very popular for antibiotics analysis due to their many advantages such as sensitivity, high sample throughput, and need of small sample volumes. However, they present some disadvantages based on enzyme activity measurement (e.g. presence of natural inhibitors, nonspecific binding of enzyme, temperature effect on enzyme activity,…). In this work, an electrochemically active derivative of quinolone was synthesized to realize a sensitive and pioneering immunosensor. Difloxacin (6-fluoro-1-(4-fluorophenyl)-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic-acid) was modified with a ferrocene group (on carboxylic fonction) to perform a competitive detection. Monoclonal IgM antibodies were immobilized on a carbon screen-printed electrode through carbodiimide immobilization after diazonium activation of the sensing surface. Electrochemical impedance spectroscopy, Differential pulse voltammetry (DVP) and Cyclic voltammetry were used to characterize each step of the immunosensor development.
A one-step competitive measurement allowed the determination of 4 quinolones in buffer, the method was based on pre-mixing 50 µL of the sample with 50µL of difloxacin modified with ferrocene. The obtained performances were compatible with the levels set by European Union (100 µg/kg).

Keywords: *quinolone, mmunosensors, electrochemistry, derivate synthesis, meat*

Acknowledgement: *This work was carried out in the frame of the TESTACOS project (cofinanced by the European Regional Development Fund (ERDF) through the Interreg V-A Spain-France-Andorra programme (POCTEFA 2014-2020))*

T18

DEVELOPMENT OF A CONFIRMATORY LC-MS/MS METHOD FOR THE RAPID, MICROWAVE-ASSISTED ANALYSIS OF EIGHT BOUND NITROFURAN RESIDUES

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Nitrofurans are a class of synthetic, broad spectrum antibiotics that were previously licensed for use as veterinary drugs for the prevention and control of disease, and as feed additives for growth stimulation. They are prodrugs, and the parent drugs are rapidly metabolised to form highly stable, protein-bound metabolites. These metabolites pose a threat to consumer safety due to their toxicological properties, and hence in 1995, the European Union completely banned nitrofurans from use in food producing animals. Due to their rapid metabolism upon administration, the parent drugs are unsuitable for detection and instead, nitrofurans are detected as their protein-bound metabolites. Since their ban, the majority of liquid chromatography tandem mass spectrometry (LC-MS/MS) methodology developed for the analysis of these residues has focused primarily on four compounds, namely furazolidone, furaltadone, nitrofurantoin and nitrofurazone, detected as their respective marker residues. The aim of this work was to extend the scope of analysis and develop a high throughput method to include four additional compounds, namely nifursol, nifuroxazide, nifuraldezone and nitrovin, detected as dinitro-salicylic acid hydrazine (DNSAH), hydroxybenzhydrazide (HBH), oxamic acid hydrazide and aminoguanidine, respectively. In this work, a microwave-assisted reaction was optimised, using Response Surface Methodology, to shorten the required derivatisation step from 16 h to 13 min. It was found that a four minute ramp from 20 °C to 65 °C, and a nine minute hold at 65 °C were the optimal parameters. The derivatised samples underwent a modified QuEChERS (Quick Easy Cheap Effective Rugged Safe)-based extraction, followed by LC-MS/MS analysis. Chromatographic separation of all eight bound nitrofuran residues was achieved using reverse phase chemistry, with an ammonium formate aqueous phase and a methanolic organic phase. This rapid approach achieves greater reproducibility (2 -26 % RSD), when compared with the conventional 16 h incubation and double liquid-liquid extraction (10 – 51 %). The limits of quantification (LOQ) for all analytes ranged from 0.02 to 0.04 μg kg^{-1}, with the exception of oxamic acid hydrazide (LOQ = 0.2 μg kg^{-1}). Analysis time has been shortened from 4 days to 1.5 days, allowing higher throughput and improved sample turnaround times.

Keywords: *nitrofurans, bound residues, rapid, microwave-assisted derivatisation, LC-MS/MS*

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T19

APTASENSOR BASED ON INTRINSIC APTAMER REDOX ACTIVITY FOR TETRACYCLINE DETECTION IN WATER AND MILK

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Tetracycline (TET) and its residues are a part of the pollutants qualified as emergent because of their proven presence in water and dairy food products posing a potential risk for public health [1, 2]. However, no analytical technique can be used to date for the *in situ* monitoring of these compounds in water. To this end, a label-free electrochemical aptasensor was developed for reagentless determination of tetracycline (TET) using ssDNA aptamer that selectively binds to tetracycline as recognition element [3]. A 5'-amino-modified 76-mer oligonucleotide was immobilized on screen-printed carbon electrode via carbodimide coupling and the binding of tetracycline to aptamer was analyzed by cyclic voltammetry. Cyclic voltammetry experiments revealed an unexpected electroactivity of immobilized aptamer, showing a quasi-reversible signal with oxidation and reduction peaks at respectively +0.19 and -0.25 V vs Ag/AgCl [4]. The binding of TET on the aptasensor induced a decrease of oxidation peak, allowing the detection of TET for concentrations ranging from 0.05µg/L to 20µg/L, with a detection limit of 0.035µg/L. The aptasensor showed high selectivity for tetracycline *versus* other structurally related tetracycline derivatives (oxytetracycline and doxycycline), even in mixtures.
The aptasensor was also used for the detection of tetracycline in milk. It was shown that a simple sample treatment with isopropanol followed by filtration was sufficient to eliminate matrix effects, allowing the determination of tetracycline in milk at concentrations compatible with legislation requirements.

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Keywords: *intrinsic aptamer redox activity, aptasensor, tetracycline, milk, water*

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T20

SCREENING FOR MIXED CHEMICAL CONTAMINANTS IN AQUACULTURE PRODUCTS USING LC-HRMS

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Aquaculture, or fish farming, is one of the fastest growing food production industries. Chemotherapeutics, such as antibiotics, anthelmintics and antifungals, have been widely used to maintain a healthy population of animals. Other chemicals including pesticides, disinfectants and discarded human pharmaceuticals could also contaminate aquaculture products. Monitoring for chemical residues in farmed fish is important to ensure quality and safety.
Our laboratory has developed and validated a method using LC with quadrupole- Orbitrap high resolution (HR) MS for screening chemical residues in aquaculture products. The initial optimization and validation of this method, including the extraction procedure and HRMS data acquisition, focused on 70 veterinary drug residues. Recently, the scope of the method has been expanded to include other chemical contaminants such as disinfectants, selected pesticide and herbicide residues, and human pharmaceuticals prevalent in surface waters. Although HRMS can potentially detect an unlimited number of chemical residues for retrospective data analysis, this process may be limited by how well compounds are recovered through the sample clean-up procedure and how MS data is acquired. For these reasons, new analytes are evaluated by fortifying fish tissues and analyzing the resulting extracts. The rapid clean-up procedure and HRMS detection provided screening limit levels between 0.5-10 ng/g for most of the additional compounds tested. Different data acquisition techniques including full-scan MS in combination with non-targeted or targeted product ions scans are used to effectively identify residues in fish tissues.
This HRMS screening method has been applied to numerous imported aquaculture samples. In addition to finding the analytes routinely detected with an established triple quadrupole MS method, other residues identified by HRMS include anthelmintic compounds and fluoroquinolone antibiotics. Characteristic metabolites of violative analytes were also identified in several samples. Data from both non-targeted and targeted data acquisition can be used to evaluate presumptive positive findings to confirm or rule out the presence of chemical contaminants. Using HRMS screening as an effective investigative tool, we are continuing to expand the scope of chemical contaminants that be detected in farmed fish.

Keywords: *HRMS, Screening, veterinary drug residues, chemical contaminants, aquaculture*

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T21

MULTI-RESIDUE SCREENING APPROACH FOR THE DETECTION OF VETERINARY DRUGS IN ANIMAL TISSUES

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Inappropriate use of drugs in veterinary practice may leave residues in edible tissues, which constitutes a potential health risk for consumers and can induce antimicrobial resistance. The EU has set maximum residue limits (MRLs) for a variety of veterinary drugs in tissues, milk and eggs [1]. Recent surveys for official control conducted in Europe show only low rates of non-compliance, so "screening" by a range of microbiological or bioassay techniques appears an effective solution. Any suspect positive sample requires quantification and identification, typically using LC-MS/MS, to confirm any non-compliance. These screening tests have the benefit of simplicity and low cost but provide limited information about the identity of the substance or its concentration, can face issues with high frequency of false positives if used outside recommended scope and the application may not work on all types of sample, leading to false negatives. LC-MS/MS is a versatile technology, now broadly accepted and recognised as the 'gold standard' for determination of chemical contaminants in food including veterinary drug residues. This can better ensure we avoid false negatives results and have a low frequency of false positives by using validated analytical techniques for screening based upon LC-MS/MS.

This poster describes the development and validation of a comprehensive screening method based on UPLC–MS/MS for the analysis of over 140 veterinary drugs, from many different classes, in a range of representative animal tissues. In order to maximise throughput and minimise costs it is desirable to aim to screen for the widest possible range of veterinary drug residues in the samples with a single analytical method. The use of multi-residue analytical methods implies analysing compounds exhibiting great differences in physicochemical properties. Here, we prepared representative samples by using a simple acetonitrile extraction procedure, using oxalic acid to avoid chelation of tetracyclines with metal ions available in the matrix and LC system. The effectiveness of a series of quick and easy to use clean-up options was evaluated. Validation was done in accordance with the guidelines laid down by Decision 2002/657/EC for qualitative screening methods and covered detection capability (CCb) and selectivity/specificity against various interferences.

[1] European Commission. 2010. Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. Off J Eur Union. L15:1–72.

Keywords: *veterinary drugs, screening, LC-MS/MS, animal tissues*

T22

DEVELOPMENT OF AN INTEGRATED APPROACH FOR THE DIAGNOSIS OF WATER QUALITY IN THE RIVER MEUSE (DIADEM PROJECT)

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The DIADeM (Development of an Integrated Approach for the Diagnosis of Water Quality in the Meuse) project proposes to develop and deploy an interdisciplinary, cross-border approach in order to improve the diagnosis and monitoring of the chemical quality of the water in the Meuse river and two affluents. The project proposes bringing together chemical and biological analyses (biomarkers) carried out on enclosed organisms of species representative of cross-border hydrosystems and mathematical models to predict the effect at a population level.
The objective of the CER Groupe is evaluating the bioconcentration factor and/or bioaccumulation of drugs residues and contaminants in water by their dosage in the tissues of the targeted organisms on the basis of UHPLC-MS /MS and APGC-MS/MS methodologies.
These analyzes will be carried out, on one hand, on the organisms exposed in artificial rivers and, on the other hand, on organisms caged on the various study sites.
An important objective will be to confirm the accumulation capacity of these molecules within the biota and to identify, the most appropriate species to express the contamination of water.
The progress made by the DIADeM project will help protect and enhance the environment through the integrated management of cross-border resources.

T23

DEVELOPMENT AND VALIDATION OF AN LC-MS/MS METHOD FOR DETERMINATION OF ANTIVIRAL DRUGS IN POULTRY MUSCLE

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The abuse of antiviral drugs targeting influenza in avian breeding increases the risk of new resistant avian influenza virus strains emergence and therefore, indirectly threatens human health.
A liquid chromatography-tandem mass spectrometry method for 14 antiviral drugs targeting influenza in poultry muscle is then currently being developed. The method includes well-known drugs (e.g. Amantadine) but also more recently developed drugs (e.g. Favipiravir). The difficulty of this work is the heterogeneity of these compounds chemistry and structure.
Concerning chromatography, a screening of various stationary phases has been done, experimenting with reversed mode or HILIC mode, however most were found to be unsuitable. Hydrophilic phases were found to not give enough retention for less polar compounds and high ion suppression for polar compounds (e.g. Laninamivir). The stationary phase selected among others was a Triart C18 (YMC®), as its structural properties were found to retain sufficiently polar compounds while having a reasonable retention (up to k=3.4) for non-polar compounds.
An appropriate gradient was also developed using standards in solvent, acetonitrile was favoured over methanol as an organic solvent considering peak shape and ionization efficiency. This resulted to a total run time of 9 minutes. Additionally, performances of two different modes of ionization: electrospray and Unispray® on Waters® Xevo TQ-XS have been compared, evaluating signal intensity and signal to noise ratio.
Regarding sample preparation, a compromise has been found in term of extractability with a protein precipitation step using an acidified mixture of acetonitrile/water. Performances of protein precipitation were found to be improved by centrifugation at cold temperature. The extract is evaporated and reconstituted in a suitable solvent that matches both analytes solubility and starting gradient conditions.
The matrix suppression was nonetheless found to be relatively high for some problematic compounds, particularly for the most polar one that are also eluting the earlier. The issue has been investigated via a screening of clean-up techniques. The use of dispersive SPE with a polymeric anion exchange sorbent seems to be the most efficient way to purify the matrix.
The limit of quantitation of the method is estimated to be between 1 and 10 µg/kg depending on the compound.
The aforementioned developed method is to go through validation according to the 2002/657/EC guidelines.

Keywords: *LC-MS/MS, antivirals, polar drugs, poultry*

T24

PRACTICAL USE OF HIGH RESOLUTION MASS SPECTROMETRY FOR CONFIRMATORY IDENTIFICATION IN TARGETED ANALYZES BASED ON MASS ACCURACY CRITERION IN SELECTED EXAMPLES OF RESIDUES OF BANNED SUBSTANCES AND VETERINARY DRUGS

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Targeted analysis using high resolution mass spectrometry is increasingly used for determination of banned substances and veterinary drugs. In order to protect consumers in the European Union, requirements for confirmatory analytical methods have been laid down in Commission Decision 2002/657/EC. For high resolution mass spectrometric detection are required at least two identification points per ion. Commission Decision states requirements for resolution (≥ 10 000 at 10 % valley), relative intensity of measured ions (> 10 % of the base peak), signal-to-noise ratio (≥ 3:1) but no requirement for mass accuracy parameter. However, selected examples of measured data by Orbitrap on our poster point to usefulness and necessity of using mass accuracy criterion in confirmatory identification. Mass accuracy describes the difference between the measured mass/charge (m/z) of an ion and the real, exact m/z of that ion. Orbitrap enables to measure normally with an accuracy of 1-3 ppm. For confirmatory identification in our work was determined retention time and m/z of product ion and at least 3 fragment ions within this range of mass accuracy. For structural characterisation of fragment ions was applied software Mass Frontier from Thermofisher Scientific.
Examples include mass spectrum of anabolic steroid hormone testosterone, mycotoxin deoxinivalenol and antibiotic trimethoprim. The results confirm the usefulness and practicality of using high resolution mass spectrometry, mass accuracy as an identification criterion and Mass Frontier as tool for structural characterisation of fragment ions for confirmatory identification of residues of banned substances and veterinary drugs.

Keywords: *high resolution mass spectrometry, mass accuracy, targeted analysis, confirmatory identification*

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T25

DEVELOPMENT OF A MULTI-RESIDUE/MULTI-CLASS METHOD FOR ANTIBIOTICS DETECTION IN ANIMAL TISSUES FOR THE CONTROL OF FOOD CHAIN

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An appropriate use of antibiotics in food-producing animals is strongly recommended and mandatory at least for three major reasons: (1) assure consumer protection, (2) guarantee animal health and limit antibiotic-resistance phenomena, (3) reduce/avoid critical impact on environment. Official controls are required to verify the respect of the correct use of antimicrobials veterinary drugs in terms of dosage, withdrawal times and residual levels.
Unfortunately, the term "antibiotics" include a very large number of compounds characterized by different chemical and physical properties, but laboratories need rapid and robust methods to screen a relevant number of samples for all these different compounds. In recent years, the implementation of ultra-highly performing Liquid Chromatography (LC) coupled to very sensitive Mass Spectrometry (MS), enabled to develop "multi-residue and multiclass" methods, *i.e.* one single analytical protocol to analyse different classes of antibiotics in one shot.
Therefore, the goal of this project was to develop and validate a multiclass screening method based on LC-MS for the determination of 75 antibiotic residues belonging to different classes of antibiotics (sulfonamides, macrolides, lincosamides, tetracyclines, quinolones, pleuromutilins, phenicols, rifamycin, diaminopyrimidine derivative) in muscle samples.
Different extraction and clean up procedures were tested to achieve satisfactory performances for the majority of the analytes; in the end, a simple and rapid method based on extraction with EDTA 0.25 M and acetonitrile, without SPE purification, was chosen.
Chromatographic separation was performed on a Poroshell 120 EC-C18 column (2.1 x 100 mm, I.D. 2.7 µm) using a gradient of aqueous solution with 0.1% formic acid and acetonitrile at a flow rate of 0.25 ml min^{-1}.
The method was validated according to the guidelines laid down by Commission Decision 2002/657/EC for screening method. Therefore, performance characteristics, such as detection capability (CCβ, < 10/25 µg/kg depending by analytes), specificity and ruggedness were evaluated.

Keywords: *multi-residue/multi-class method, antibiotics, screening*

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T26

DEVELOPMENT OF IC-ELISA FOR THE DETECTION OF TYLOSIN RESIDUES IN FOODSTUFFS OF ANIMAL ORIGIN

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Tylosin (TYL) is one of the most commonly used macrolide due to its effect on a wide range of Gram-positive bacteria and selected Gram-negative bacteria, pathogenic Mycoplasma and Chlamydia. For this reason, TYL is approved for prevention and treatment of respiratory and enteric infections of cattle, sheep, swine and poultry, moreover, it is highly effective against disease of honey bees. The unregulated usage of antibiotic could result in food contamination that would cause antimicrobial resistance and allergic reactions. Therefore, TYL has been prohibited as feed additive and allowed only as medicament in Europe and Russia. According to Decision №28 of Eurasian Economic Commission Council the maximum residue level for tylosin is 100 μg/kg for meat, fish and by-products, 200 μg/kg for eggs and 50 μg/kg for milk. Consequently, effective and rapid analytical method for detection tylosin residues in meat and by-products, fish, milk, eggs, honey, feed and feed additives is required. A simple indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) method for tylosin determination was developed in this study. Sera containing polyclonal antibody were obtained from male chinchilla rabbits engrafted with 0.1 mg of TYL immunogen and Freund's adjuvant as emulsion in saline. Booster injections were repeated every month for one year and blood was taken in one week after each immunisation. Serum was separated and tested for sensitivity. Good linearity was achieved in the range from 1 to 250 μg/kg with the half maximal inhibitory concentration (IC_{50}) and limit of detection (LOD) values of 30 μg/kg and 1 μg/kg, respectively. The coefficient of variation was less than 15% over the range of TYL concentrations studied. The method revealed less than 0.1% of cross-reactivity with some antibiotics, such as tilmicosin, lincomycin, clindamycin, pirlimycin, spiramycin, erythromycin, valnemulin, tiamulin, streptomycin and spectinomycin. Sample preparation for different types of products (beef, pork, kidney, liver, milk, eggs and honey) was optimized and recoveries for all used matrices were calculated for different concentration ranges. The developed technique is simple and specific and can be applied in routine analysis of tylosin residues in foodstuffs of animal origin.

Keywords: *tylosin, ELISA, macrolides' residue, food safety, polyclonal antibodies*

T27

APPLICATION OF NON-TARGET ANALYSIS FOR IDENTIFICATION OF THERMAL DEGRADATION PRODUCTS OF OXYTETRACYCLINE IN PACIFIC WHITE SHRIMP

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The detection of antibiotic residues in the aquatic environment has numerous consequences on the emergence of antibiotic resistant bacteria, and from a food safety perspective, the presence of antibiotic residues in aquaculture products can pose a health risk through alteration of intestinal microflora or triggering allergy symptoms in sensitive individuals. The degradation of antibiotic residues during cooking can also represent a health risk, as newly formed degradation products could have a higher toxicity compared to the parent compound. In the shrimp industry, oxytetracycline is one of the most used antibiotics, with residues usually below regulatory limits. Most methods target only the extraction of the parent oxytetracycline, but the application of non-target extraction methods presents a lot of advantages, as it allows not only for the extraction of oxytetracycline but also other possible compounds of interest, such as thermal degradation products. In this study, a non-targeted workflow based on a simple water/acetonitrile/methanol (0.1 % formic acid and 0.1% EDTA) extraction following analysis with LC-QTOF-MS was used to study the fate of oxytetracycline in pacific white shrimp muscle and shells after cooking. Oxytetracycline was less degraded in shrimp shells, 30% compared to 60% in muscle, and calcium content was found to have an influence of the stability of the compound in the sample studied. Degradation products unique to each matrix will be further described.

Keywords: *veterinary drugs, non target, high resolution mass spectrometry, thermal degradation*

Acknowledgement: *NSERC, FRQNT*

T28

VALIDATION OF A BIOCHIP MULTI-ARRAY TECHNOLOGY FOR THE SCREENING OF 14 SULFONAMIDE AND TRIMETHOPRIM RESIDUES IN MILK AND ITS APPLICATION FOR MONITORING OF MILK SAMPLES IN TEHRAN, IR IRAN

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Milk is one of the main sources of supply of energy, protein and fat. Antimicrobial compounds are used in food production to treat or prevent animal diseases. The antibiotic residue in milk causes hypersensitivity reactions, and reduces quality of milk and other dairy products. Because of these concerns, many countries have banned or restricted the use of antimicrobial compounds in food-producing animals and have established maximum residue limits (MRL) for antimicrobial residues in foods. There are several methods for screening of antibiotic residues in milk. The Evidence Investigator™ Biochip Array Technology (Randox, UK) has been used as a screening method for simultaneous analysis of sulfonamides and trimethoprim residues in honey. In this study, the microarray kit I (AM I of Evidence Investigator™ system) for the simultaneous detection of 14 Sulfonamides (Sulphadiazine, Sulphadimethoxine, Sulphaquinoxaline, Sulphmethazine, Sulphmethoxazole, Sulphmethiazole, Sulphisoxazole, Sulpyridine, Sulphamerazine, Sulphamonomethoxine, Sulphamethoxypyridazine, Sulphachlorpyridazine, Dapsone, Sulphdoxine) and Trimethoprim residues in milk was validated according to the European decision EC/2002/657 as well as the European guideline for the validation of screening methods for veterinary medicines (2010). After validation, it was applied for monitoring of 14 sulfonamides and TMP residues in 53 pasteurized milk samples collected from Tehran, IR Iran. The method was found to be rapid and able to screen simultaneously 14 sulphonamides and TMP in milk with no sample preparation procedure (or just one-step centrifugation). The false-positive rate of less 5% was obtained for all sulfonamides and TMP which is satisfactory. The result showed that for all antibiotics, the positivity threshold T was much higher than cut-off value Fm. Thus, all detection capabilities (CCβ) were equal to validated concentration and the CCβ were below the regulatory limit (MRL) for all antibiotics. The results of the analysis of 53 milk samples showed that 71.7% of samples were not contaminated with any antibiotic residues. In contaminated samples, concentration of trimethoprim and sum of sulfonamides were below MRL set by European commission (100 µg/kg for the sum of all sulfonamides and 50 µg/kg for trimethoprim in milk).

Keywords: *validation, biochip, multi-array technolog,y sulfonamide and trimethoprim, residues milk, IR Iran*

T29

A HIGHLY SENSITIVE AND BROAD RANGE ELISA KIT FOR THE DETERMINATION OF QUINOLONES IN ANIMAL TISSUES

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Quinolones are one of the most used classes of antibiotics, as well in human medicine as in veterinary medicine, thanks to their broad spectrum activity against a wide range of bacteria and to their physicochemical properties. However, there are recent concerns about the emergence of quinolone-resistant bacterial strains and the impact on the environment of the overuse of these drugs. In addition, fluoroquinolones carry the risk of serious adverse effects as infections, tendinopathy, neuropathy, and have multiple drug-drug interactions. Regarding the use of these antibiotics in animals production, the EU Regulation 37/2010 defines the maximum residue levels in tissues deriving from different species. In 2018, the European Medicines Agency finalized a review of serious, disabling and potentially permanent side effects with quinolone and fluoroquinolone antibiotics given by mouth, injection or inhalation. Moreover, a public health concern is that the use of fluoroquinolones in livestock selects for bacterial resistance that can be transmitted into the food chain.
In this work, the I'screen Quino ELISA kit for the determination of quinolones in animal tissues is presented. The assay has very high cross-reactivity toward a large number of different molecules of the family: ≥100% for enrofloxacin, oxolinic acid, norfloxacin, danofloxacin; 95% for ciprofloxacin; 81% for flumequine; 49% for difloxacin; 43% for sarafloxacin; 20% for marbofloxacin.
The sensitivity of the assay, in terms of the lowest amount of contaminant it can detect with a probability of 95% (CCb), was investigated for sarafloxacin, one of the quinolones with lowest cross-reactivity, in shrimp, fish and muscle (swine, bovine and chicken), testing 20 samples for each matrix. The CCb obtained were 2 ppb for shrimp, 3 ppb for fish and 5 ppb for muscle, indicating that the I'screen Quino is a very sensitive and reliable kit for the screening of quinolones.

T30

FIPRONIL IN EGG - AN ENDURING SCANDAL – ANALYSIS AND STATISTICS

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In the middle of the year 2017 a worldwide scandal started in Belgium with the found of the pesticide fipronil in eggs. Fipronil was uses in a cleaning solvent for barnstables and detected by the measurement of a food control agency.
Over a short period in our laboratory the sample number increased to 250 samples of egg and egg products a day. In the first 3 month samples mainly arrived from Europe. Later fipronil was analyzed in samples of different matrices from all over the world.
Until now nearly 18.000 samples were analyzed, about 10.000 samples during the second half of 2017 and half of them only in August 2017. The number of samples decreased from August 2017 with nearly 5000 samples per month to 150 samples in June 2019.
Statistical data of the scandal are shown from the view of a routine laboratory.
Today nearly 20% of the samples are positive for the metabolite. Over all fipronil was detected in almost 0,5% of all samples and 25% were positive for the metabolite fipronil sulfone.
88% of the positive analyzed fipronil sulfone levels ranged from 3 to 30 µg / kg, 10% of the samples had contents between 30 and 100 µg / kg and 2% between 100 and 300 µg / kg. The highest analyzed content in a sample was 5200 µg / kg.
In our poster a short overview about the analytical method for the determination of fipronil and its metabolites is given.
The large quantity of samples resulted in an automation of the sample preparation, where only the weighing is done manually. After this sample extracts were prepared automatically by a robot and analyzed by LC-MS/MS. The Method was validated according to Commission Decision 2002/657/EC an meet all requirements of the European Regulations (EC) 396/2005 and 1127/2014 setting MRL of pesticides (incl. fipronil) in or on food and feed of plant an animal origin.

Keywords: *fipronil, LC-MS/MS, egg scandal*

T31

VETERINARY DRUG ANALYSIS IN ROUTINE CONTROL OF FOOD - LC-HRMS AND LC-MS/MS - COMPARISON OF RESULTS

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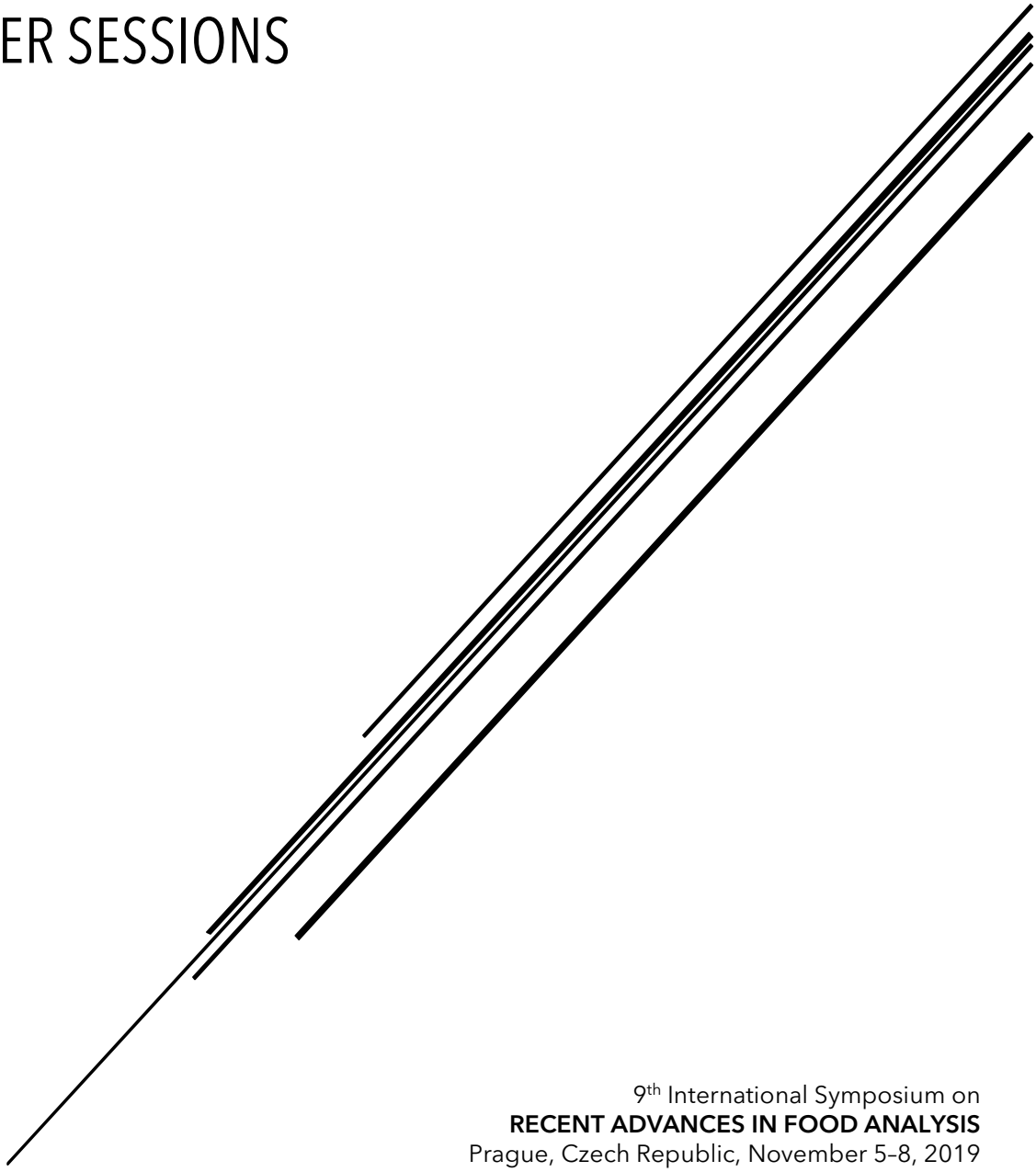
Veterinary drugs are widely used for animal breeding to prevent or treat diseases or to promote growth. Abuse of veterinary drugs and its residues in food becomes an increasing problem due to the risk to human health.
In the EU the use of veterinary drugs is regulated by Commission Regulation (EU) 37/2010 establishing Maximum Residue Limits (MRL) and listing prohibited substances. For routine control of veterinary drug residues in food sensitive and robust analytical methods are needed.
To cover the whole spectrum of relevant analytes a screening and quantification method for the simultaneous determination of about 120 veterinary drug residues in food (amphenicoles, benzimidazoles, quinolones, beta-lactams, macrolides, nitroimidazoles, sulfonamides, tetracyclines and triphenylmethan dyes) by LC-HRMS was developed.
The method was validated according to the requirements of the Commission Decision (EU) No 2002/657/EC and is successfully applied in egg, meat, fish and milk.
Reproducibility (CV for meat: 1.1 – 36 %) and repeatability (CV for milk: 0.7 – 19 %) are within an acceptable range and the LOQ meets the requirements with regard to the MRL.
Positive results of the Thermo Scientific Q Exactive Orbitrap™ LC-HRMS were confirmed by measurement with a Sciex API 5500 QTrap™ LC-MS/MS system. Since 2013 in our laboratory about 600 samples of several matrices have been measured by LC-HRMS and LC-MS/MS. Comparison of about 800 results show good accordance between both techniques.
Egg and egg products are most often positive (270 of 800 results), followed by fish (about 120 results), meat (about 100 results) and milk and milk products (about 80 results).

Keywords: *Orbitrap, HR-MS, LC-MS/MS, multi-method, vet. drugs*

SENSORY ANALYSIS

POSTER SESSIONS



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U1

ROMANIAN CONSUMERS PERCEPTION ABOUT SENSORY QUALITY OF BLACK ANGUS

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The *Black Angus* meat is considered worldwide as the ideal choice for a successful, tasty and at the same time healthy steak. Although Angus meat is available on the Romanian market from 15 years ago, there are only a few consumers who use it frequently (4.9 kg/head/year compared to pork meat 36.1kg/head/year) according to the annual balance (NIS 2017).
The beef cuts used in this research were bought from a Romanian farm of *Black Angus*. The sensory characteristics of the *Black Angus* beef cuts (Brisket and tenderloin) matured for 21 days were evaluated by voluntarily beef consumers (n=18). Each sample was cut into 1.25 x 1.25 x 2.50 cm pieces (cut across the grain), cooked up until the internal temperature reached 71°C (medium done). Randomly identified three-digit-codes for each cut were labeled on the side of the porcelain plate. Samples were evaluated immediately after cooking, and assessed randomly by the evaluaters. Participants evaluated the beef quality for consumption. Briefly, the test included evaluation of tenderness, juiciness, taste/flavour and overall appreciation of beef samples on a scale of 1 to 8, where 1 = not at all tender, extremely tough, dry, not flavourfull and dislike extremely and 8 = extremely tender, juicy, flavourful and extremely like (ASMA, 2015). The panel was also asked to record their perception about quality of consumption for each sample on the following scale: unsatisfactory, good everyday quality, better than good everyday quality and permium quality (ASMA, 2015). The data were analyzed on Microsoft Office Excel and Consumer Check.
The consumers panel found that the beef muscle was more tender, juicy, tasteful and flavorful than the Brisket (Anova, $P<0.05$). Evaluation of muscle resulted in significantly higher scores overall quality (6.17), taste (5.94), juiciness (6.39) and tenderness (6.50) than Brisket overall quality (4.33), taste (4.61), juiciness (4.06) and tenderness (3.61).
As previous research already demonstrated, there is a complex relationship between the three key eating quality traits of beef (tenderness, juiciness and taste/flavor). This study is a preliminary consumer testing about the sensory perception of beef. The data from the current study indicate that Romanian consumers score higher the beef muscle as opposed to Brisket. One explanation might be the difference of fat distribution in muscle and Brisket. However, further research is needed to evaluate if the Brisket was scored less due to the visual cue of the fat (many panelist left the fat layer untouched), or due to the taste.

Keywords: *Black Angus, beef consumers, sensory evaluation, quality for consumption*

U2

THE CONCURRENT USE OF HEDONIC TEST, J.A.R. AND RANKING TEST ON ROMANIAN BRINE CHEESE INCREASE PRODUCT DISCRIMINATION

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Romanian brine cheese (telemea) is highly appreciated by consumers due to its high nutritive value, rich flavour, versatility in preparation of a variety of dishes. It is prepared from milk from various species but mostly from cow milk and buffalo milk. Various brands of brine cheese are available on the Romanian market. They present slightly different sensory characteristics especially regarding the taste (intensity of salty and / or sour taste) and texture which further leads to consumer preference. It was the purpose of this study to evaluate the consumers' perception about the sourness and texture of brine cheese. The study was performed at USAMV Cluj-Napoca, Laboratory of Sensory Analysis of Foods. Four different brine cheeses available on supermarket were prepared by labelling with three-digit codes (201, 426, 553, 636) and sampling 2 square pieces (1cm /edge). Each consumer (n=100, age 18-55 years old) received the samples randomly. Consumers evaluated the sourness and creaminess of samples on 5 point hedonic test (e.g. hedonic scale for evaluation of sourness: 1 – very unpleasant, 2 – unpleasant, 3 – indifferent, 4 – pleasant, 5 – very pleasant) and 5 point Just About Right test (e.g. J.A.R. scale for evaluation of sourness: 1 – not at all sour, 2 – slightly sour, 3 –ideal for me, 4 – too sour for me, 5 – extremely sour). Data were interpreted using Consumer Check Software. The values of hedonic scores for sour taste ranged from 3.65±0.96 to 4.06±0.83, while creaminess 3.21±1.00 to 3.60±0.85. Although the hedonic scores for both sourness and texture were in the range of pleasant area of the scale (above 3), the general J.A.R. scores for each attribute were slightly lower than the value "ideal for me" (2.44-3.12). A close look at the distribution of the J.A.R. scores shows that above 40% of the consumers considered the samples either too sour or too creamy. PCA analysis revealed that sample 201 was perceived completely different than all samples tested, while samples 636 and 462 were perceived similar in terms of sour taste and creaminess. Sample 636 was placed first on ranking test although it did score the lowest on hedonic test, but it was the first on J.A.R test. Samples 201 and 462 were both on the second place in ranking test. In conclusion, an ideal brine cheese in the mind of consumers is slightly sour and harder in texture.

U3

SENSORY EVALUATION OF COLD PRESSED SUNFLOWER OIL

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Sunflower oil is highly appreciated by East European consumers due to its high availability, convenient price and good flavor to either fried or cooked products. According to FAO data (2019), sunflower oil consumption continues to grow worldwide. Even more, according to the same source, Romania was the sixth oil-seed producer in the world in 2013. Cold pressed sunflower oil won a positive image in the mind of health-conscious consumers. It was the purpose of this study to evaluate the consumers' acceptability of cold pressed sunflower oil. The study was performed at USAMV Cluj-Napoca, Laboratory of Sensory Analysis of Foods, on voluntarily consumers (n=75) selected based on their availability and oil consumption habits. The samples - provided by a local producer - were prepared in plastic transparent cups (20 ml oil) and were labeled with three-digit codes. The sensory characteristics evaluated on the 9 point hedonic scale were overall appreciation, aspect, color, viscosity, smell, taste, aroma, after taste. Participants were asked to record the intensity of their perception about some positive and negative sensory characteristics like: sweetness, acidity, bitterness, astringency, rancid, roasty and other sensations using a 5 level intensity scale (not at all, slightly, moderate, much, intense). The data were analyzed on Microsoft Office Excel and Consumer Check. Most of the participants declared that they use sunflower oil for cooking (86.84%) and only about 9% use sunflower oil for salads. All sensory characteristics evaluated on hedonic scale recorded high, positive scores (above 7.74±0.95). The consumer's panel compared the cold pressed sunflower oil with the refined version which they remembered from daily-use at home and found that the former was very tasty and rich in flavor. The negative sensory attributes of cold pressed sunflower oil were very low (below 1.5 on the 5 level intensity scale). Many small producers invest and develop the technology to create high quality cold-pressed oils for niche consumer segments, especially health oriented consumers. Therefore, due to this abundance on the market of cold pressed oils, research oriented to develop a lexicon for these products will be in the benefit of both producers and consumers.

U4

SALT CONTENT REDUCTION IN HOT-SMOKED HORSE MACKEREL (TRACHURUS TRACHURUS) – ITS EFFECT ON SENSORY PROPERTIES AND ACCEPTABILITY

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Smoking is one of the oldest methods of food preservation. It can be applied to preserve fish or other food products by exposing them to smoke from smouldering wood or other plant materials. The whole process usually consists of a combination of salting, drying, heating and smoking steps. Nowadays salting and smoking are mainly used only for imparting flavour, the preservative effect being secondary. Furthermore, reducing salt intake is an important public health target and Food industry and catering services are searching for means to reduce the salt content in their products
This study aims to ascertain consumers' acceptability of smoked horse mackerel fillets (*Trachurus trachurus*) concerning salt reduction.
Thirty-six fishes were filleted and *fillets skin*-on were further separated in four batches: brined (21% salt) for 1 minute (B1) and 2 minutes (B2); dry-salted for 15 minutes (D15) and 30 minutes (D30) and kept at 4 - 5ºC for 24 hours. Smoking (70 °C for 3 hours) took place in a mechanical smoker (Maxi AFOS MK, England) followed by a heat shock of 90 °C for 10 minutes.
Salt content (NaCl), moisture, water activity (a_w), were determined (three fillets for each determination). A one-way ANOVA followed by Tukey HSD test was applied. A quantitative descriptive analysis test, considering eight attributes (colour uniformity, brightness, smooth smoke, hardness, succulence, characteristic (to smoked), pleasant fishy and salty taste), was carried out by the 7 semi-trained panelists. Product acceptability was performed to 30 people using a nine points hedonic scale.
In samples B2 and D30, non-significant differences ($p>0.05$) were found in NaCl content (3.41±0.03% and 3.33±0.10%, respectively) but significant differences ($p<0.05$) were observed in NaCl content of B1 and D15 samples, 3.09±0.04% and 2.35±0.07%, respectively. Non- significant differences in moisture content were detected in B2 and D30 samples (56.81±0.21% and 57.16±0.40%, respectively). B1 samples presented the lowest moisture content (55.40±0.54%) whereas D15 presented the highest (58.70±0.28%). Concerning a_w the same behaviour was observed, sample B1 showed the lowest values (0.92±0.01), followed by B2 and D30 (0.93±0.01 and 0.93±0.01, respectively), and D15 samples presented the highest a_w value (0.94±0.01). Significant differences in a_w were only observed between the B1 and D15 samples ($p<0.05$). Regarding sensory evaluation, there was some dispersion among the panelist's perception. This dispersion was attributed to the overall similarity of samples' characteristics.
Regarding consumers' acceptability, non-significant differences were detected despite of the different salt contents. This means that salt reduction of hot-smoked horse mackerel can be applied without deceiving consumer expectations, therefore the process dry-salting for 15 minutes will be chosen for further salt reduction and acceptability experiments.

Keywords: *salt reduction, smoked fish, consumers, acceptability*

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U5

HS-GC-IMS AS A SCREENING TOOL TO DISCRIMINATE VIRGIN OLIVE OILS QUALITY GRADES

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Sensory attributes evaluated by Panel Test in virgin olive oils are among the parameters to classify the product in the respective quality grades (extra virgin, EVOO; virgin, VOO; and lampante, LOO). Their presence and perceived intensity is due to the occurrence of specific volatile compounds depending on diverse factors, including the variety and the cultivation area of the olives, as well as other agronomic, environmental and technological variables. The development of rapid screening instrumental methods, based on the analysis of volatile compounds, to support the Panel test, represents one of the most current challenges for researchers in the sector and it is foreseen in the framework of the EU H2020 OLEUM project, as part of the "Quantitative Panel Test". The main objective of these analytical approaches is to pre-classify oils amply belonging to one product category (e.g. extra virgin or lampante olive oils), in such a way reducing the number of samples that need to be assessed by the Panel test to those closer to the border between two different categories. This would reduce the daily work of the panels (both official and professional), so increasing the efficiency of the quality controls. In this experimental work, a FlavourSpec HS-GC-IMS (Headspace Gas Chromatography Ion Mobility Spectrometry) from G.A.S. Dortmund to analyze 167 samples of virgin olive oil, most of which directly collected from olive oil companies among the years 2017 and 2018 within the OLEUM project, was used, as targeted approach. The 3D data matrices composed with 15 volatile compounds, previously selected as markers of positive and/or negative sensory attributes in virgin olive oils, were used to developed chemometric models by using PLS Toolbox for MATLAB. Specifically, two different types of chemometric techniques have been used: PLS-DA to discriminate the samples according to the quality grades (EVOO *vs* non-EVOO and LOO *vs* non-LOO) and PLS, to estimate the sensory attributes (the most perceived defect and the positive attribute of fruity). Furthermore, the analytical performance of the HS-GC-IMS method was evaluated, calculating the intra and inter-day repeatability, in terms of RSD% of the area of two specific volatile markers in a single sample for each quality grade. For intra-day repeatability, seven replicates of a single sample for each quality grade in a single day were analyzed, while for inter-day repeatability each sample has been analyzed in seven different days. Another parameter evaluated for the performance of the method was the linearity of the 15 examined volatile compounds, expressed in terms of range and R^2. Finally, through the calibration curves the 15 volatile compounds were quantified in the virgin olive oil samples and their concentrations discussed in respect to their occurrence in the different quality grades.

Keywords: *olive oil volatile compounds sensory analysis HS-GC-IMS chemometric analysis*

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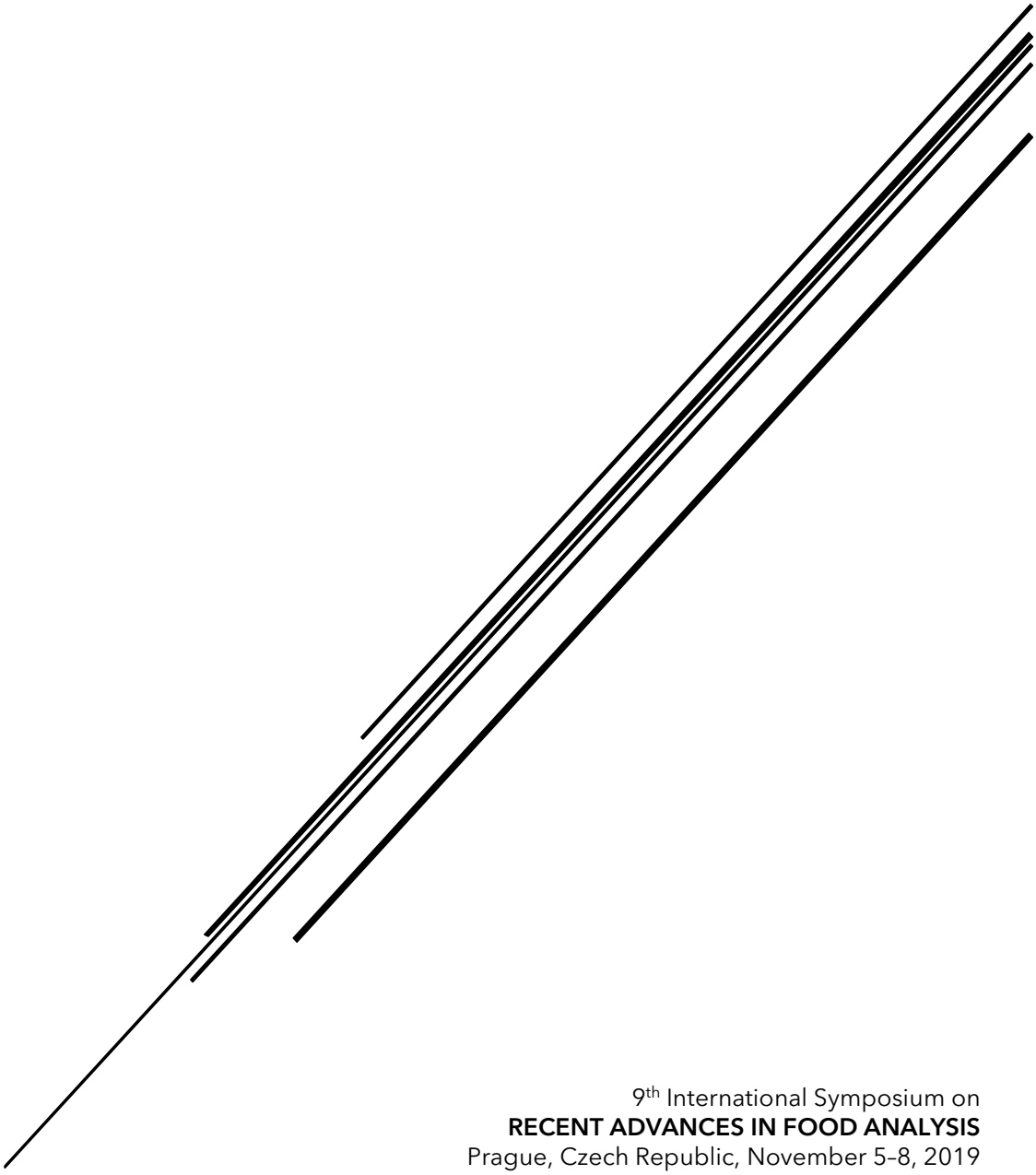
SMART SENSORS

POSTER SESSIONS



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V1

DETECTION OF WATER ADDITION IN FRESH, FROZEN AND THAWED BIGEYED TUNA (THUNNUS OBESUS) BY NEAR INFRARED SPECTROSCOPY

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Seafood is a healthy protein and lipid source, widely consumed worldwide (Sriket et al., 2007). Being an increasingly valued product, it is susceptible to abusive industrial processing along the whole supply chain, namely induced water uptake by injecting water and additives above the regulations. European Regulation (1169/2011, Annex VI) states that in prepared fishery products, "which have the appearance of a cut, joint, slice, portion, filet or of a whole fishery product", it is mandatory the declaration of the presence of added water if it makes up more than 5% of the weigh of the finished product. Nevertheless, this declaration is not always included and this, together with recent food fraud scandals (RASF, 2014) are leading to a decrease of trust in the seafood value chain.
The more expensive a seafood product is, the more susceptible to this type of fraud in which consumers are purchasing water at "seafood" price, such as octopus (Mendes, et al., 2017a; Mendes, et al., 2017b; Mendes et al., 2018). Also, tuna and tuna like species are especially prone to being mislabeled (Sotelo, et al., 2018).
To date, there are few smart sensors for detecting this type of fraud. Sensors based on Near infrared spectroscopy (NIRs), together with machine learning classification techniques, can be powerful tools for detecting water addition in seafood products in real time and in a non-destructive way.
In the present work we used bigeye tuna (*Thunnus obesus*) as model for testing this technology in water addition detection, in three different stages: fresh, frozen and thawed and as spectrometer, a low-cost handheld device (MicroNIR OnSite, Viavi), working from 950-1650 nm. We scanned 60 tuna portions of about 300-400 g: 10 were used as control and 50 were injected with different solutions of water and additives. For every three conditions (fresh, frozen and thawed) 8 scans per portion were acquired.
In order to clean the acquired signal from the noise, spectra were submitted to several pre-processing methods, such as 1st and 2nd Savitzky-Golay derivatives, standard normal variate (SNV) and combinations of all of them. Then, data were mean centered and afterwards, three different classification machine learning methods, such as partial least squares discriminant analysis (PLS-DA), k-Nearest Neighbors (kNN) and Support Vector Machines (SVM) were tested in order to create models able to distinguish between injected and non-injected portions at any of the three tested stages.
From all the tested techniques, PLS-DA gave the best results, with an accuracy in the cross-validation of 0.88 for the fresh stage, 0.94 in the frozen and 0.76 in the thawed stage.
These results show the potential of the use of NIR spectroscopy together with machine learning for detection of water injection in tuna in any kind of storage condition (with different water injection treatments), which opens a world of possibilities for increased performance of food fraud detection.

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V2

RAPID AND NONDESTRUCTIVE DETERMINATION OF QUALITY CHARACTERISTICS IN CHERRY TOMATO USING HYPERSPECTRAL IMAGING AND CHEMOMETRICS

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Cherry tomato has currently become widely consumed. It possesses a number of beneficial nutrients such as vitamins A, C, and E; lycopene; β-carotene; and other bioactive components. During postharvest storage period, quality of fruit changes rapidly because of respiration and metabolic activities. Firmness, soluble solids content (SSC), acidity (pH) and color (Lab) are the primary quality attributes of cherry tomatoes which are associated with changes in structure and composition of the cells, and they directly affect consumer acceptance. These quality attributes of cherry tomatoes are often obtained by instrumental measurement. However, the instrumental method of quality evaluation is highly time-consuming, needs to destroy the fruit, and requires extensive labor-intensive work. Because of these limitations, increasing number of researches are focused on developing sensor-based technologies and nondestructive methods to evaluate the quality of fruit. The objectives of the present study are to evaluate the use of Vis/NIR hyperspectral imaging in measuring the quality characteristics of cherry tomatoes. In total, 600 cherry tomatoes were analysed. Tomato samples were supplied by a local industry in Almería (Spain) every week during seven months. The sampling process included two stages: hyperspectral analysis and quality parameters measurement. The quality parameters were used as a Reference value in constructing the regression models and measuring the models performance to predict the quality parameters in hyperspectral approach. The quality parameters of tomatoes were represented by the SSC, pH, fruit firmness and color. On the other hand, hyperspectral images of tomatoes were collected using Specim FX-10 camera working at 400-1000 nm and Specim FX-17 camera working at 900-1700 nm. Image correction was performed to get the reflectance value of each pixel in full wavelength spectral band.
Partial least square (PLS) was applied to build the regression models to predict these quality characteristics from their spectra. The effects of different preprocessing techniques, including smoothing, first- and second-derivative Savitzky–Golay (S-G) and standard normal variate (SNV) on prediction performance were also evaluated. In the end, validation analysis was performed to evaluate the method in predicting the tomato quality regarding the quantitative parameter. VNIR spectra-based models performed the best at estimating the quality characteristics in cherry tomatoes because of high fitting correlation coefficients (R^2_{pred}), low Root Mean Square Error of Prediction (RMSEP) values, and the high Residual Predictive Deviation (RPD). Excellent prediction for SSC (RPD value greater than 3.0) and good prediction for acidity (RPD value between 2.0 and 2.5) were obtained using PLS models. Consequently, the simplicity of the proposed method suggests that it can be used as a tool for the non-destructive estimation of quality parameters in the tomato industry.

Keywords: *cherry tomatoes, hyperspectral imaging, quality characteristics, PLS models*

Acknowledgement: *The authors gratefully acknowledge the supply of cherry tomatoes from the local industry FERVA SAT (Almería, Spain).*

V3

A PORTABLE BACTERIOPHAGE-BASED ELECTROCHEMICAL BIOSENSOR FOR DIRECT AND RAPID DETECTION OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) IN FOOD AND ENVIRONMENTAL SAMPLES

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Biosensors combines biological components with physicochemical detectors, which can detect pathogens (ie Shiga Toxin-Producing *Escherichia coli*, STEC) and other significant analytes. Immunosensors have gained popularity due to bioaffinity and commercial availability but suffer from a number of limitations such as cross-reactivity among species (ie polyclonal antibodies), interference such as background noise, and high production cost.
The purpose of this study was to directly detect viable STEC cells in complex matrices using a portable STEC-specific bacteriophage-based electrochemical biosensor without enrichment and conduct cost analysis against commercially-available STEC screening methods.
An electrochemical biosensor, comprised of biotinylated bacteriophages, was developed to capture and detect viable STEC cells by a sandwich-type recognition approach. Fresh ground beef (FGB) and pasteurized apple juice (PAJ) inoculated with STEC strains, and natural environmental water samples (25 g or mL) were prepared. Samples (50 mL) were then incubated on bacteriophage-functionalized electrodes (12 min, room temp) before sequentially adding bacteriophage-gold nanoparticles solution (20 mL), H_2O_2 (40 mM), and 1, 1'-ferrocenedicarboxylic acid for amperometric test (100 mV/s). Delta currents were analyzed using ANOVA and LSD ($p<0.05$). A product cost structure was defined to determine the total cost per assay and compare it with current STEC screening methods.
The detection limits (DLs) of FGB were 1 log (O157) and 2 log (O26 and O179) CFU/g with R^2 values of 0.98, 0.95, and 0.76, respectively. The DLs of PAJ were 1 log (O157) and 2 log (O26 and O179) CFU/mL with R^2 values of 0.94, 0.95, and 0.83, respectively. Negative controls and natural environmental samples had delta currents below the positive detection threshold. The estimated cost of a single test was $3.28, which is 75% and 77% lower than that of molecular-based and immunoassay-based methods, respectively.
The application of the bacteriophage-based biosensor in complex matrices revealed its superior sensitivity and specificity even without enrichment. Low cost and on-site screening capability make it as an excellent alternative to current STEC detection methods.

Keywords: *biosensor, portable, on-site*

Acknowledgement: *We would like to acknowledge Produce Safety and Microbiology Unit, USDA ARS. U.S. Patent Application has been filed.*

V4

EXPLORING THE FORENSIC APPLICATION OF A NOVEL, RAPID, ECONOMIC AND EASY-TO-USE HANDHELD NIR SPECTROMETER FOR CHEMICAL IDENTIFICATION, ACTIVE INGREDIENT QUANTIFICATION AND COUNTERFEIT DETECTION

Yannick Weesepoel*[1], **Tjerk Venderink**[1], **Peter Keizers**[2], **Frank Bakker**[2], **Marcel Heerschop**[3], **Annette van Esch**[4], **Fionn Wallace**[4], **Annemieke Hulsbergen- van den Berg**[4], **Arian van Asten**[5]

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The application of a small, smart-phone operated, hand-held short-wavelength range near-infrared (SW-NIR) spectrometer was explored for several forensic screening and security applications aimed at foods, chemicals, drugs and medicines. Seven SW-NIR devices were used in this study to investigate the reproducibility between scanners for the several cases investigated. Multivariate statistics were used to assess the viability of the specific SW-NIR application per case. The SW-NIR proved to be very effective for chemical identification of organic materials (such as skimmed milk powders, cocaine, cutting agents, knitting explosives, etc.), whilst for inorganic materials (such as pyrotechnics, gun powders, salts) no useful spectral signatures were obtained. The active compound cocaine could be quantified in seven mixture types independent of the cutting agent used with an accuracy of 5 % (*w/w*) through specific SW-NIR variable selection. A similar case was established for skimmed milk powders and addition of illegal nitrogen enhancers (i.e. melamine) and low-value fillers (i.e. whey proteins). MDMA tablets with different tabletting materials were investigated and compared to similar tablets with different active compounds. Poor quality amoxicillin capsules could be distinguished based on active substance content. Finally, identification counterfeit spirits and identification of the presence of methanol and other higher alcohols were successful. In conclusion, this study demonstrates the potential of SW-NIR to assist law enforcement and inspection officials with on-site, rapid, easy-to-use chemical identification and classification.

Keywords: *near-infrared, drugs of abuse, food adulteration, on-site detection, spectroscopy*

V5

GOLD NANOPARTICLES ACTING AS PEROXIDASE-MIMICKING NANOZYMES FOR BIOLOGICAL AND CHEMICAL ANALYSES IN FOOD: CHALLENGES AND OPPORTUNITIES

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Gold nanoparticles have demonstrated excellent, intrinsic peroxidase-mimicking activity (so-called nanozymes). When merged with their ease of synthesis, biocompatibility, tunability, and low cost, the property enables them excellent candidates for applications in biological and chemical analyses. Despite these unique advantages, rational design of efficient peroxidase-mimicking nanozymes remains highly desirable yet challenging. These aspects will be reviewed in the presentation. Our recent research progress on fundamental properties and practical applications of the nanozymes for the detections of biological and chemical contaminants in food will also be reported.

V6

LOOKING FOR AFLATOXIN B CONTAMINATION WITH A LOW COST OPTICAL APPARATUS AND MACHINE LEARNING APPROACH

Francesca Romana Bertani[(1)], **Annamaria Gerardino***[(1)], **Luca Businaro**[(1)], **Eugenio Martinelli**[(2)], **Arianna Mencattini**[(2)], **Davide Di Giuseppe**[(2)], **Michele Solfrizzo**[(3)], **Lucia Gambacorta**[(3)]

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Aflatoxin detection currently relies mainly on chemical methods usually based on chromatography approaches, and recently developed immunochemical based assays that are fairly accurate, however, they are time-consuming, expensive and destructive. Non-destructive, optical approaches are recently being developed in order to assess the presence of contamination in a cost and time-effective way, maintaining high levels of accuracy and reproducibility, but are usually based on the benchtop and expensive instruments. Here we will present the evolution of results of the analysis of fluorescence spectra of contaminated almond samples during the development of an optical multi-sensor device in the framework of PhasmaFOOD project. The aim of the project was to develop a low cost and portable instrument comprising multispectral and imaging capabilities, conjugated with a cloud Reference database and analysis toolbox for food features analysis. One of the use cases of the project is the fast, reliable and non-destructive detection of mycotoxin (in particular aflatoxin) contamination in food products. For this use case, we used in particular fluorescence spectroscopy and different approaches to data analysis. After the first feasibility tests in the range of mg/g contamination range with a simple and effective analysis that led to highly reliable results, the work was focused on the detection limits with samples (almond) in the range of 0-291 ng/g acquired with a simple portable device and excitation light at 365 nm wavelength. An ad hoc processing strategy based on a feature selection steps coupled with a nonlinear classifier has been developed and test with two different datasets collected one month from the other another. The system performances have been evaluated training the classification model with one dataset and testing its with the other. The results have shown an accuracy higher than 80% with a threshold lower than 10ppb as contamination level.

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*

V7

SMARTPHONE-BASED PORTABLE INSTRUMENTS FOR FOOD SAFETY APPLICATIONS

Hyun Jung Min[(1)], **Xiyao Wang**[(1)], **Iyll-Joon Doh**[(1)], **Bruce Applegate**[(1)], **Amanda Deering**[(1)], **J. Paul Robinson**[(1)], **Euiwon Bae***[(1)]

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Background: Due to the economy of scale, smartphones are becoming more affordable while their computing powers are increasing dramatically every year. In this work we developed a smartphone into a portable instrument for analyte quantitation utilizing the characteristics of typical smartphone imaging systems specifically designed for use as transducers for different applications. Three applications were included in this work: quantitative colorimetric analysis, ultra-low radiant flux detection, and portable spectrometry. Method: For colorimetric applications, quantitative analysis of lateral flow assays (LFA) for *E. coli* O157:H7 and *Salmonella* were conducted for pure cultures and artificially contaminated food samples. For low-light detection applications, bacteriophage ϕV10 nanoluc was utilized to induce a luminescent signal from *E. coli* O157:H7. A series of detection assays using the smartphone including serial dilutions of pure cultures and artificially contaminated ground meat were compared to a Referenceluminometer. For portable spectrometry, protein concentrations were estimated by the Biruet assay and smartphone spectrometry measurements were challenged against various milk samples for protein concentrations. Result: Smartphone LFA systems were able to detect 10^4- 10^5 CFU/ml samples from pure and food matrices while the low-light application was able to detect the presence of low concentrations of *E. coli* O157:H7 within 12 hr of phage addition to ground beef. For smartphone spectrometry, protein concentrations were accurately estimated within of actual concentrations.

Keywords: *smartphone, portable instrument, lateral flow assay, luminescence, colorimetry*

Acknowledgement: *This material is based upon work supported by the U.S. Department of Agriculture, Agricultural Research Service, under Project No. 8072-42000-077. This work was also supported by the USDA National Institute of Food and Agriculture, Hatch project number 1004830. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.*

V8

WAX PRINTING APPLICATION ON A HYBRID PAPER-3D PRINTED LAB-ON-A-CHIP INJECTOR WITH SMARTPHONE-BASED COLORIMETRIC READ-OUT

Aristeidis Tsagkaris*[1], **Davide Migliorelli**[2], **Daniel Filippini**[3], **Jana Pulkrabova**[1], **Jana Hajslova**[1]

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An ever-increased use of paper for the fabrication of microfluidic devices has been noticed due to the inherent merits of paper, namely, portability, cost-effectiveness, capillarity and disposability. In this context, microfluidic paper-based analytical devices (μPADs) feature alternative characteristics which have been widely applied for improving point of care testing (POCT), mostly, in the medical field [1]. However, this concept has also the potential to improve food testing status providing in-situ detection of food contaminants [2]. An important challenge that has to be faced is to retain the μPADs fabrication cost as low as possible. A step towards this direction is to apply the "wax printing" concept in which minimal resources are required (paper, a commercial printer and a hot plate). The printer prints patterns of solid wax on the surface of a paper, and a hot plate melts the wax so that it penetrates the full thickness of the paper. This process creates complete hydrophobic barriers on paper that can be exploited in various ways such as hydrophilic channels, fluid reservoirs, or reaction zones. Up to date, we are developing a hybrid paper-3D printed lab-on-a-chip (LOC) assay for on-site screening of organophosphate (OP) and carbamate (CM) insecticides. This is a competitive assay (OPs and CMs inhibit AChE activity) based on acetylcholinesterase (AChE) activity which is related to color production on paper strips and the monitoring of this color intensity with a smartphone reader [3]. In this study, wax printing was applied to investigate the assumption that by printing hydrophobic wax channels, the color intensity of the AChE paper assay may be increased and also the necessary enzyme solution for strips preparation may be reduced. Reasonably, the hydrophilic region modification required assay re-optimization in terms of enzyme concertation and the volume of samples and reagents. Importantly, wax thickness and pattern were tested and optimized conditions are presented. In conclusion, wax printing can be beneficial for paper-based assays requiring minimal instrumentation and simple fabrication.

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Keywords: *lab-on-a-chip, acetylcholinesterase, smartphone, wax printing, pesticides*

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V9

INTEGRATING DIFFERENT SPECTROSCOPY SENSORS TO IMPLEMENT A VERSATILE MULTIMODE ANALYSIS INSTRUMENT: TECHNICAL CHALLENGES AND SOLUTIONS

Francesca Romana Bertani[1], **Annamaria Gerardino***[1], **Luca Businaro**[1], **Peter Reinig**[2], **Jens Knobbe**[2], **Hans-Georg Dallmann**[2]

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In the frame of the EU project PhasmaFOOD a portable photonic multisensor device for the detection of food contaminations, spoilage and fraud has been developed. It integrates different capabilities: spectroscopic detection (from 400nm to 1890 nm, including UV excited fluorescence, visible reflectance, NIR reflectance), imaging (RGB camera), smart signal processing, data analysis and comparison with updated models on cloud platform hosting data set for training and calibration of food analysis algorithms and it supports interface with a smartphone/tablet and mobile application (iOS and Android). Several technical issues have been faced in the development of such an integrated device and its performances have been tested in different laboratory environment. The PhasmaFOOD device incorporates the sensing unit, including optical components, detectors and illumination sources, which conduct the measurements on the food samples, as well as an electronic subsystem in order to control, collect and partially process the aforementioned sensory measurements. A mechanical enclosure for the sensing device, particularly designed with emphasis and attention to achieving the highest possible compactness, ensures that the PhasmaFOOD sensing device will be user-friendly and easy to use. We will here illustrate technical choices made to match functional requirements, taking into account mechanical, optical, electrical, electronic operational requirements. We will detail the choice of detectors (UV-VIS spectrometer, NIR spectrometer, CMOS camera), illumination sources (UV led, White LED, NIR microlamp), optical components (lenses, filters) and the definition of an acquisition protocol, the problems solved and the open questions.

Keywords: *mutimodal analysis, NIR spectroscopy, fluorescence spectroscopy, RGB imaging*

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*

V10

MOBILE, MULTIANALYTE BIOSENSING FOR FOOD SAFETY MONITORING

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Rapid and reliable on-site monitoring of multiple substances from food samples is of paramount importance due to the increased complexity of food supply chain. For example, different antibiotics are frequently used to treat cows against diseases and for prophylaxis during the dry period. However, high level of antibiotics in milk or meat present significant health risks to humans, as well as induce uprising of antibiotic resistant bacterial strains. Antibiotics can also prevent further processing of milk into yoghurt or cheese, thus causing economic damage in the order of EUR 200 million annually in EU alone. For this reason, the allowed antibiotic concentrations are regulated in EU and worldwide and it is necessary to develop methods for their rapid detection along the whole supply chain. On site monitoring on-farm, during transport and processing prevents mixing of contaminated volumes with larger milk pool and is thus of significant economic importance. In cereals mycotoxins destroy large quantities of cereals if not detected in time. Biomensio is developing a mobile biosensing platform for rapid detection of 20-30 analytes from a single droplet of sample. Our technology is based on mass-sensitive microarray (MSMA) chip, which is comprised of 64 individual sensors on a single chip. Thanks to microfabrication technologies MSMA can be produced in high scale and high quality at low cost. Our sensing platform is suitable for rapid real-time, label-free measurement from any liquid or liquified sample. We present here applications for monitoring antibiotics from milk, mycotoxins from grain samples and drugs-of-abuse from saliva. In addition to measurement platform, we are developing powerful data analysis and cloud systems for efficient data management.

LAST MINUTE

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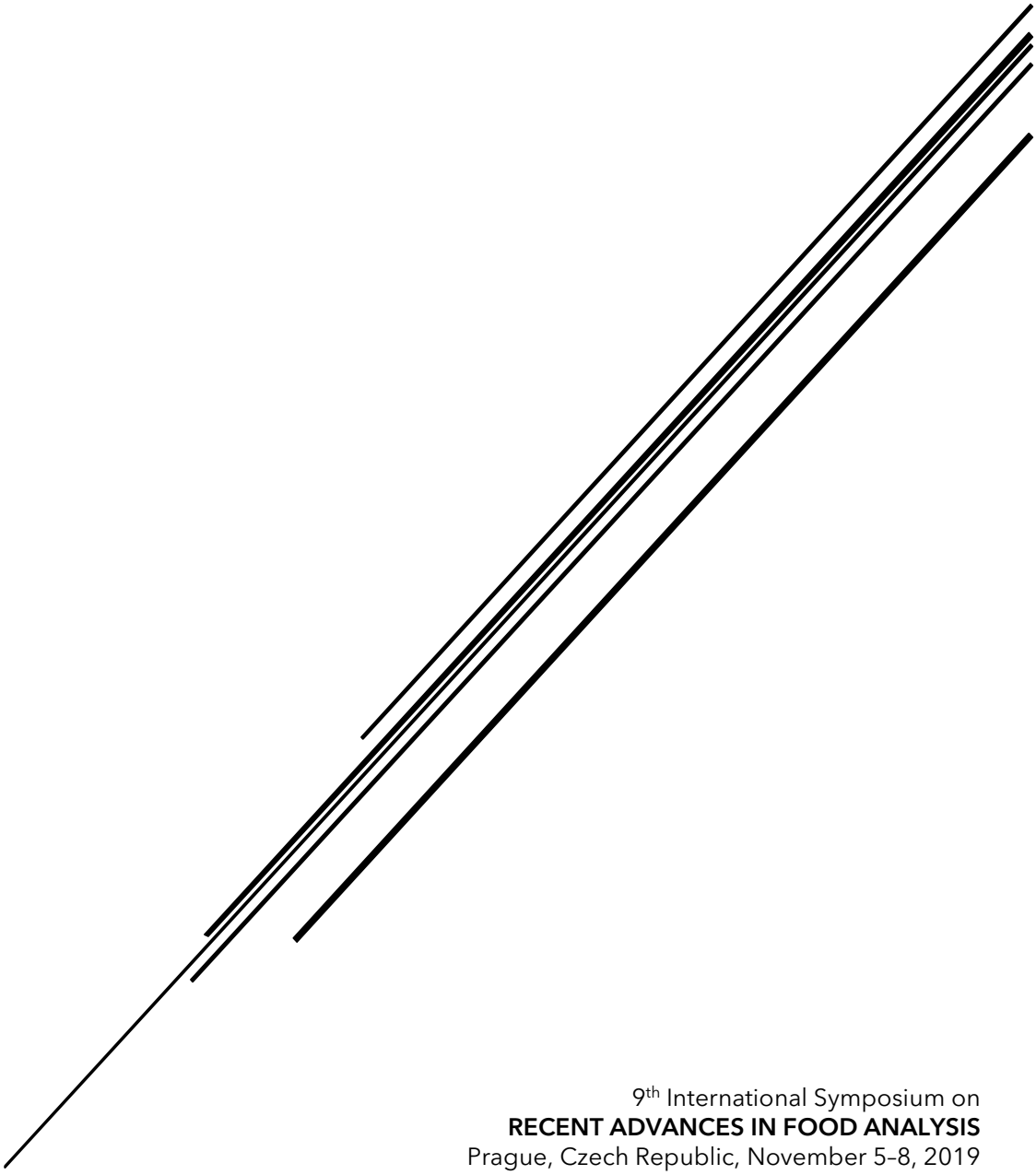

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X1

AUTOMATED EXTRACTION OF GLYPHOSATE/AMPA/GLUFOSINATE IN RED WINE PRIOR TO LC-MS/MS ANALYSIS WITHOUT DERIVATIZATION

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This application describes the rapid purification of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate from red wine using a new highly selective solid phase extraction material, AFFINIMIP® SPE Glyphosate. The automation of this efficient SPE clean up and concentration process with the Gilson 241 ASPEC helps eliminate users variability and facilitate training in high throughput laboratories. The benefit of this process is that it allows to purify samples for LC-MS/MS analysis without the need for prior derivatization while providing reproducible results with recoveries ranging from 70% to 96%.

Keywords: *glyphosate, red wine, automated extraction, LC-MS/MS analysis without derivatization*

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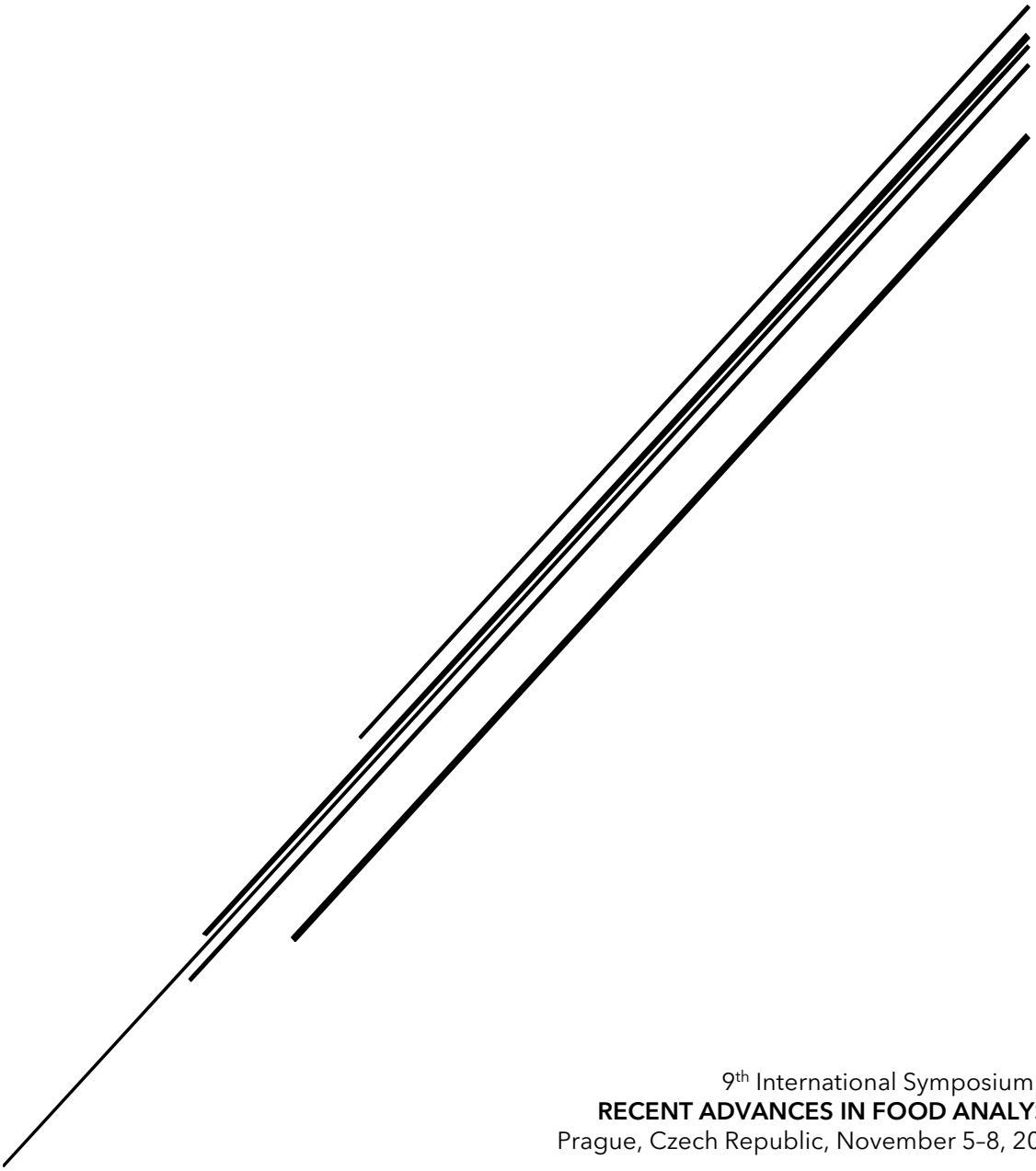

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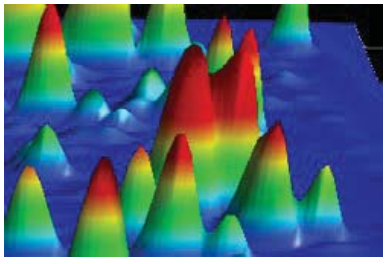


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