Ensuring the Integrity of the European food chain

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FoodIntegrity Training Week: SYMPOSIUM for post-graduate students on food fraud

PROGRAM & ABSTRACTS

6-7 September 2018
Prague, Czech Republic

Organized by

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

UNIVERSITY OF
CHEMISTRY AND TECHNOLOGY
PRAGUE

PROGRAM

Location:

University of Chemistry and Technology, Prague, Technicka 3, 166 28, Prague 6, Czech Republic, Hall BII (2nd floor)

Thursday, September 6, 2018

11:30-12:30		Registration for the symposium and welcome refreshment
12:30-12:40		**Welcome and opening of the symposium** Jana Hajslova, University of Chemistry and Technology, Prague, Czech Republic
Session 1		*Chair: Saskia van Ruth, Wageningen University, Netherlands*
		Food authentication for fraud detection: rapid, non-destructive techniques
12:40-13:10	L1	Guest speaker: **Developments in rapid, non-destructive techniques for food authentication** Saskia van Ruth, Wageningen University, Netherlands
13:10-13:30	L2	**Evaluation of handheld Micro-NIR for extra virgin olive oil authentication** Jing Yan, Wageningen University and Research, Wageningen, Netherlands
		Food fraud drivers
13:30-14:00	L3	**Food fraud in Australia: a strict liability offence and historical food safety regulation driver** Janine Curll, Monash University, Melbourne, Australia
		Food authentication for fraud detection: metabolomics
14:00-14:30	L4	Guest speaker: **Food metabolomics revealing the mysteries from farm to fork** Josep Rubert, University of Trento, Trento, Italy
14:30-14:50	L5	**Authentication of wheat species: development and application of an untargeted metabolomics approach** Laura Righetti, University of Parma, Parma, Italy
14:50-16:00		Coffee break & **Poster session** (B32)
Session 2		*Chair: Josep Rubert, University of Trento, Trento, Italy*
		Food authentication for fraud detection: simplified strategies (1)
16:00-16:30	L6	Guest speaker: **Innovations in detecting food fraud using Rapid Evaporative Ionisation Mass Spectrometry (REIMS)** Olivier Chevallier, Queens University Belfast, United Kingdom
16:30-16:50	L7	**Detection of undeclared foreign proteins in meat by means of REIMS** Vit Kosek, University of Chemistry and Technology, Prague, Czech Republic
16:50-17:10	L8	**An innovative one-run method to assess chocolate authenticity** Michaela Rektorisova, University of Chemistry and Technology, Prague, Czech Republic
17:10-17:30	L9	**Simplified approach to food adulteration detection by LC-MS/MS** Ewa Wielogorska, University of Chemistry and Technology, Prague, Czech Republic
17:45-19:00		Symposium networking drink (Respirium)

Friday, September 7, 2018

9:00-10:30		**Excursion in the UCT Prague brewery**
10:30-11:00		Coffee break & **Poster session** (B32)
Session 3		*Chair: Olivier Chevallier, Queens University Belfast, UK*
		Food authentication for fraud detection: molecular biology methods
11:00-11:20	L10	**Fish species identification by PCR using parvalbumin gene as a platform** Diliara Akhatova, University of Chemistry and Technology, Prague, Czech Republic
11:20-11:40	L11	**DNA analysis for the monitoring of meat adulteration** Eliska Fialova, University of Chemistry and Technology, Prague, Czech Republic
		Food authentication for fraud detection: simplified strategies (2)
11:40-12:00	L12	**Does food supplement based on *Tribulus Terrestris* contain boldion as a natural component?** Kamila Hurkova, University of Chemistry and Technology, Prague, Czech Republic
12:00-12:20	L13	**Recognition of meat origin based on histidin dipeptides pattern** Monika Jiru, University of Chemistry and Technology, Prague, Czech Republic
12:20-12:40	L14	**Synthetic food additives in soft drinks and fruit wines** Ales Krmela, University of Chemistry and Technology, Prague, Czech Republic
12:40-13:00	L15	**Critical assessment of CBD oils quality** Marie Fenclova, University of Chemistry and Technology, Prague, Czech Republic
13:00-13:15		Closure of the symposium Award for the best presentation
13:15-14:00		Lunch (B32)
14:00-15:00		**Optional laboratory tour**

LECTURES

L1
DEVELOPMENTS IN RAPID, NON-DESTRUCTIVE TECHNIQUES FOR FOOD AUTHENTICATION

Saskia van Ruth[1*]

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Food fraud is a form of criminal behaviour, no matter the definition of crime. Its consequences are devastating. The interaction between motivated offenders, and the opportunities presented by victims and lack of control measures favour occurrence of food fraud. Control measures help to counteract fraud opportunities and motivations. Analytical testing is one of those control measures. Traditional measurements have focused on the analysis of one or a few product characteristics. However, nowadays-analytical techniques generating detailed analytical fingerprints are used to determine the identity of foods, and we have many spectrometry and spectroscopy based techniques available. In the last decade we have seen developments in the area of rapid and non-destructive techniques, which are often based on spectroscopy. This development will be illustrated with a number of examples from practice.

Keywords: food authentication, analytical techniques, spectroscopy, BARDS

L2
EVALUATION OF HANDHELD MICRONIR FOR EXTRA VIRGIN OLIVE OIL AUTHENTICATION

Jing Yan[1*], **Louka van Stuijvenberg**[2], **Saskia van Ruth**[3]

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Introduction: Olive oils are among the most fraud-vulnerable products on the market. Especially analytically challenging is the differentiation between extra virgin olive oil (EVOO) and its lower grade counterparts, refined and pomace olive oil (ROO and POO). Recent hardware development of handheld Micro-NIR spectroscopy system allow development of application to discriminate EVOO from the other lower quality counterparts. **Objective:** The aim of this study was to evaluate handheld MicroNIR spectroscopy technique (Micro-NIR 1700 ES) for the rapid and non-destructive analysis of EVOO authentication.
Material and Methods: 180 samples were collected and measured in duplicate by MicroNIR and GC-MS for fatty acids (FAs), including 90 authenticated EVOO, 40 ROO, 10 POO, 20 rapeseed oil, 15 sunflower oil and 5 peanut oil. Pearson bivariate correlation and PLS regression were used to explore the fundamental relations between MicroNIR spectra and FAs, meanwhile, the contents of FAs of each samples were predicted based on MicroNIR spectra data by PLS regression. Afterwards, PLS-DA combined with several pre-processing methods (mean-center, auto-scale, MSC, SNV, 1st derivative) were engaged to optimize the classification model which could discriminate EVOO from other categories.
Results: PCA shows that EVOO can be visually separated from other categories. A good relation between MicroNIR spectra (two spectral range from 1137.291 to 1298.956 cm^{-1} and from 1378.871 to 1552.313 cm^{-1}) and FAs were found according to Pearson correlation and PLS regression methods. Furthermore, the trained PLS-DA classification model based on MicroNIR spectroscopy fingerprint showed 100% accuracy for leave-one-out cross validation and 91% accuracy for external validation. Whereas FAs based PLS-DA trained model only achieved 88% correct classification for leave-one-out cross validation and 72% accuracy for external validation.
Conclusion: PLS-DA classification model based on the handheld MicroNIR spectral fingerprints has the ability to identify the authenticity of premium quality olive oil. Handheld MicroNIR spectroscopy technique is promising for the rapid screening.

Keywords: authentication, handheld device, MicroNIR spectroscopy, non-destructive, PLS-DA

L3
FOOD FRAUD IN AUSTRALIA: A STRICT LIABILITY OFFENCE AND HISTORICAL FOOD SAFETY REGULATION DRIVER

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International food fraud policy discourse involves much discussion about what food fraud looks like, how it occurs, why it occurs, where it occurs and options for preventing it in globalised food supply mazes. This paper focuses on the modern history of its legal and regulatory control in Australia. Although globalisation of the food supply network has transformed food fraud risk profiles and the range of harms to safety and public health, food fraud is not a new phenomenon for regulatory control. Food fraud control has been a driver of food legislation for safety and public health for centuries. Before the risk to safety and public health from microbial hazard contamination was well characterised for systematic control, assuring food authenticity dominated regulatory control mechanisms in the interest of health. This paper reveals Australian food law reform in 2000 shifted from proactive 'technocratic' regulation of food adulteration to prohibitions on false, misleading or deceptive conduct. Food fraud is a strict liability offence in Australia. This means, its significance to protect health justifies regulatory repercussions without any burden on regulators to prove intent. An adapted John Spink and Douglas Moyer's Food Protection Matrix conceptualising where threats to safety and public health manifest is provided. With reference to Australia's food offence architecture, the adapted Food Protection Matrix demonstrates the dichotomy between intentional and unintentional offences: Food safety, food defence, food fraud and food quality. The paper concludes with an Australian centric food fraud definition coherent within those laws.

Keywords: food fraud, food authenticity, food fraud regulatory control, food fraud law, Australian food law

L4
FOOD METABOLOMICS REVEALING THE MYSTERIES FROM FARM TO FORK

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Food authenticity becomes a necessity for global food policies since food placed in the market without fail has to be authentic. It has always been a challenge, since in the past minor components, called also markers, have been mainly monitored by chromatographic methods in order to authenticate the food. Nevertheless, nowadays, advanced analytical methods have allowed food fingerprints to be achieved. In other words, metabolomics is becoming more and more relevant. Metabolomics is the study of small molecules in biological systems, which aims to provide comparative semi-quantitative information about all metabolites in the system. Metabolomics is an emerging and potentially powerful tool in food authenticity. These sophisticated methods based on different separation techniques or standalone have been recently coupled to high-resolution mass spectrometry (HRMS) in order to verify the authenticity of the food. During this talk, several concerns will be voiced from me thod development to data interpretation. At the same time, the use of the new generation of HRMS detectors will be discussed highlighting several critical parameters, such as resolving power, sensitivity, robustness, extended dynamic range, and scan speed, among others. The purpose of this oral presentation is to summarize and describe the most recent metabolomics approaches in the area of food metabolomics, and to discuss the strengths and drawbacks of the HRMS analytical platforms combined with chemometrics.

Keywords: Metabolomics, Method development, Mass spectrometry, Biomarkers, Data handling
Acknowledgement: I am grateful to UCT team for the kind and warm invitation. I would also like to thank TRIANGLE project, 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.

L5
AUTHENTICATION OF WHEAT SPECIES: DEVELOPMENT AND APPLICATION OF AN UNTARGETED METABOLOMICS APPROACH

Laura Righetti[1*], Josep Rubert[2], Kamila Hurkova [3], Chiara Dall'Asta[4], Jana Hajslova [5], Milena Stranska-Zachariasova[6]

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Due to favourable climate condition, Italy is a prominent producer of different wheat varieties. There are huge quantities of wheat by-products. Several wheat baked goods are produced, but the most typical Italian food are made of durum and common wheat flour, like pasta and pizza. Because of the great importance of wheat in Italian food market, authenticity represents an essential quality parameter not only for the producers and regulatory bodies, but also for consumer. The aim of our study is to test the effectiveness of an untargeted metabolomics for the discrimination of Triticum species using liquid chromatography-High Resolutions Mass Spectrometry. Chemometric evaluation revealed an optimal sample clustering according to the wheat species and the presence of several significant markers able to discriminate the groups. Among the metabolites resulted statistically significant, alkylresorcinols, glycerophospholipids and galactolipids could be further used for the discrimination of common and durum whole grain flour. The results demonstrate that untargeted lipidomics, in conjunction with chemometric tools has potential as a screening tool for the detection of wheat fraud.

Keywords: authenticity, triticum spp, durum wheat, common wheat, untargeted lipidomics

L6
INNOVATIONS IN DETECTING FOOD FRAUD USING RAPID EVAPORATIVE IONISATION MASS SPECTROMETRY (REIMS)

Olivier Chevallier[1*], Connor Black[2], Birse Nicholas[3], Niladri Chatterjee [4], Chris Elliott[5]

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REIMS enables simultaneous acquisition and classification of samples using an electrosurgical knife. Known for its applications in cancer surgery, REIMS has more recently found applications towards the detection of food fraud. Our studies have been focused on investigating various aspects of fish and meat fraud. Briefly, 478 samples of five different white fish species were subjected to REIMS analysis using a monopolar electrosurgical knife. Chemometric models were generated using the mass range m/z 600-950 of each sample. The analysis of 99 validation samples provided a 98.99% correct species classification within seconds. Significant time comparisons between REIMS and polymerase chain reaction (PCR) were observed when analysing six mislabelled samples demonstrating how REIMS can be used as a complimentary technique to detect fish fraud. Additionally, the species and geographic origin of different shrimp samples and the catch method of haddock samples were found to be capable of detection using REIMS. The analysis of beef burgers adulterated with various offal cuts has been conducted to assess what limits of detection are achievable using REIMS. Levels ranging from 5-10% were identified. REIMS could provide a paradigm shift across authenticity applications by providing real-time, reliable, and simple method for the analysis of food products.

Keywords: mass spectrometry, food authenticity
Acknowledgement: Julia Balog, Sara Stead, Steven Pringle (Waters), Zoltan Takats (Imperial College London), Christophe Cavin (Nestle), MAria Riina, Francesca MArtucci, Pier Acutis (Istituto Zooprofilattico Sperimentale), Young's, Morrisons

L7
DETECTION OF UNDECLARED FOREIGN PROTEINS IN MEAT BY MEANS OF REIMS

Vit Kosek[1*], Leos Uttl[2], Monika Jiru[3], Connor Black[4], Monika Tomaniova[5], Christopher Elliot[6], Jana Hajslova[7]

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Meat adulteration is a significant economic problem as it can result in substantial economic gains and loss of consumers' trust in the food industry. Addition of a bulking agent masking the addition of water into minced meat is a fraudulent practice that is very difficult to detect. The quality of the meat can be assessed by measurement of total net protein, however the methods used to measure such property are not able to cope with the quite sophisticated modern-day adulteration practices. In our study, we assessed the potential of recently introduced Rapid Evaporative Mass Spectrometry (REIMS) technology to discover undeclared additives in chopped pork and chicken meat-based products such as sausages and burgers. The REIMS technique was able to discover such adulterants with a high degree of confidence when more than 2.5 % of these substances were added. The results could be obtained within a few minutes. In this context REIMS can be classified as a rapid screen ing method which could be employed as a front-line testing method to ensure the quality and authenticity of meat products.

Keywords: meat authentication, rapid evaporative ionization mass spectrometry, ambient mass spectrometry, meat processing additives
Acknowledgement: This research was supported by the European Union´ s Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop) and by the Czech Republic National Agency for Agricultural Research (Project no. QJ1530272). 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).

L8
AN INNOVATIVE ONE-RUN METHOD TO ASSESS CHOCOLATE AUTHENTICITY

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Chocolate authentication usually involves a combination of various analytical procedures, thereby making the analytical process time-consuming and costly. Thus, we developed an innovative method for the simultaneous determination of the key chocolate quality features by utilizing the high potential of supercritical fluid chromatography coupled to quadrupole-time of flight mass spectrometry (SFC-QTOF-MS). By combining hexane and water extracts from sequential extraction, a single eight-minute analytical run enabled us (i) to determine cocoa butter equivalents (CBEs) and milk fat content according to triacylglycerols, (ii) to calculate dry non-fat cocoa solids based on theobromine and caffeine content, and (iii) to screen present sugars. In addition to several time-scheduled targeted MS/MS functions optimized for the individual analytes (parallel reaction monitoring), the MS method comprised a full MS scan to detect the presence of any unpredicted compounds. For 40 chocolate samples collected at the Czech market, the results obtained by the newly developed method and those standard ones (LC-UV for non-fat cocoa solids, and GC-FID for CBEs) were in good agreement. The only limiting factor could be a higher uncertainty of TAG profile determination; thus, our method is suggested for the fast, cost-effective and efficient screening of chocolate quality and authenticity when only samples found suspicious for the presence of CBEs are referred to the standard method to determine the exact levels of CBEs.

Keywords: high-resolution mass spectrometry, parallel reaction monitoring, supercritical fluid chromatography, cocoa butter equivalents, cocoa solids
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), "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015), and specific university research (MSMT No 21-SVV/2018).

L9
SIMPLIFIED APPROACH TO FOOD ADULTERATION DETECTION BY LC-MS/MS

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Food fraud has been a global issue affecting both consumers safety and trust in the supply chain. As such development of novel, fast and rugged analytical is of high importance. However, detecting and quantifying food adulteration is a challenging task due to a large variety of products affected, constantly changing modus operandi of fraudsters as well as limitations of available methodologies. Accordingly, a simplified, multi-tier testing regime is proposed as the best solution to both screen and confirm food fraud. Development and validation of a combination of a FTIR screening assay and a targeted LC-MS/MS, biomarker based method will be presented as a powerful tool to address issues of adulteration and mislabelling on the example of herbs and seafood.

Keywords: food, fraud, LC-MS/MS

L10
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, has 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 mislabelling can result as fraudulent substitution of meat with high value with some less expensive fish. Moreover, proper labelling 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 was developed: black seabream (*Sponyliosoma cantharus*), Atlantic mackerel (*Scomber scombrus*) and iridescent shark (*Pangasianodon hypophthalmus*). 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.

Keywords: fish, PCR, DNA, species identification, food adulteration
Acknowledgement: This research was financed by the Czech Ministry of Agriculture, grant No. MZE RO0318.

L11
DNA ANALYSIS FOR THE MONITORING OF MEAT ADULTERATION

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Nowadays, many different methods which allow us to differentiate animal species in meat products exist. One of the possibilities is to use polymerase chain reaction (PCR) based on DNA analysis. DNA is a universal molecule present in most cells of the animal organism, analysis of samples of various animal species and tissues can be therefore carried out under the same conditions. The aim of this work was to develop protocols for PCR authentication of beef, pork, chicken and horse meat and then experimentally verify. Initially, primers complementary to the chosen sequence of mtDNA for cytochrome b were used for multiplex qualitative analysis of animal species. As a second target, the chromosomal DNA was used. Single copy chromosomal genes as cattle cyclic-GMP-phosphodiesterase, pig beta-actin, interleukin-2 for chicken and myostatin from mammal and poultry were analysed by multiplex qPCR (mqPCR). The functionality of methods was proved by mixed DNA samples and by mixed meat with and without heat treatment. Both methods, mPCR and mqPCR, were verified on several commercially available samples include sausage, meatloaf and salami typical for Czech products. The discrepancies between results of DNA analysis and content of the sample declared by producer were determined.

Keywords: DNA, PCR, authentication, quantitative PCR, multiplex PCR
Acknowledgement: This study was supported by a grant MZe (NAZV) QJ1530272: Complex strategies for effective detection of food fraud in the chain production-consumer.

L12
DOES FOOD SUPPLEMENT BASED ON *Tribulus Terrestris* CONTAIN BOLDION AS A NATURAL COMPONENT? (CASE STUDY AIMED AT FRAUD DETECTION)

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Tribulus terrestris is a plant growing worldwide. It has been extensively used in traditional Chinese medicine because of various health benefits including prevention of heart diseases. In Europe and the USA it is mainly used for increasing muscle strength and improving sexual function. The bioactive phytochemicals contained in *Tribulus terrestris* belong mainly to phenolic compounds, saponins, sterols or alkaloids. However, some food supplements based on this plant were found to contain boldion, precursor of anabolic-androgenic steroid (AAS) boldenone. The producers claim that this is one of natural components, originated through plant metabolism. According to the World Anti-Doping Agency (WADA), AASs belong among the most abused drugs in competitive sport. For this reason, natural occurrence of boldion in *Tribulus terrestris* was monitored in our study. U-HPLC-HRMS/MS method for boldion analysis was developed and validated. LOQ 0.01 mg/kg and linear range from 0.01 to 10 mg/kg were achieved. Altogether 22 samples of different parts of *Tribulus terrestris* plant were analyzed: stem with leaves (n=11), fruit (n=5), admixture of stem, leaves and fruit (n=4), admixture of stem, leaves, fruit and root (n=1), powdered root (n=1). Methanolic extracts of all samples were subjected to targeted analysis of boldion, as well as to screening of bioactive compounds present in *Tribulus terrestris* according to literature. Under the conditions of our method, boldion was not detected in any part of the plant. On the other hand, the presence of the number of bioactive components namely steroidal saponins, protodioscin and protogracillin and flavonoids were identified.

Keywords: *Tribulus terrestris*, food supplement, boldion, U-HPLC-HRMS/MS, bioactive compounds
Acknowledgement: This work was funded 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).

L13
RECOGNITION OF MEAT ORIGIN BASED ON HISTIDIN DIPEPTIDES PATTERN

Monika Jiru[1*], Ales Krmela[2], Vladimír Kocourek[3], Milena Stranska Zachariasova[4], Jana Hajslova[5]

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Meat and meat products are occasionally subjected to counterfeiting, mislabelling and/or similar fraudulent activities. Substitution of declared ingredients of minced meat with other cheaper ones is one among many forms of economic fraud. Histidine dipeptides, carnosine, anserine and balenine are contained in meat species in different ratios, so their analysis can be a criterion for identifying compliance with a label. For the determination of target analytes (carnosine, balenine, anserine), a rapid and sensitive analytical U-HPLC-MS method employing a new single quadrupole mass analyzer (QDa) was optimized and validated. Within the method validation, repeatability of the method expressed as relative standard deviations (RSDs, %) was determined. The method repeatability (n=9) was in the range 7-9 % and limits of quantification were in the range 3.5 – 7.5 µg/g. The optimized method was than successfully applied for analysis of different meat and meat mixtures. In the first part of our study 18 samples of beef (n=6), pork (n=6) and chicken (n=6) meat were analysed to recognize the variability of histidine dipeptides composition in each species. The results we obtained were comparable with similar studies. In chicken meat, anserine (3.8-7.7 mg/g) dominated over carnosine (1.3-2.8 mg/g), while the latter dipeptide was predominant in pork (2.0-4.7 mg/g) and beef (2.4-2.6 mg/g). Dipeptide anserine in pork and beef occurred at approximately one order of magnitude lower concentration level than in chicken meat. As regards balenine, its concentration in pork was ten times higher than in beef. To assess the sensitivity of the proposed method to distinguish intentional addition of cheaper ingredients in meat products, in the second part of our study prepared admixtures of pork with chicken meat (0-50 %) meat as well as admixtures of beef with pork meat (0-50 %) were analysed. In case of pork-chicken admixtures, the addition of 0.5% chicken to pork meat was distinguished based on the carnosine/anserine ratio. On the basis of anserine/balenine ratio the addition of pork meat to beef can be revealed from 2 %. Finally, 8 real meat products were analysed and dipeptides ratios were compared with manufacturer´s declaration. All analysed meat products complied with the information on the packaging label.

Keywords: histidine dipeptides, meat authentication, mass spectrometry, liquid chromatography
Acknowledgement: This work has received funding from the National Agency for Agriculture Research (NAZV-QJ1530272), the Operational Programme Prague – Competitiveness (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by European Union´s Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop).

L14
SYNTHETIC FOOD ADDITIVES IN SOFT DRINKS AND FRUIT WINES

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Food additives, designated by characteristic E code, represent a wide range of chemicals, of both natural and synthetic origin, which are intentionally added to foodstuffs for enhancement of their properties, such as shelf-life, colour or flavour. Despite these benefits, food additives are a potential source of concern for some population groups suffering from specific health disorders. Therefore, the use of some additives is regulated; maximum limits have been established (Commission Regulation No 1129/2011). Regardless it is compliance with limits, declaration at the product label or illegal use detection, effective analytical control is necessary. Until now, a number of methods suitable for additives determination has been developed, many of them employ HPLC technique coupled with conventional detectors like UV/VIS or tandem mass spectrometers (MS). Presented study demonstrates the use of relatively cheap and easy to operate single quadrupole mass spectrometer as a tool for quick additives monitoring. A number of beverages available at the Czech market, such as fruit wines and soft drinks were analyzed, revealing a wide use of synthetic food additives. Fruit wines usually contained a number of additives such as preservative sorbic acid, sweeteners acesulfam K and dye azorubine. In case of soft drinks, the use of synthetic food additives was not an exception. They often contained synthetic sweeteners in addition to the presence of monosaccharides, and synthetic dyes complemented the use of plant extracts. On the other hand, some of the analyzed samples did not contain synthetic food additives at all, proving that their use is not always necessary.

Keywords: food additives, mass spectrometry, food fraud
Acknowledgement: This work was funded 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).

L15
CRITICAL ASSESSMENT OF CBD OILS QUALITY

Marie Fenclova[1*], Frantisek Benes[2], Petra Peukertova[3], Lucie Drabova[4], Jana Hajslova[5]

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CBD oils represent a group of hemp (*Cannabis sativa var. sativa*)-derived food supplements, rich in the content of non-psychoactive phytocannabinoid cannabidiol (CBD). As they are prepared by enrichment of the edible oils by an extract of hemp, the CBD oils may contain not only the health beneficial CBD but also, based on the hemp variety used for extraction, various amount of psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC). Moreover, the fatty matrix of CBD oils may be a source of lipophilic environmental contaminants, such as polycyclic aromatic hydrocarbons (PAHs). Due to high popularity of CBD oils, the quality of those preparations available at the European retail market was assesses. (i) CBD and Δ^9-THC content determined by UHPLC-HRMS/MS with the producer's declaration and (ii) PAHs content determined by HPLC-FLD with the maximum limits of 2 µg/kg for benzo(a)pyrene (BaP) and 10 µg/kg for the sum of 4 carcinogenic PAHs benzo(a)pyrene, chrysene, benzo(a)anthracene and benzo(b)fluoranthene (**PAH4**) according the EU Regulation No 1881/2006, were compared. As the first sample set, 23 oils with the CBD declaration 1.7–25% were analysed. The determined CBD content was lower than declared in 6 samples, the rest (74%) fulfilled or even exceeded the declaration. Although any of the producers declared the Δ^9-THC presence, it was detected in all of the samples at concentrations 14-2908 mg/kg. Therefore, the amount of Δ^9-THC in the CBD oil daily dose (recommended by producers) was compared with the EFSA acute reference dose (ARfD) of 1 µg Δ^9-THC/kg b.w. Considering the consumer as an average 70 kg weighing person, the ARfD was exceeded by 48% of the samples with the highest Δ^9-THC dose being even 2627% of the ARfD. The PAHs were also detected in all of the samples. 70% of them exceeded the legislation limit either for BaP or **PAH4** (or both) with the maximum almost 10-fold higher than the limits. The CBD oil producers were informed about the results; after one year, another 35 oils (CBD declaration 0.3– 17%) including 9 samples being the same origin as in the first year were analysed. The CBD concentration fulfilled / exceeded the declaration for 86% of the samples; it was lower only in 2 samples and for 3 samples the declaration was missing. Δ^9-THC was detected in 80% of the samples (12–3363 mg/ kg), most of them without the declaration. The ARfD for Δ^9-THC was exceeded by 43% of the samples, the highest Δ^9-THC dose reached 1614 % of the ARfD. PAHs were again detected in all of the samples, exceeding the BaP and/or **PAH4** limits in 63% of them. The highest concentrations were 7- and even 19-fold higher than the limit. Comparing the same samples in both testing years, improvement in fulfilling the declared CBD content was observed. But almost no improvement was made for Δ^9-THC where the declaration was still missing despite the presence of this psychoactive compound. The levels of PAHs even increased above their limits in some cases.

Keywords: CBD oil, Cannabis sativa, cannabidiol, tetrahydrocannabinol, polycyclic aromatic hydrocarbons
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), by the "National Program of Sustainability I" - NPU I (LO1601 - No.: MSMT- 43760/2015) and also by pecific university research (MSMT No 21-SVV/2018).

POSTERS

P1
DIRECT ANALYSIS IN REAL TIME-HIGH RESOLUTION MASS SPECTROMETRY AS AN APPROACH TO THE DISCRIMINATION BETWEEN WILD-TYPE AND FARMED SALMONS

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In the last years, the constant request of seafood items worldwide has involved an increasing growth of the incidence of products obtained by aquaculture systems on the market. However, a parallel fraudulent commercialization of farmed products as wild-type ones has become a recurrent phenomenon. A careful characterization of the lipid component of seafood products based on chromatography-mass spectrometry techniques has been reported as a promising approach to a reliable distinction between farmed and wild-type products. In this context, a novel and fast method based on Direct Analysis in Real Time (DART) coupled to High Resolution Mass Spectrometry (HRMS) based on a single stage Orbitrap mass analyzer, integrated by Principal Component Analysis (PCA), was developed during our study and applied to discover spectral features useful to discriminate wild-type from farmed salmons belonging to *Salmo salar* species. In particular, normalized intensities obtained for the 30 most intense signals (all referred to fatty acids, FA) detected in negative ion DART-HRMS spectra of the lipid extracts of salmon fillets [26 wild-type from Canada, 74 farmed from Canada (25), Norway (25) and Chile (24)] were considered as the variables for PCA. The scatterplot referred to the first two principal components showed a clear distinction between wild-type salmons and farmed ones, which gathered as a unique cluster, despite the remarkable differences in their geographical origin. In accordance with previous studies based on more complex and time-consuming analytical approaches, three saturated (14:0, 16:0 and 18:0) FA, along with unsaturated ones having 20 or 22 carbon atoms, were found as the main discriminating variables for wild-type salmons, whereas FA with compositions 18:1, 18:2, 18:3 and several oxidized forms arising from them were found to have a significantly higher incidence in farmed salmons. The evaluation of relative abundances of DART-HRMS signals relat ed to specific FA appears then very promising for the differentiation of wild-type salmons from farmed ones, a very relevant issue in the context of consumers' protection from adulterant seafood substitutions.

Keywords: direct analysis in real time-high resolution mass spectrometry (DART-HRMS), food authenticity, salmon, fatty acids, principal component analysis (PCA)
Acknowledgement: The present research has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration, under grant agreement No. 613688 2 "Food Integrity".

P2
WINE QUALITY MONITORING AND AUTHENTICITY DETERMINATION

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Wine quality and authenticity has been an issue for a long time. Different approaches have been applied to control/monitor it. We are looking at it from an industry perspective, being a company based in the Netherlands and operating in the large-volume-wine industry mainly in Europe for more than 20 years. Our clients are across the whole industry: producers, suppliers, bottling companies, supermarkets, consumer associations, etc. We help them to make sure they are dealing with wines of a high and stable quality, and authentic. This is done by using a three-component approach: tasting, monitoring of basic chemical parameters (ethanol, total acidity, etc.) and analysis of aroma compounds with proton-transfer-reaction mass spectrometry (PTR-MS). We apply fingerprint approach to compare a wine with its peers by using multivariate analysis. This 3-component approach has proven to be a powerful tool to monitor consistency of quality of large volumes over a long period of time and detect most wine deviations. Moreover, over the years we have collected a database with thousands of commercially available wines which gives us a solid reference.

Keywords: wine, quality, authenticity, PTR-MS

P3
SPECTRAL FINGERPRINT DATA ANALYSIS USING HEADSPACE – GAS CHROMATOGRAPHY – ION MOBILITY SPECTROMETRY FOR DETECTING CONTAMINATION AND AUTHENTICATING GEOGRAPHICAL ORIGIN OF PALM OIL

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Increasing consumer demands for improved traceability, sustainability and authenticity within vegetable oil supply chains is a major challenge for the vegetable oils sector. Although regulations and legislation have been implemented to ensure the safety and reliability of products, in many cases these are based on potentially fallible audit trails from suppliers rather than on analytical data generated by downstream users. Therefore, there is an urgent need for the development of state-of-the-art methods to accompany current administrative controls, to ensure the authenticity of vegetable oils and their associated products. We have assessed the use of Gas Chromatography – Ion Mobility Spectrometry (GC-IMS) for palm oil analysis – with a particular focus on authentication of geographical origin and detection of adulterants and contaminants (e.g. presence of animal fats, colourings/dyes, process contaminants and lower grade oils). GC-IMS is an emerging technology which is pioneered by our sponsor company, IMSPEX. It combines the sensitivity of a GC with the sensitivity and selectivity of IMS, enabling rapid headspace analysis volatile organic compounds (VOCs) present in a sample, without any sample pre-treatment. Analysis typically lasts between 3-15 minutes (depending on study interest) and data analysis is then undertaken using an in-house software package LAV Suite v2.0.0 (G.A.S. GmbH, Dortmund, Germany). Principal Components Analysis (PCA) is applied to data using the integrated Dynamic PCA function, for classification of samples according to a certain factor (i.e. origin or grade). Identification of VOCs present in a sample, particularly those associated with adulterants or contaminants, is conducted using the GC-IMS Library Search v1.0.3 with column normalisation to NIST 2014. In this presentation, we demonstrate the potential of GC–IMS as a rapid, sensitive and cost-effective method for palm oil analysis, particularly for detection of adulterants and contaminants. The greater aim of our work is to develop optimised GC-IMS methods for implementation within global palm oil supply chains to aid traceability and authenticity, whilst accompanying current administrative controls.

Keywords: palm oil, gas chromatography, ion mobility spectrometry, authenticity, supply chains
Acknowledgement: We gratefully acknowledge funding and support from the European Social Fund via the Welsh Government, IMSPEX Diagnostics, University of South Wales and KESS2. We would also like to thank Wageningen University for providing us with palm oil samples.

P4
METABOLOMIC FINGERPRINTING APPROACH USED FOR DETECTION OF GINSENG PRODUCTS ADULTERATION

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Food supplements are widely used by consumers around the world and represent one of the relatively expensive commodities. They are also very often the target of counterfeiting practices. For this reason, it is necessary to have an effective analytical tool to control the authenticity of food supplements and to detect their counterfeiting, which may be, for example, in the form of a lower content of the active substance than the declaration on the packaging or complete substitution of the ingredient used versus the claim on the packaging and so forth. This study has been focused on the authentication of ginseng-based food supplements. For this purpose various ginseng species (*Panax ginseng, Panax japonicus*) were investigated by metabolomic fingerprinting strategy employing liquid chromatography coupled with high resolution mass spectrometry. In addition to ginseng, *Gynostemma Pentaphyllum* was further analyzed, because it contains the same characteristic compounds (ginsenosides) which are present in ginseng. Due to this fact *Gynostemma Pentaphyllum* could be easily mistaken for ginseng (ginseng substitute). Advanced statistical methods allowed identification of characteristic markers of *Gynostemma Pentaphyllum*. The model mixtures of this plant and ginseng were prepared and, using the selected analytical strategy, 1% addition of *Gynostemma Pentaphyllum* to ginseng was recognized.

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

P5
VALIDATION OF LC-MS-BASED METHODS FOR VERIFICATION OF FOOD AUTHENTICITY

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In 2013, the European Parliament published a list of ten foods that are highly at risk of food fraud, with olive oil ranked first and fish ranked second on the list. As fraudsters continuously adapt to food monitoring, the verification of food integrity has become a major analytical challenge. Due to its multiplexing capacity, mass spectrometry is gaining increasing attention from food surveillance. In recent years, several methods have been developed that use liquid chromatography coupled to mass spectrometry (LC-MS) to identify species-specific marker peptides in food. These marker peptides can then be used to verify food authenticity. Although these methods have great potential, none of them have been standardised or validated in inter-laboratory studies yet. In order to meet the requirements of the official authorities responsible for food surveillance in Germany and Europe, these methods must be validated and transferred to the ''Official Collection of Methods of Analysis and Sampling (ASU)'' and to CEN. Therefore, the Federal Office of Consumer Protection and Food Safety (BVL) constituted a new working group consisting of experts from this field. Currently, several LC-MS methods regarding fish identification, nut allergen detection, transglutaminase detection in meat, or the detection of plant proteins in meat are being reviewed and validated in inter-laboratory studies. In addition to the validation of existing methods, general guidelines for laboratory comparison studies and the marker peptide search will also be developed in the working group. Here, the organisation of the group and the basic design of pilot studies at the example of fish species identification will be presented. The aim of these pilot studies, each with at least four laboratories participating, are the standardisation of the sample preparation for mass spectrometric analyses, the standardisation of the search for marker peptides with high specificity and sensitivity, and the evaluation of the results with different mass spectrometry equipment under a uniform procedure.

Keywords: method validation and standardisation, official collection of methods, LC-MS, protein analysis

P6
MONITORING FOOD AUTHENTICITY USING AN ADVANCED GLYCAN ARRAY PROFILING PLATFORM

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Constant advancements enabling the fraudulent manufacturing of food produce 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. Products based on cereal grains can potentially provide positive health effects and the benefits of wholegrain products are well documented, however a consensus regarding definitions of wholegrain content is not present. This uncertainty can be exploited by producers, such that a product carrying a 'wholegrain' label, may in fact contain very low levels of the grain components that confer health benefits. A recent recommendation (2017) issued by the Healthgrain Forum suggested that 'wholegrain' products should contain at least 30% wholegrain by dry weight. Such quantitative descriptions are necessary – but to maintain consumer confidence suitable monitoring technology is required to ensure they are adhered to. The work being conducted describes a new analytical platform that enables the wholegrain content of diverse foods to be analysed rapidly and in detail. The method combines the high-throughput capability of microarrays with the specificity of monoclonal antibodies, exploiting the fact that some polysaccharides only occur in certain anatomically distinct regions of grains. Once identified, these polysaccharides can be used as markers for the presence of germ, bran, endosperm etc., even when these components are homogenised via milling or product formulation. 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]. In this project we are working with specialist millers and wholegrain interest.
[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, authenticity, food, wholegrain
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P7
AUTHENTICATION OF BANANAS USING HYPERSPECTRAL IMAGING: GEOGRAPHICAL ORIGIN AND MANAGEMENT SYSTEM

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Nowadays, bananas are one of the most produced and exported fruits in the world. Its vast consumption is boosting the economic development of exporting regions, while it also promotes the health of consumers worldwide. Europe is one of the world's leading banana importers, also making them susceptible to fraudulent practices in the trade. Retailers and consumers are very concerned about the origin and cultivation practices of bananas since these factors are closely related to the quality and price thereof[1]. According to media reports, illegal operators deliberately alter the information of the origin of production and the organic certification labelling to obtain higher profits. In response to potential food fraud risks, the portable Specim IQ® camera was used to capture hyperspectral images of 245 banana samples originating from Costa Rica. The hyperspectral imaging (HSI) technique in the range of 400-1000 nm was used to detect Cavendish and Gros Michel banana powders from different geographical origins (i.e. Corbana and Sixaola) and management systems (i.e. conventional and organic)[2]. A group of Cavendish banana samples from fourteen locations in Corbana plantations were used to determine the effect geographical origin. Organic Gros Michel and conventional Cavendish bananas from three locations in Sixaola plantations were used to determine the effect of management system. To analyze spectral data quickly and intelligently, a novel software package, perClass Mira, was introduced and the classification models for geographical origin and management system of bananas were established. The results demonstrate the potential of using a novel and portable HSI device for the authentication of bananas. Further analyses using chemometric techniques are the next steps to provide more in-depth information about this HSI technique[3].

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2. Zhang, X., Liu, F., He, Y., & Li, X. (2012). Application of hyperspectral imaging and chemometric calibrations for variety discrimination of maize seeds. Sensors, 12(12), 17234-17246.
3. ElMasry, G., Kamruzzaman, M., Sun, D. W., & Allen, P. (2012). Principles and applications of hyperspectral imaging in quality evaluation of agro-food products: a review. Critical reviews in food science and nutrition, 52(11), 999-1023.

Keywords: food fraud, banana, hyperspectral imaging

P8
EVALUATION OF BENCHTOP NIRS AND PORTABLE NIRS FOR CLASSIFICATION AND QUANTITATION OF HIGH OLEIC ACID PEANUT

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High oleic acid peanut are increasingly used in product processing and gradually become the main raw material because they have more benefits than the normal one, such as extended shelf time for products and high health value for human beings. The conventional method (gas chromatography) for detecting fatty acid is time-wasting, high-cost, tedious and ineffective. By contrast, NIRS makes use of the group vibration to analyze the fatty acid quickly, non-destructively and conveniently. The current study aimed to evaluate the benchtop NIRS and portable NIRS for classifying high oleic acid peanut and quantifying the major fatty acid in order to purchase raw material and breed variety fast and efficiently . The sample set included 106 high oleic peanut varieties and strains and 102 normal ones. Principal component analysis was conducted to explore the datasets and linear discriminant analysis was performed to build classification models. The result showed that the accuracy for distinguishing the high oleic acid peanut and normal one was 97.54% by benchtop NIRS and 96.65% by portable NIRS. Partial least square was used to build quantitative model for detecting major fatty acid in peanut. For high oleic acid peanut, the R of calibration model of benchtop NIRS is 0.88, 0.87 and 0.71 for oleic acid, linoleic acid and palmitic acid. The R of calibration model of portable NIRS is 0.80, 0.83 and 0.71 for oleic acid, linoleic acid and palmitic acid. This study indicated that benchtop NIRS and portable NIRS have great classification and prediction performance for this application.

Keywords: peanut, fatty acid, high oleic acid, benchtop NIRS, portable NIRS
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P9
FRAUD VULNERABILITY IN THE DUTCH MILK SUPPLY CHAIN

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Food fraud happens anywhere in the world. It brought not only large economic losses to companies, but also had an influential impact on human health and public confidence. The raised awareness has strengthened the demand to evaluate the food fraud vulnerability. SSAFE had developed a Food Fraud Vulnerability Assessment (FFVA) tool in 2015 to help all actors across the food supply chain to conduct fraud vulnerability assessments. Milk is a common target for food adulteration. The current study aims at understanding the fraud vulnerabilities of the Dutch milk supply chain, and profiling the critical risk factors in the chain, with the application of SSAFE FFVA tool. The three main tier groups (farmers, processors and retailers) in the Dutch milk chain were investigated and the risk factors for each tier group were identified. The FFVA tool provides a practical approach to elucidate the vulnerability of food industry or certain actors in the food supply chain. The outcome of the current study helps to understand the potential fraud threat in the Dutch dairy chain and to develop the corresponding fraud mitigation schemes. Furthermore, it provides feasible way to ensure the integrity of local and global food system.

Keywords: fraud vulnerability, milk adulteration, milk supply chain
Acknowledgement: The authors acknowledge Sino-Dutch Dairy Development Center for funding the project.

P10
MINIATURIZED DEVICES FOR DNA PURIFICATION THROUGH MICROSCALE SOLID-PHASE EXTRACTION AND FOR ISOTHERMAL DNA AMPLIFICATION FROM FOOD SAMPLES

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Miniaturized devices for DNA-based analysis have several advantages over conventional methods, such as the smaller volumes required, lower cost, portability and improved performance, being faster and more sensitive. DNA extraction and purification are critical steps of DNA analysis, which should ensure an efficient recovery of DNA from the sample while removing other compounds that might be present and can interfere with the following steps of the analysis. Microscale solid-phase extraction (μSPE) is one of the most common methods used on miniaturized devices for DNA purification, allowing to put in contact a higher volume of initial binding material with the solid phase and recover the DNA in a lower volume during the elution step. This characteristic allows to concentrate the DNA when minute amounts are present in the food samples, especially in processed food samples (e.g. olive oil, wine). Regarding DNA amplification, polymerase chain reaction (PCR) is the most common method used but, in the last years, alternative isothermal methods have been developed and explored. Among them, loop-mediated isothermal amplification (LAMP) has been demonstrated to have several advantages over PCR, such as being performed at constant temperature, higher tolerance to the presence of inhibitors and possibility of naked-eye detection. These advantages also make this technique more suitable to be integrated in a miniaturized device. At the International Iberian Nanotechnology Laboratory (INL) our research group is working on the development of tailored, miniaturized and automatized devices to perform the steps of DNA analysis from complex food samples. Two miniaturized devices for the DNA purification step are currently being developed and optimized: one is a washable and reusable system designed to contain a commercial disposable silica membrane while the other is a disposable system with a chamber containing thousands of functionalized micropillars. Both systems are being optimized in order to obtain the best DNA yield and a highly efficient protocol has already been developed with one of the prototypes for DNA purification from olive oil, which is considered as one of the food products most at risk of food fraud. For the DNA amplification step, we are currently developing a miniaturized device to perform LAMP reaction. This device includes a high-performance closed-loop Peltier module for temperature control connected to a precision platinum sensor for temperature monitoring. Preliminary results have shown the potential of this system to carry out this isothermal amplification technique.

Keywords: Miniaturized DNA analysis, food authenticity, μSPE, LAMP
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