ANALYTICAL

ANA 1a: Proposed Updates to AOCS Official Methods, including Green Chemistry
Chairs: Susan Seegers, Bunge Oils, USA; and Cynthia Srigley, US Food and Drug Administration, USA

Update of AOCS Ce 6-86 Antioxidants Method and an overview of the need for methods updates. Mark W. Collison*, *Archer Daniels Midland Co., USA*

AOCS Method Ce 6-86 was written at a time when HPLC was not a well developed technique. It is based on a method from the late 1907's, requires the use of very low performance columns and poor choice of mobile phases. The method has been updated to allow use of modern columns and a section has been added to describe how to use the method to determine the absence of antioxidants as well as their quantitation. I am Editor in Chief of the AOCS methods, I will also briefly discuss the need for AOCS methods updates.

Simultaneous GC-FID and MS Analysis of Trans-Fatty Acids in Human Plasma Heather C. Kuiper*, Emily J. Mueller, and Hubert W. Vesper, *CDC, USA*

Trans-fatty acids (TFA) are geometric isomers of naturally occurring cis-fatty acids, formed industrially via partial hydrogenation of vegetable oils or naturally in ruminant animals. High TFA consumption has been associated with increased risk of cardiovascular disease. Here we combine two commonly used detection techniques to assess TFA and regular fatty acids (FA) in human plasma. We developed and validated a new isotope dilution-GC-negative chemical ionization-MS method for the quantitation of 27 FA, including four major TFA, in 100 µl of human plasma. FA were derivatized with pentafluorobenzyl-bromide, resolved on a 200 m Select-FAME column with hydrogen carrier gas, and analyzed in selected ion monitoring mode using negative chemical ionization. We compared our detection method with FID, which is commonly used in FA analysis, by adding a 3-way splitter and FID detector to our GC-MS system, directing 2 parts flow to the FID and 1 to the MS. This system provides us with simultaneous selective and non-selective detection within the same chromatographic run. Some FA may not be fully separated with current GC methods and co-elutions can be difficult to detect with a non-specific FID. Our combined MS and FID approach provides an additional level of m/z specificity. Additionally, with this combined system, we observed better sensitivity for TFA using the MS detector as compared to FID. MS provides sensitivity and selectivity benefits for TFA analysis in biological samples. However, this method with FID detection could be suitable for samples where sensitivity is not concern, such as some food analyses.

The Certo Fatty Acid Extraction Method Adam H. Metherel*, *University of Toronto, Canada*

Many of the methods for extraction and isolation of lipid-soluble molecules, such as the fatty acid-bound phospholipids, triacylglycerols and cholesteryl esters, are analytically challenging as they require large solvent volumes and tedious, multi-step processes. We have developed a rapid one-step method for the extraction of these molecules from numerous food and tissue matrices that requires significantly less organic solvents and sample amounts, and that can be performed in a fraction of the time compared to standard methodologies. The method uses a column containing a filter that selectively retains the aqueous phase and allows the lipid-containing non-aqueous phase to pass through and be isolated. The method has been laboratory tested and comparison to one of the most

popular lipid extraction methods – 'the Folch method' – yielded statistically equal fatty acid compositions when determined in adipose, liver, brain, dog food, chicken and salmon. We continue to assess additional food and tissue matrices to broaden the applications of the Certo method. Our novel, one-step approach for analyte extraction and isolation de-skills the process and allows laboratory technicians to significantly minimize the volume of chemicals required, lower the time required for analysis, and as a result lower overall cost.

Oxidation and Its Challenges: Peroxide Value Determination in Solid Non-Oil Matrices B.J. Bench*, *Tyson Foods, USA*

Most pet food products consist of rendered animal protein meals and fats as one of the major building blocks in pet food diets. As pet food ingredients start to degrade via the lipid oxidation phenomenon it undergoes changes affecting odor, flavor, nutritional quality and palatability. Several parameters can be measured as means of determining oxidative stability and shelf-life of rendered protein meals and finished pet food products. In pet food products containing oils and fats, peroxide value is a popular oxidative stability measurement. However, it is not possible to use peroxide value alone to determine the actual quality of protein meals as hydroperoxides decompose rapidly during storage. The current American Oil Chemists' Society method (AOCS Official Method Cd 8b-90) for measuring peroxide value does not provide insight into fat extraction procedure for solid matrices. Laboratories are using a multitude of methods ranging from heated to cold solvent extraction, manual vs automated equipment, along with numerous ways to mix solvents with solid samples. This presentation will review current methodologies employed to determine peroxide values in rendered products and the influence of the multiple methods employed by commercial, industrial, and academic laboratories.

ANA 1b: Selected Analytical Presentations by the Dutton Award Winner
Chairs: Luigi Mondello, University of Messina, Italy; and Walter Vetter, University of Hohenheim, Germany

Evolution of Comprehensive Two-Dimensional Gas Chromatography for Non-Target Analysis Applications John Dimandja*, *University of Georgia, USA*

The technology of comprehensive two-dimensional gas chromatography (GCxGC) has increased in scope since its invention nearly 30 years ago. At first a proof-of-concept tool in academic laboratories, GCxGC (particularly with MS detection) is now a viable method development technology that is commercially available by several companies worldwide. Due to the emergence of the "omics" era, the need for the comprehensive screening/fingerprinting of complex samples has been the focus of many investigations in several different fields (environmental, forensics, food and fragrance, bioanalytical, etc.). In this presentation an overview of the figures-of-merit of the GCxGC technology will be given, and the application of GCxGC to the non-targeted analytical method development will be described. Two specific applications of GCxGC to oil analysis in foods will be given. One example will describe a method for the fingerprinting of fatty acid methyl esters in food contaminating bacteria. The other example will present a method for the enhanced profiling of virgin olive oil minor polar compounds (MPCs) for product characterization. Some concluding remarks regarding the future prospects of bi-dimensional chromatographic techniques will also be discussed.

Fast GC and GC×GC Approaches to Detailed Fatty Acid Fingerprinting in Natural Fats and Oils Luigi Mondello*, *University of Messina, Italy*

Objective. Intensive research has been carried out for reducing the analysis time in GC by speeding up the oven heating rates and cooling time, increasing inlet pressure, split ratio, and sampling rate for data collection. The feasibility of rapid separations through reduced ID columns was earlier investigated, and subsequently exploited in comprehensive gas chromatography techniques (GC×GC) for detailed analysis and group-type 2D mapping of natural fats and oils.

Methods used. Fast GC: 10 m × 0.1 mm I.D. × 0.1 µm df narrow bore column. GC×GC: cryogenic interface. Detection: FID, quadrupole MS. Stationary phases: 5% diphenyl, 95% dimethylpolysiloxane, and polyethylene glycol phases, both apolar-polar and inverted GC column sets.

Results. Analysis times were drastically reduced on going from conventional to fast GC, with the same or even improved resolution even in the separation of compounds having only slight chemical differences, such as ω7 and ω9 single bond homologous isomers in lipid samples. GC×GC applications carried out on FAME samples of vegetable and animal origin (including fish oils rich in PUFAs) rendered highly structured 2D chromatograms, from which exponential functions could be derived for FAME groups characterized by the same carbon chain length and number of double bonds.

Conclusion. The effectiveness and predictive potential of fast GC and GC×GC was demonstrated, as an adequate methodology for the separation and identification of very complex samples. The automation of spectra simultaneous search with linear retention indices acts as an additional filter to support the identification in the absence of reference material.

Unexpected Reduced Peak Widths of Partly Transferred Peaks after Heartcut Two-Dimensional Countercurrent Chromatography Walter Vetter*, Marco Müller, Medisa Muric, and Lisa Glanz, *University of Hohenheim, Germany*

Objective Countercurrent chromatographic (CCC) is an all-liquid based method well-suited for the isolation of natural products. Isolation of structurally related compounds with high chemical purity can become challenging, especially in the field of lipids, due to the requirement of a biphasic solvent systems in which analytes distribute evenly into both phases. Under these conditions, isolation of minor compounds is often hampered by overlapping major compounds in natural samples. Methods used. In this study we used heartcut two-dimensional CCC (2D CCC) to transfer the analyte completely to the second dimension while the interfering compound was only partly transferred. The same solvent system and the same columns (same coil i.d. and length) were used in both dimensions. Results. Heartcut 2D CCC effectively enabled to remove the interfering major compound. Moreover, the peak width of partly transferred peaks in the second dimension was generally (up to 25%) smaller than at the end of the first dimension despite the double column length. This unexpected positive effect of 2D CCC on peak resolution was verified by means of three examples. This effect was traced back to the smaller transfer volume. Conclusion. Typically, heartcut 2D techniques (e.g. in GC and HPLC) take advantage of two different (orthogonal) separation modes in the two dimensions. However, our results clearly show that heartcut 2D chromatography has a beneficial effect even when the same column used in both dimensions.

Studies in Multidimensional Gas Chromatographic Separations for Triglyceride and Fatty Acid Analysis Philip Marriott*, *Monash University, Australia*

Objective: Triacylglyceride (TAG) and fatty acid (FA) analysis in food products range from oil seeds, to fish, and animal fat products. To investigate the role and utility of advanced separations methods based on multidimensional gas chromatography (MDGC) we study enhanced separations for TAG and FA analysis in a range of samples. Methods Used: Methods are based on MDGC approaches and comprehensive two-dimensional gas chromatography (GC×GC) with both flame ionisation and mass spectrometry detection. A variety of different stationary phases are employed, especially for FA methyl ester analysis, including newer ionic liquid phases which provide useful moderation of relative retention. Results: TAG and FA range from samples predominant in just a few major components, to exceptionally complex mixtures of individual FA, where structural variations comprise different chain length, various degrees of unsaturation at variable positions, chain branching, in some cases varying substitutions (e.g. hydroxylation) in 'bacterial' FA samples, cyclic structures, and oxo groups. Analysis is confounded by TAG and FA producing gas chromatographic (GC) peaks that comprise overlapping components – difficult to identify purely on retention times; often their mass spectra (MS) have ill-defined MS specificity. Advanced GC strategies for TAG and FA with various GC column phases demonstrate specific structural information for individual components. GC×GC provides a unique structured retention, where increasing unsaturation is immediately recognised by altered ^{2}D retention. MDGC of TAG illustrates compositional heterogeneity of poorly resolved first dimension peaks. Conclusion: Advanced GC separation methods provide new capability for

profiling TAG and FA, with improved speciation of individual compounds.

NMR Analysis as a Tool to Ensure Authenticity of Lipids Torben Küchler*, *Eurofins Analytik GmbH, Germany*

Food authenticity and the detection of food fraud was a rising topic through the last years. In the field of fat and oil analysis, several methods are known since decades to uncover e.g. illegal admixtures or heat-treatment of cold-pressed oils. Chromatographic methods are still state-of-the-art for lipid analysis, but NMR technique can give additional information about lipids or can simply solve analytical questions with much less effort. In this presentation, some examples are shown for use cases of NMR in fat and oils analysis but also for food authentication by analyzing the lipid fraction.

ANA 1c/PCP 1a: Protein Assessment Methods

Chairs: Janitha Wanasundara, Agriculture and Agri-Food Canada, Canada; Sneh Bhandari, Merieux NutriSciences, USA; and Denis Chereau, IMPROVE, France

From Protein Digestibility Corrected Amino Acid Score (PDCAAS) to Digestible Indispensable Amino Acid Score (DIAAS) Hans H. Stein*, and Hannah M. Bailey, *University of Illinois, USA*

The PDCAAS system has been used for more than 25 years to evaluate quality of food proteins, but was recently suggested being replaced by the DIAAS system. Work to determine DIAAS in a number of food proteins have been conducted and further work is ongoing. It has been demonstrated that DIAAS values for cereal grains are low (between 29 and 77 for children older than 3 years) with the first limiting amino acid always being lysine. Legume proteins generally have greater DIAAS than cereal proteins with a range from 75 to 100 with soy proteins generally being close to 100 and with the sulfur containing amino acids being the first limiting. Most animal proteins including milk and meat proteins have DIAAS that are between 120 and 140 for children older than 3 years. It has also been demonstrated that generally, the PDCAAS system overvalues low-quality proteins and undervalues high-quality proteins. The DIAAS system allows for calculating the complementary effect of proteins to provide a balanced meal consisting of several protein sources. Because of the high DIAAS scores for animal proteins, these proteins may complement the poor protein quality of cereal grains to provide adequate mixed meals, as for example when milk and corn flakes or meat and rice are consumed together. In conclusion, transitioning from PDCAAS to the more accurate DIAAS system will be possible as DIAAS values for additional proteins becomes available and the DIAAS system allows for calculating the protein value of mixed meals.

Protein Contents and Quality Assessment Methods in Relation to Regulations for Nutritional Labeling and Protein Claims Sneh Bhandari*, *Merieux NutriSciences, USA*

The current trend in development of a great numbers of protein enhanced food and dietary products has further raised the importance of accurate and precise estimation of protein quantity and nutritional quality. Kjeldahl and combustion (Dumas) are widely accepted methods for total protein estimation with a general recognition in their lack of selectivity to rule out potential adulteration. Protein digestibility-corrected amino acid score (PDCAAS) is a measure of nutritional quality of a protein in diet of adults and children over 1 year of age and the estimation depends on protein's amino acid score and true digestibility value. Protein efficiency ratio (PER) is a quality measure for proteins in foods for infants through 12 months of age. A minimum nutritional quality threshold needs to be met for a protein to qualify as a significant source of protein.

How to Assess Protein Functionality? Frederic Baudouin*, *IMPROVE, France*

Novel sources of protein have gained much attention in the recent years due to the increasing global demand for protein and the growing market for vegan products. Plants, algae, yeast and by-products from the food industry such as oil meals are promising sources of novel proteins. To demonstrate their capacity to replace conventional food ingredients, producers must be able measure objectively the functionality of their products. Functional properties of proteins are very diverse in nature and strongly depend on the conditions of the test. Assessing protein functionality and

comparing protein from different source is therefore challenging. This presentation reviews the different methods available to measure protein functionality (emulsifying, foaming, gelling, water-holding…) and how factors such as pH, temperature or concentration may affect each test. Proteins from novel and conventional sources will be presented as illustrations. The need to harmonize methods will be emphasized.

Legumes - Trypsin Inhibitors, Phytohaemagglutnins (lectins) and Tannins

Shridhar K. Sathe, Sahil Gupta, Valerie D. Zaffran, Sangokunle Oluwatoyin, and Tengfei Li, *Florida State University, USA*

Legumes are important sources of dietary proteins in human and animal nutrition. Trypsin inhibitor activity (TIA), phytohaemagglutinin (lectin) activity (PHA), and tannins are often present in several legumes. TIA, PHA, and tannins with their literature reported anti-nutritional and toxicological effects may impede effective utilization of legume nutrients. Therefore, accurate detection and quantification of TIA, PHA, and tannins is essential for assessing their possible nutritional and/or physiological effects in human and animal nutrition. The primary focus of this presentation will be to address the challenges encountered by the commonly utilized methods for the quantitative determination of legume TIA, PHA, and tannins.

ANA 2a: Polar Lipids, including Phospholipids

Chairs: Francesca Giuffrida, Nestec SA, Switzerland; and Bernd W.K. Diehl, Spectral Service AG, Germany

Advances in Preparative Separation of Gangliosides from Porcine Samples via High-Speed Counter Current Chromatography (HSCCC) Nuanyi Liang*[1], Lucie Necasova[2], Yuanyuan Zhao[2], and Jonathan M. Curtis[1],[1]*Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada;* [2]*University of Alberta, Canada*

Gangliosides are glycolipids that play critical roles in cell-cell communication and intracellular function modulation. Due to their great diversity, their structure-function relationships in many biological contexts are still to be elucidated. Therefore, it is helpful to find ways to purify individual gangliosides, in order to study their specific functions. Also, from an analytical perspective, purified compounds are important for structural analysis and the absolute quantitation. Since few individual gangliosides are commercially available, this research aimed to develop methods for preparative separation of specific gangliosides classes, species or analogues. The high-speed counter-current chromatography (HSCCC) method was used to ensure good separation of gangliosides with a high sample-loading capacity and high recovery of the target analyte. This involves evaluation of partition coefficients, separation factors, and the relative retention of stationary phases for selection of the best solvent systems for HSCCC separation. LC-MS/MS analysis was used to monitor the CCC separation of gangliosides and confirm their purity and molecular structures. Thus far, to our knowledge, this is the first to report using liquid-liquid chromatography to achieve the satisfactory separation of highly similar individual ganglioside species (GM1d36:1 and d38:1, which only differ in their fatty acid moieties) at the mg level. Separation among species GM1, GD1a, GD1b and GT was also achieved. Thus, HSCCC was shown to be a suitable technique to improve the large-scale purification of gangliosides individuals in a labour and cost-efficient manner. This will make possible further investigations of their structure-dependent biological functions, such as in studies of Alzheimer's and cancers.

Analyzing thousands of individual cellular lipid species without HPLC separation Xianlin Han*,*University of Texas Health Science Center at San Antonio, USA*

We have developed a technology platform which has been referred to as multi-dimensional mass spectrometry-based shotgun lipidomics (MDMS-SL). MDMS-SL is comprised of the components of multiplexed sample preparations (including chemical derivatization), intrasource separation, MDMS identification, two-step quantification, and bioinformatic data analysis. This technology platform represents one of the most well-developed, powerful, and relatively high throughput approaches in the lipidomics field. The technology enables us to identify and quantify over 95% of total cellular lipid mass, nearly fifty lipid classes, and thousands of individual lipid molecular species in an unbiased manner from a minimal amount of source materials. This technology has been successfully applied to numerous biological and biomedical studies. Collectively, we present how thousands of individual cellular lipid species are identified in MDMS-SL without HPLC separation. Acknowledgement: This work was partly supported with NIGMS Grant R01 GM105724, NIA Grant R01 R01AG061872, Methodist Hospital Foundation, and intramural institutional research funds. References 1. Yang, K., Cheng, H., Gross, R.W., and Han, X. (2009) Automated lipid identification and quantification by multidimensional mass

spectrometry-based shotgun lipidomics. Anal. Chem. 81, 4356-4368. 2. Han, X., Yang, K., and Gross, R.W. (2012) Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. Mass Spectrom. Rev. 31, 134-178. 3. Wang, M., Wang, C., Han, R.H., and Han, X. (2016) Novel advances in shotgun lipidomics for biology and medicine. Prog. Lipid Res. 61, 83-108. 4. Han, X. (2016) Lipidomics: Comprehensive Mass Spectrometry of Lipids. pp 496, Wiley, Hoboken, New Jersey.

Analytical Tools Allowing You to Push the Boundaries of Lipid Analysis Balji Ubhi*,*Sciex, USA*

Abstract not available.

Polar Lipid Quantification in Human Milk Francesca Giuffrida*[1], Emmanuelle Bertschy[2], Isabelle Tavazzi[3], and Cynthia Marmet[3],*[1]Nestec SA, Switzerland; [2]Nestlé Research, Vers-chez-les-Blanc, Switzerland; [3]Nestlé Research Vers-chez-les-Blanc, Switzerland*

Human milk (HM) is considered the optimal form of nourishment for infants during the first six months of life (WHO) and among its macronutrients, the lipid fraction is crucial, representing almost 50% of the calories supplied to the newborn infant. In HM the lipid fraction is mainly composed of triacylglycerols which represent ~98%, while polar lipids such as phospholipids and glycosphingolipids represent less than 2%. Analytical methods to quantify major gangliosides, cerebrosides and phospholipids in human milk were developed and validated. Gangliosides and cerebrosides were separated by reversed phase chromatography and quantified by the standard addition approach using high resolution mass spectrometry; phospholipids were separated by hilic chromatography and quantified by external calibration curve using high resolution mass spectrometry. The analytical methods were validated in terms of trueness and precision according to FDA guidelines. Validation data showed repeatability (CV (r)) and intermediate reproducibility (CV (iR)) values lower than 17% and trueness values ranging between 95 and 105%.

Normal Phase HPLC of Hydroxylated Neutral Lipids and Polar Lipids Compatible with UV, ELSD and Radio Detectors Hari Kiran Kotapati* and Philip D. Bates, *Washington State University, USA*

The main objective of our research is to develop a normal phase HPLC method to completely separate hydroxylated and non-hydroxylated triacylglycerols (TAGs), diacylglycerols (DAGs) and major polar lipid classes from plants for online liquid scintillation counting within 14C based metabolic flux experiments. PVA coated silica stationary phase was employed to separate the target lipid classes in the normal phase separation mode. Previously, we were able to demonstrate the separation of hydroxylated and non-hydroxylated neutral lipid classes, on the PVA coated silica stationary phase, under normal phase conditions using UV and radio detection methods. Here, we present a method for the separation of major polar lipid classes under normal phase conditions that uses UV and ELSD detectors. This method for the polar lipid separation was combined with the recently published method for the separation of hydroxylated and non-hydroxylated neutral lipid classes, and a single method for neural and polar lipid classes was developed. The HPLC method was combined with β-ram detector for continuous flow liquid scintillation counting to efficiently quantify the 14C labeled lipids obtained from in vivo metabolic flux experiments. This HPLC method was used to analyze the non-labeled individual lipid classes, and also the 14C labeled lipid extracts from in vivo metabolic labeling experiments conducted

in developing Arabidopsis thaliana seeds. In the metabolic labeling experiments, the samples collected from different time points during the course of a continuous 14C metabolic labeling experiment were analyzed to determine the biosynthetic precursor-product relationships of major lipid classes.

Multinuclear NMR Spectroscopy, a Holistic Quality Test on GPC Bernd W.K Diehl*,*Spectral Service AG, Germany*

Glycerophosphocholine (GPC) is a new type of dietary supplement. Its detailed analysis is a challenge for classical methods like chromatography; item of the presentation is not only to the qualitative and quantitative determination of the substance in finished formulations, but also to the determination of the stereochemistry and the origin of the raw material. However, multinuclear NMR spectroscopy gives a concrete and valid answer to all these questions. The characterization can be done via 1H as well as over 13C, 31P and even over 15N NMR spectroscopy. The determination of the GPC content is possible even to the trace amounts by 31P NMR, a well-known analytical method, analogue to the lecithin analysis. A real challenge is the determination of the enantiomeric purity. GPC from natural sources, namely from vegetable or animal lecithin, always shows the natural stereochemistry. Two methods, including chemical / enzymatic treatment and derivatization with chiral defined reagents, discriminate the L and D forms in NMR spectra down to 0.1% detection limit. Finally, distinction to synthetic material is possible due to the natural asymmetric distribution of the 13C isotopes in the glycerol or choline structural part. The 13C NMR spectroscopy gives an accurate proof of origin using multivariate data evaluation.

ANA 2b: Advanced Methods of Analysis, including Automation

Chairs: William Byrdwell, USDA, ARS, BHNRC, FCMDL, USA; and Arun Moorthy, National Institute of Standards and Technology, USA

Covalent Adduct CI-MS/MS for FAME Double Bond Position Assignment without Standards on Shimadzu Triple Quadrupole J. Thomas Brenna*[1], Hui Gyu Park[2], Dong Hao Wang[3], Zhen Wang[4], Riki Kitano[5], and Kumar S.D Kothapalli[3],*[1]Cornell University, USA; [2]Dell Medical School/Dell Pediatric Research Institute, USA; [3]Dell Medical School/Dell Pediatric Research Institute, United States; [4]Cornell University/University of Texas at Austin, USA; [5]Shimadzu Scientific Instruments, Inc., USA*

Double bond location, along with geometry and chain branching, are the key structural elements determining fatty acid function, but are not available from conventional electrospray MS/MS or by any widely available MS technique applied to fatty acid methyl esters (FAME). Nearly 20 years ago we introduced CACI-MS/MS based on an ion-molecule reaction between an CH3CN-derived ion and FAME, and developed it for location of double bonds in most unsaturated FAME at GC rates. The 3D ion traps optimal for low volatility regent gases originally used are no longer commercially available. We report modification of a Shimadzu model GCMS TQ8050 triple quadrupole MS that enables high sensitivity CACI-MS/MS. A specialized flow inlet was developed for the conventional chemical ionization ion source. The m/z 54 ion CH2=C=N=CH2+ was similar or greater intensity to that obtained in internal ionization ion traps. CACI-MS/MS of the M+54 ions were tested for more than a dozen homoallylic FAME with 1-4 double bonds produced mass spectra comparable or superior to that obtained previously. Several novel conjugated trienes from pomegranate seed oil produced strong diagnostic ions permitting assignments; monoenes from cherry and rambutan seeds were easily assignable. Several novel branched chain monoenes produced by the action of FADS2 on the corresponding branched chain fatty acid produced strong signals as well. This novel source enables structural assignments without standards for nearly all unsaturated fatty acids, offering the sensitivity advantages of a triple quadrupole MS for high sensitivity applications.

Lipidomics Workflows – from Sample Preparation to Data Analysis Sheher Mohsin**,Agilent, USA*

Lipidomics is a part of metabolomics that has emerged as an important field of research in recent years. The complete workflow for lipidomics from sample preparation to analyses has not been optimized for high throughput applications. Several methods exist for Liquid-Liquid extractions that work very well and have stood the test of time. The disadvantage is that liquid-liquid extraction procedures are manual, time consuming and depend on the skill set of the analyst. We have developed a method using solid phase extraction to prepare samples for lipidomics that reduces time and is more reproducible. The extraction procedure also includes steps for preparing the sample for proteomics and metabolomics studies if desired. Separation for global lipidomics is another challenge because of the range of polarity of lipids. Traditionally, two methods are needed - A normal phase method for the non-polar lipids and a reverse phase method for polar lipids. Super critical fluid chromatography (SFC) is well suited for global analyses of the lipidome. We have developed a separation method for global lipidomics that separates lipids by classes. Using the SFC method, the whole lipidome can be investigated within a run time of less than 14 minutes. The complete workflow from reproducible extractions to SFC

separation and data analysis was used to analyze lipids in plasma and in cerebellar tissue samples. A new lipid annotator tool that uses an identification algorithm based on a combination of Bayesian probability, probability density, and non-negative least squares fit was used to make lipid Ids.

Comprehensive Multidimensional LCMS Analysis of Milk: Working Toward LC3MS4 = LC1MS2 x (LC1MS1+LC1MS1) William C. Byrdwell*, *USDA, ARS, BHNRC, FCMDL, USA*
Milk fat triacylglycerols (TAGs) contain a wide range of fatty acids (FAs), from C4 to >C18. Therefore, the set of TAGs that come from those FAs are highly complex, and contain many isomers of TAGs. One-dimensional reversed-phase HPLC is simply not adequate to completely resolve all milk TAG molecular species, including isomers. Recently, we have used comprehensive two-dimensional (2D)-LC for TAG analysis, combining RP-HPLC and silver-ion UHPLC. Unfortunately, the short-chain FAs in milk TAGs are saturated (no sites of unsaturation), so silver-ion chromatography is ineffective for their further separation. Thus, a different second-dimension separation is necessary for separation of overlapped short-chain TAGs, while still keeping the silver-ion chromatography that is effective for unsaturated TAGs. We report here a new approach to multi-dimensional LC, in which a single first-dimension separation is split to two second-dimension separations, one for unsaturated TAGs, and one for short-chain milk TAGs. A pair of contact-closure (CC) controlled UHPLC switching valves were coupled to a timed CC circuit to allow a second 2D separation to be performed in parallel with the silver-ion chromatography first 2D separation, both in series with RP-HPLC as the first dimension. Electrospray ionization (ESI) mass spectrometry (MS) was used in parallel with atmospheric pressure chemical ionization (APCI) MS (LC1MS2) to monitor the first dimension RP-HPLC, while atmospheric pressure photoionization (APPI) MS (LC1MS1) monitored silver-ion UHPLC and ESI-MS monitored UHPLC using a C4 or C8 column (LC1MS1), to produce LC3MS4 = LC1MS2 x (LC1MS1 + LC1MS1). Results from preliminary experiments are presented.

Simultaneous Analysis of Desired Aroma-active Compounds and Undesired Food-borne Toxicants Michael Granvogl*,*Technical University of Munich, Germany*
In the past, many studies have been undertaken to elucidate the key odorants of food and to identify formation pathways of the so-called “food-borne toxicants”. But, up to now, analytical approaches including the quantitation of desirable aroma-active compounds in combination with undesirable toxicologically relevant substances by sensitive methods are scarcely available. The lecture will present recent studies, which were combining the analysis of important aroma compounds and of selected food-borne toxicants (e.g., acrylamide, acrolein, crotonaldehyde, styrene, etc.) formed during food-processing, e.g., deep-frying of potato chips and donuts in different edible oils. Odorants were identified by gas chromatography-olfactometry as well as GC-MS and quantitated by stable isotope dilution analysis (SIDA). For the toxicants, new quantitation methods using stable isotopically labeled standards (e.g., [13C3]-acrolein or synthesized [13C4]-crotonaldehyde) were developed and formation pathways were proven by labeling experiments. In summary, it will be shown that lowering the amounts of undesirable compounds in combination with the maintenance of an overall aroma, which is well accepted by consumers is a challenging task, but mitigation strategies of the "bad guys" can be advised after getting the knowledge of their formation pathways.

On the Reliability of Identifications using Mass Spectral Library Searching Arun S. Moorthy*, *National Institute of Standards and Technology, USA*

Compound identification using mass spectral library searching is ubiquitous across several industrial and research applications. The spectrum of an unknown compound (a query) is searched against a library of spectra for known compounds, and a hitlist of potential identifications for the query is generated. Recently, several applications call for automated identification of compounds using mass spectral library searching (e.g. lipidomics, proteomics, metabolomics). These workflows assume that the first hit retrieved in the hit list is the correct identification of the query. Unfortunately, the first hit is correct only about 70% of the time. In this seminar, we discuss the fundamentals of mass spectral library searching and propose methods to test the reliability of an identification generated through automated searching.

A Fast Analytical Approach for Simultaneous Determination of Biphenyl, 2-Phenyl phenol and Anthraquinone in Coconut Oil Using Stable Isotope Dilution and Gas Chromatography-Tandem XueQing Wei*, ZhiMin Jiao, Ruifeng Zhang, Chuan Zhou[1], Wen Ming Cao[2], Hai Zhang, Yang Zhao, and Xuebing Xu[3],*[1]Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd, China, China; [2]Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China; [3]Wilmar Global Research and Development Center, China*

Coconut oil was easily detected to contain biphenyl, 2-phenyl phenol, anthraquinone and other harmful substances due to the factors of pesticides abuse, environmental pollution and heating treatment. A fast, simple and cost-effective GC-MS/MS method was established for simultaneous determination of biphenyl, 2-phenyl phenol and anthraquinone in coconut oil with stable isotope dilution assays （SIDAs）. Samples were extracted by acetonitrile and frozen for 2 hours. The extracts were filtered using 0.22-micron syringe filters for GC-MS/MS analysis. Matrix effects were efficiently compensated by D_{10}-biphenyl and D_8-anthraquinone as the [D]-labelled internal standards. The procedure was validated by spike recovery experiments at three different concentration levels, and the mean recoveries ranged from 86.3% to 95.3%, with RSD varied from 1.4% to 1.8%. The result showed a good linear relationship (R^2>0.999) between signal intensity and concentration in the range of 0.01 - 5 mg/kg. The detection limit of each compound was 0.01 mg/kg. The application of frozen to the pretreatment was proven to be very convenient and effective, and required no post-extraction sample clean-up steps. Finally, the method was successfully applied to analyze coconut oil samples. The method was simple, rapid, high sensitive and suitable for the simultaneous determination of biphenyl, 2-phenylphenol and anthraquinone in coconut oil.

ANA 2c/LOQ 2b: Chemical and Sensory Methods to Predict Food Stability
Chairs: J. David Pinkston, Archer Daniels Midland Co., USA; and Lan Ban, Kemin Food Technologies, USA

The Effect of Rosemary Extract and Phospholipase A2 on the Color Stability and Lipid Oxidation of Fresh Pork Sausage James Whalin*, Ling Liu, and Mark P. Richards, *University of Wisconsin-Madison, USA*

The objective of this study was to determine if a combination of rosemary extract (RE) and phospholipase A2 (PLA2) could stabilize color and limit lipid oxidation in pre-rigor pork sausage as well or better than synthetic (Syn) antioxidants. We hypothesized the combination of PLA2 and RE would stabilize color and lipid better than either PLA2 or RE individually and as well as synthetic antioxidants. Sausage was manufactured from sows within one hour post-exsanguination. Sausages were stored in the dark at -20°C (up to 245 days) prior to light display for nine days of refrigerated storage. Color stability was measured based on redness. Peroxide values (PVs) were measured spectrophotometrically and headspace hexanal were measured via gas chromatography (GC) as markers of lipid oxidation. Sausage with RE and RE+PLA2 exhibited better color stability than the synthetic antioxidants. However, the synthetic antioxidants had less lipid oxidation as measured by headspace hexanal. RE and PLA2 had lower PV than RE alone at one time point of cold storage. In conclusion, RE and PLA2 decreased lipid oxidation (compared to RE) and enhanced color stability (compared to Syn) and offer an alternative to synthetic antioxidants in pork sausage.

The Effect of Volatile and Non-volatile Degraded Products on the Performance of Frying Oil Junmei Liang*[1], Fuhuan Niu[2], and Yuan Rong Jiang[3], *[1]Wilmar (Shanghai) Biotechnology Research & Development center Co., Ltd, China; [2]Wilmar (Shanghai) Biotechnology Research & Development center Co., Ltd; [3]Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Frying is a very popular food preparation method in both food and catering industry. Deep fried food is always popular by their special flavor, delicious taste and crispy texture in the surface of fried food. Frying oil is one of the most important factor affecting the fried food quality and sensory. During deep frying, different reactions happened including autoxidation, polymerization and degradation. Both the volatile compounds and non-volatile compounds formed during frying. These compounds affect both the sensory of fried food and the quality of the frying oil. In this presentation, the detailed composition in the polar compounds will be analyzed, including both volatile compounds and the non-volatile polar compounds include free fatty acids, diglycerdies, oxidized triglycerides mono, dimers and polymers. The difference of the composition in degraded compounds with different frying oils will be compared. By compared the degraded products in the different oils, the effect of fatty acids profile will also be discussed. The effect of the total polar compounds composition of the frying oil on the sensory and quality of the different frying oil will be discussed.

Improvement of Flavour and Stability of High-oleic Sunflower Oil by Onion Frying Chang Chang*, and Xingguo Wang, *Jiangnan University, China*

High-oleic sunflower oil has been received more attentions by its prolonged stability and positive impacts on low-density lipoprotein cholesterol, but flavour qualities of its fried foods and itself were not satisfactory. The objective of this study was to improve sensory characteristics of high-oleic sunflower oil with an advantageous oxidative stability by onion

frying. Aroma compounds formed in high-oleic sunflower oil during the processes of heating and onion frying were investigated, as compared with palm oil and soybean oil. Onion frying developed four times more volatiles in the high-oleic sunflower oil than the simple heating process. Other than aldehydes, hydrocarbons, alcohols, and ketones resulting from lipid oxidation, onion frying also brought furans, furanones, furfurals, and phenols into the frying oils via Maillard reaction of amino acids with reducing sugars and aldehydes. In comparison with palm oil and soybean oil, high-oleic sunflower oil exhibited the highest flavour intensity after continuous frying of 16 h at 160 °C, especially on some representative volatiles [e.g., 2-n-octylfuran, dihydro-5-pentyl-2(3H)-furanone, and furfural]. Moreover, total polar compounds and fatty acids profiles of the heated and onion fried oils were comparatively studied. A great loss of flavonoids in onions took place during frying to effectively prevent oxidative deterioration of the oils by inhibiting the formation of polar compounds and trans fatty acids under deep frying. In conclusion, sensory quality of high-oleic sunflower oil was significantly optimized by onion frying with an unignorable bonus on oxidative stability under frying conditions.

Correlation of Oxidative Shelf-life to Test Conditions and Physical Stability of Antioxidants Chia-Yu F. Shen*, Kristen Robbins, and Lan Ban, *Kemin Food Technologies, USA*

Oxidative stability of foods and beverages is related to their intrinsic physical and chemical properties. Effective and clean-labeled antioxidants are strongly demanded by market-driven customers to improve the unsatisfactory oxidative stability. Antioxidants have been fast screened by accelerated tests, including oxidative stability index (OSI), Oxipres and elevated temperature storage stability. However, those accelerated tests may not truly reflect the antioxidants performance. In this study, selected antioxidants were evaluated in bulk oil and a low moisture food by OSI and Oxipres respectively. Those accelerated tests were compared to ambient storage condition which monitored oxidative byproducts and sensory change. The study reveals interesting observations. First, accelerated tests cannot reflect the actual antioxidants performance in foods. The combination of rosemary extract and ascorbic acid performed the best in improving shelf-life in biscuits, but Oxipres showed tocopherols the best. The discrepancy possibly came from different antioxidants chemical stability. Second, physical stability is crucial for ascorbic acid in extending bulk oils shelf-life which cannot be captured by OSI. Third, only ambient oxidative byproducts well-correlated to ambient sensory evaluation. Abusive storage conditions resulted in poor correlation between the two. In summary, sensory acceptance scores well-correlated to chemical markers in biscuit at ambient condition, and the physical stability of an insoluble antioxidant closely correlated to its shelf-life extension capabilities. Limited correlation was seen between highly abusive acceleration tests (Oxipres and OSI) and actual antioxidant performance in extending oxidative shelf-life in food systems.

Evaluation of Oxipres™ Apparatus to Study Oxidative Stability and Antioxidant Activity Cindy Tian*, *Kalsec, Inc., USA*

When studying the stability of oils and oil containing foods, accelerated tests are regularly used, where the rate of oxidation is enhanced either by using higher temperature or by addition of prooxidants. Oxipres™ apparatus, which shares similar principle as the ASTM Oxygen Bomb method, provides a fast way to examine stability of diverse applications compared to other methods such as Accelerated Shelf Life Testing (ASLT) and Oxidative Stability Index (OSI). As a preliminary

work to develop an Oxipres™ system to screen natural antioxidants, different matrices were studied to determine the optimum condition for each. Bulk oil, salty cracker, cookie, and dressing were selected to represent different food types in terms of different oil, protein, sugar and water content. Testing conditions including temperature, oxygen pressure, and sample size were studied. Temperature showed the biggest impact on induction time, whereas O2 pressure and sample size affected various matrices differently. All above factors also showed influence on the oxidation rate and total oxygen consumption. Initial work on the development of model systems for screening antioxidant activities will also be discussed.

ANA 3a: Rapid Methods of Analysis, including Portable Devices
Chairs: Magdi Mossoba, US Food and Drug Administration, USA; and Hongshun Yang, National University of Singapore, Singapore

Rapid Evaluation of Extra Virgin Olive Oil Authenticity: A Targeted FT-NIR Spectroscopic Procedure Magdi Mossoba*, Sanjeewa R. Karunathilaka, Kyungeun Lee, Zachary Ellsworth, Lea Brückner, and Betsy J. Yakes, *US Food and Drug Administration, USA*

Economically motivated adulteration of extra virgin olive oils (EVOO) has been a concern for regulatory agencies. In 2016, the U.S. Food and Drug Administration was directed by Congress to analyze imported olive oils and determine if they were adulterated or misbranded. To screen and assess EVOO authenticity, we propose a rapid (1.18), refined olive oils (FDI1.18). Based on validated official methods, only a few products (3%) were determined to be adulterated, thus the remaining 52% were mostly indicative of lower quality olive oils and inconsistent with the extra virgin label declaration.

Effects of Molar Mass and Ester Functionalities on Terahertz Spectra of Oils Svajus J. Asadauskas*, Mindaugas Karaliunas, and Gintaras Valušis, *FTMC, Lithuania*

Since terahertz radiation easily penetrates through various plastic and other polymeric barriers, Terahertz Time-Domain Spectroscopy (THz-TDS) can provide an excellent tool for Quality Control and non-destructive monitoring of packaged oils. A plastic cuvette was filled with various hydrocarbon and ester-based liquids, including a number vegetable oils. THz-TDS scans were recorded in the interval from 0.1 to 2 THz. The spectra demonstrated significant differences in refractive index (RI) and absorption intensity. RI increase from 1.38 to 1.5 clearly correlated with the molar mass of pure hydrocarbons and mono-functional esters, such as acetates and palmitates. In contrast, multi-functional esters, including vegetable oils, did not follow this correlation. Effects of addition of free oleic acid or water were also investigated, but no clearly evident relationships were identified. Nevertheless, the ability of THz-TDS to non-invasively detect different oils without a need to open plastic vessels, piping or other containers appears to be very promising for various engineering and technological aspects of oleochemical industry.

Synthesis of Immuno Magnetic Nanopartical for Quantification of Aflatoxin B1 in Oil Seeds Hongshun Yang*, Xi Yu, and Suan Liang Isaac Foo, *National University of Singapore, Singapore*

Oil seeds are susceptible to Aflatoxin B1 (AFB1) contamination if they are stored improperly and AFB1 is a dangerous mycotoxin which harms human health severely. In this report, immune magnetic nanoparticle was synthesized and a highly sensitive method which employs liquid solid extraction combined with this synthesized nanoparticle was developed for the extraction and quantification of AFB1 in oil seeds. Self-synthesized antibody functionalized magnetic nanoparticles (MNPs) were firstly characterized using FTIR and TEM before being used to extract the AFB1 in oil seeds. After the extraction process, the AFB1 was detected and quantified using HPLC with fluorescence detection. The extraction method was optimized based on several crucial factors which affect its efficiency, namely, the low temperature clean-up duration, volume of magnetic fluid added and vortex duration. the optimum conditions were found 20 h, 1 mL and 5 min, respectively. Regression analysis from the constructed calibration curve gave a correlation coefficient of 0.9946, showing satisfactory linearity over a concentration range of 0.1 - 100 ng g-1. The method is convenient,

highly efficient and accurate thus promising in food safety analysis.

The Development of a Robust Spectrometer for Online and Real-time Monitoring of Oil Quality Jonathon D. Speed*, *Keit Spectrometers, UK*

The online monitoring of chemical processes from heavy industry through to food and drink is an exciting tool and powerful part of modern-day process control and optimisation. Inferential sensors (such as pH and temperature probes) can be robust and reliable but are lacking in the depth of information they afford. Mid infrared spectroscopy (FTIR) is a powerful tool in the laboratory for oil analysis, but conventional spectrometers are sensitive to vibration, and the fibre optic-based probes they use are extremely fragile and not suitable for industrial processes. Here we present the trials and tribulations of the development of a spectrometer designed specifically for the manufacturing and industrial environment. The flexible, fragile fibre probe has been replaced with a solid "light pipe", and the moving mirrors – critical to conventional Michaelson based interferometers – have been replaced with static optics. This has come at a cost of decreased resolution, and initially a decrease in signal to noise ratio. We discuss the work that was done to improve performance – both from a spectrometer design but also from a chemometric and analytical chemistry route – and show failures as well as successes. We present the results of monitoring oils both for degradation and for contamination, and some of the tricks of the trade with relation to chemometric modelling – particularly with non-linear behaviour and when Beer's Law breaks down.

ANA 3b: General Analytical

Chairs: Torben Küchler, Eurofins Analytik GmbH, Germany; and Pierluigi Delmonte, US Food and Drug Administration, USA

Direct Quantification of Valuable Furan Fatty Acids in Fish Oils by NMR Walter Vetter*[1], Veter Gottstein[1], Johannes Günther[2], Marco Müller[1], and Katharina Wasmer[1], *[1]University of Hohenheim, Germany; [2]Core Facility Hohenheim, Germany*

Objective Furan fatty acids are valuable naturally occurring antioxidants which are ubiquitously found in food albeit at low amounts. Due to the low concentrations, the lack of reference standards and their sensitivity to degradation during sample preparation, furan fatty acid analysis (typically by GC/MS) is a challenge. As a consequence, the data on furan fatty acids is scarce compared to the proposed benefits that their presence may add to food. The richest food source for furan fatty acids is fish. Methods used. For the first time furan fatty acids were directly quantified by quantitative 1H-NMR (qNMR). The method was developed with isolated standards of alkyl esters of one monomethylated furan fatty acid (9M5) and one dimethylated furan fatty acid (11D5). These two (groups of) compounds showed the unique presence of singlets originating from the protons on the methyl substituents on the furan backbone (δ = 1.89 ppm and 1.83 ppm in CDCl3). These signals are not known to occur in other lipid compounds. Measurements were performed on a 600 MHz instrument equipped with a cryoprobe. Results. The limit of detection was ~23 µg for 11D5. Analysis of three fish oil samples by direct qNMR and GC/MS after extraction, transesterification, and silver ion enrichment were very similar. Advantages and disadvantages of qNMR and GC/MS will be discussed. Conclusion. Quantitative NMR is suited for the analysis of furan fatty acids in fish oils. Oils can be directly analysed after dilution in solvent and addition of the internal standard.

Critical Evaluation of Olive Oil Triglyceride Composition by Ultra High Performance Liquid Chromatography for the Detection of Added Seed Oils Pierluigi Delmonte*, and Andrea Milani, *US Food and Drug Administration, USA*

Several methodologies have been developed for detecting the addition of seed oil to olive oil based on the evaluation of the triglyceride (TAG) composition. These methodologies are primarily based on the different amount of saturated and unsaturated fatty acids (FA) esterified to position 2 of glycerol, compared to positions 1 and 3. The efficacy of these methodologies is limited by the incomplete chromatographic separation of TAGs provided by reversed phase HPLC. In this work we improved the separation of TAGs by utilizing Ultra High Performance Liquid Chromatography (UHPLC) and multiple reversed phase separation columns in series. Identification of TAGs was achieved by LC-GC in offline mode; the UHPLC eluent was divided in time fractions, which were analyzed by GC-FID after converting TAGs to fatty acid methyl esters. Two different methodologies were developed: one method maximizes the separation of TAGs utilizing several separation columns in series, the other balances chromatographic resolution and separation time in order to be suitable for routine analysis. The quantification of individual TAGs allowed more precise detection of seed oil addition to olive oil as compared to the delta-ECN42 determination which relies of the measurement of unseparated groups of TAGs.

Practical analyzing method of triacylglycerol isomers by using supercritical fluid chromatography Koji Masuda*[1], Kosuke Abe[2], and Yoshihiro Murano[1], *[1]The Nisshin OilliO Group, Ltd., Japan; [2]Nisshin Global Research Center Sdn. Bhd, Malaysia*

Triacylglycerol (TAG) isomers have been reported to function differently in terms of physical and nutritional properties. Therefore, analysis of TAG isomers is important for controlling the physical properties and understanding digestion and absorption in detail. However, methods to analyze TAG isomers in vegetable oils and biological samples are still under development. Recently, methods using recycle HPLC and silver ion column-HPLC were reported, but recycle HPLC method required significant amount of time for analysis. Moreover, each method has inability to analyze regioisomers and enantiomers at the same time. Thus, we aimed to develop a practical analyzing method capable of simultaneously quantitatively analyzing TAG regioisomers and enantiomers. We used supercritical fluid chromatograph – tandem mass spectrometer (SFC-MS/MS) with chiral columns. Acetonitrile and methanol was used as modifiers. Peak separation of sn-OPO and rac-POO standards was confirmed within 40 min. In addition, linear calibration curves with standards were confirmed and could be used to quantify sn-OPO, sn-POO and sn-OOP in extra virgin olive oil and palm oil, etc. We succeeded in the peak separation of TAG regioisomers and enantiomers, and then we also succeeded to quantify of TAG regioisomers and enantiomers in vegetable oil by SFC-MS/MS. As a result, we developed a practical analyzing method for TAG isomers using SFC-MS/MS.

C9-C11 Unsaturated Aldehydes in Oxidized Oils as Prediction Markers of Growth Performance in Non-ruminant Animals Jieyao Yuan*[1], Brian Kerr[2], and Chi Chen[1], *[1]University of Minnesota, USA; [2]USDA-ARS, USA*

Oxidized oils from rendering and recycling are commonly used as an economic source of lipids and energy in feeding non-ruminant animals. Oxidized oils can negatively affect animal growth performance. However, the correlations between growth performance and chemical profiles of oxidized oils were not well examined. In this study, broilers and pigs were randomly assigned to a diet containing fresh soybean oil or thermally oxidized soybean oil (OSO) from 6 heating conditions, including 45°C-336h; 67.5°C-168h; 90°C-84h; 135°C-42h; 180°C-21h; and 225°C-10.5h. Animal performance was determined by average daily gain, average daily feed intake, and gain to feed ratio. The chemical profiles of OSO were evaluated by common indicative tests of edible oils, including peroxide value, thiobarbituric acid reactive substances, p-anisidine value, free fatty acids, oxidized fatty acids, unsaponifiable matter, insoluble impurities and moisture. Among these quality indicators, p-anisidine value had the best inverse correlation with growth performance of broilers and pigs. Because p-anisidine value reflects the level of carbonyl compounds, the profiles of aldehydes in OSO were characterized by the liquid chromatography-mass spectrometry (LC-MS)-based chemometric analysis, and their levels were further quantified. Among 17 aldehydes identified in OSO, the levels of C9-C11 unsaturated alkenals, especially 2-decenal, 2-undecenal, 2,4-decadienal, and 2,4-undecadienal, had better inverse correlations ($r^2>0.8$) with growth performance than C5-C8 saturated alkanals, suggesting that chain length and unsaturation level affect the toxicity of aldehydes. Overall, C9-C11 unsaturated aldehydes could be a more robust prediction

markers of growth performance than other common quality indicators when feeding oxidized oils to non-ruminant animals.

Characterization of the Key Odorants of Steam-treated Canola Oil Eliciting the Desired Sensory Properties Compared to Canola Oil Eliciting a Fishy Off-flavor Michael Granvogl*[1], and Katrin Matheis[2], *[1]Technical University of Munich, Germany; [2]Chair for Food Chemistry, Technical University of Munich, Germany*

The molecular sensory science concept was applied to clarify the formation of a (i) fusty/musty off-flavor appearing in native cold-pressed canola oils and of a (ii) fishy off-flavor appearing in steam-treated canola oils. Therefore, for each type of oil, a positive control (PC) eliciting the desired sensory attributes and a sample with the respective off-flavor (OF) was used. Sixteen compounds significantly increased in the native cold-pressed OF. Investigation of the corresponding rapeseeds, from which OF was pressed, showed the same odorants above their respective odor thresholds as found in OF, only differing in their concentrations. Consequently, not the pressing process, but the seed quality determined this off-flavor formation. Interestingly, most compounds responsible for the fusty/musty off-flavor are caused my microorganisms, such as 2- and 3-methylbutanoic acid and 2-phenylethanol (Ehrlich degradation products), as well as 2-methoxyphenol or 4-methylphenol. Analysis of 7 further OFs (OF1-7) and 5 further PCs confirmed these odorants as general marker compounds for the fusty/musty off-flavor. The data were statistically evaluated via principal component analysis showing a clear discrimination of both oil groups. The same approach was used to characterize the fishy off-flavor in steam-treated canola oils. However, differences were only found for 3 compounds, which alone did not mimic the distinct off-flavor. Thus, additional techniques such as ion exchange chromatography and SPME/GC-MS were applied, revealing trimethylamine as the main reason for the fishy off-flavor. This finding was finally confirmed by an excellent accordance of the original steam-treated canola oil with the respective recombinate.

ANA 3c/LOQ 3a: Advanced Analytical Techniques for Lipid Oxidation

Chairs: Matthew Fhaner, University of Michigan-Flint, USA; and Rick Della Porta, Pepsico/Frito-Lay, USA

New Method for the Investigation of Oxidation Stability of Fats, Oils and Complex Food Products Carolin Edinger*, *Anton Paar ProveTec GmbH, Germany*

The quality of fats and oils strongly depends on their oxidation stability. In this contribution a new method for evaluating the oxidation stability of fats and oils by determining the induction period is introduced. Under accelerated conditions (elevated temperature and pure oxygen pressure) a sample of 5 mL/4 g is examined in a sealed stainless steel test chamber. Typical conditions of the method are temperatures between 80 °C – 140 °C and an initial oxygen pressure of 700 kPa. These conditions initiate a rapid oxidation process, which is monitored by recording the pressure until a predefined pressure drop. It was found that the elapsed time until the pressure drop is directly related to the oxidation stability of the sample. Correlation and precision studies demonstrate the method's effectiveness. Due to the defined oxygen volume in the closed test chamber, the oxygen consumption can be calculated. Furthermore, we observed Arrhenius behaviour with regard to the applied temperature, enabling the user to determine the activation energy of a specific oxidation process. Beneficially, the oxidation stability of complex food products can be investigated since even solid samples can be measured without prior sample preparation. The significantly reduced measurement time and a high repeatability of the method represent its major advantages, allowing for quick and direct measurement of the oxidation stability for research, process and test bench control.

Analysis of Polar Compounds Generated during Thermal Process of Oils and its Biochemical Function Evaluation Chen Cao*, Yongjiang Xu, and Yuanfa Liu, *Jiangnan University, China*

Objectives: during thermal process of oil, a wide variety of chemical reactions occur, which lead to the formation of kinds of compounds with high molecular and polarity, for instance oxidize triglycerides (ox-TG), oxidized triglycerides dimer (TGD), oxidized triglycerides oligo (TGO), diacylglycerol and some free fatty acids. The analysis of polar compounds generated during thermal process of oils and its biochemical function evaluation are necessary. Methods: we used HPLC, LS-MS to separate and analyze the polar compounds, established saturated – unsaturated fatty acids systems to investigate the connections between the final products and the fatty acids composition. Studied their biochemical functions in vitro and in vivo. Results: 1. The polar compounds in the frying oil can induce gene mutation and chromosome variation. At the same time, the intake of polar compounds interferes with the metabolism of lipid, causing liver function damage and affecting the health of the body. 2. The results of cell experiments showed that the toxicity of oxidized triglycerides in polar compounds was greater than others. 3. New types of fatty acids, such as epoxy fatty acids and hydroxyl fatty acids, are produced during the frying process. By the ESR method, the thermal oxidation mechanism of the frying oil is explored to explain the complex reaction process in the frying system. Conclusions: this study help us to have a better understanding of polar compounds generated during thermal process of oils.

Electrochemistry as an Analytical Tool for Monitoring Antioxidant and Omega-3 Fatty Acid Levels during Degradation Matthew Fhaner*, *University of Michigan-Flint, USA*

Electrochemical methods have been utilized for investigating antioxidant systems due to fast analysis times, low cost, direct quantitative

capabilities, and useful limits of detection and linear range. Specifically, voltammetric methods such as square-wave voltammetry (SWV), provide a direct correlation between analytical signal and analyte concentration making it possible to monitor antioxidant levels during degradation of lipid products. Our group has worked to correlate voltammetric oxidation currents of antioxidants with changes in lipid profile of omega-3 fatty acids as monitored via gas chromatography with flame ionization detection (GC-FID). Over a three week degradation study, sesamol, rosemary extract, and butylated hydroxytoluene were supplemented into stripped commercial fish oil and exposed to elevated temperatures. Antioxidant levels were assessed via SWV oxidative peak currents while two specific fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were monitored using GC-FID. It was determined that SWV does not act as a predictive tool for how well an antioxidant species will protect a specific fatty acid. SWV was successful in providing insights as to the rate of antioxidant depletion over the course of the study, albeit no significant difference was observed between the antioxidants used. An unexpected finding was that SWV was able to monitor oxidation peaks for EPA and DHA and this data mirrored that of the well-established GC-FID FAME method that was run in parallel to SWV studies. The results suggest SWV may fit a niche need for real-time analysis of antioxidant levels and potentially fatty acid quality in commercial and industrial applications.

Application of Flow Cytometry as Novel Technology in the Study of Lipid Oxidation in Oil-in-Water Emulsions Peilong Li*[1], D. Julian J. McClements[2], and Eric A. Decker[2], *[1]Dept. of Food Science, University of Massachusetts, Amherst, USA; [2]University of Massachusetts Amherst, USA*

The body of literature on the impact of emulsion particle size on oxidation rates is unclear. This could be because emulsions are typically polydisperse and the oxidation rate of individual droplets is impossible to discern. Flow cytometry is a technique for studying individual cells and their subpopulations using fluorescence technologies. It is possible that individual emulsion droplets could also be characterized by flow cytometry as a novel approach for studying lipid oxidation. Typical emulsion droplets are too small to be visualized by flow cytometer so emulsions were prepared to have droplets > 2 µm; weighting agent and xanthane gum were added to minimize creaming during storage. A radical-sensitive lipid-soluble fluorescence probe (BODIPY 665/676) was added to the lipid used to prepare the emulsion so that the susceptibility of individual emulsion droplets could be determined. The results showed that in a polydisperse emulsion system, small droplets were oxidized faster than large droplets. Using mixtures of emulsions with and without prooxidants, it was possible to see the transfer of prooxidants between droplets, a process that is influenced by surfactant and salt concentrations. For example, surfactants micelles can transport prooxidants to neighboring non-oxidized droplets when surfactant concentration was higher than critical micelle concentration (CMC). Transfer of prooxidants was promoted by adding salt which could be attributed to the effect of salt on CMC. This study showed the good potential for applying flow cytometry on oxidation of individual emulsion droplets.

ANA 3.1/EAT 3.1/IOP 3.1: Analysis of Food Applications of Low Saturated Fats/Oils; and PUFA, and Fat-Soluble Vitamins with Emphasis on Nutrition Labeling

Chairs: Jillonne Kevala, Food and Drug Administration, USA; and Serpil Metin, Cargill R&D, USA

Modernizing the Nutrition Facts and Supplement Facts Labels Jillonne H. Kevala*, *Food and Drug Administration, USA*

Objective: To modernize the Nutrition Facts and Supplement Facts labels (NFL and SFL) to reflect current scientific information on nutrition and enhance its presentation to consumers. Methods: Review and analysis of information from sources including the U.S. Department of Health and Human Services' and the U.S. Department of Agriculture's, "Dietary Guidelines for Americans 2015–2020"; the Report of the Dietary Guidelines Advisory Committee on the Dietary Guidelines for Americans (February 2015); The Institute of Medicine, which reviewed several reports on individual nutrients, from 1997 to 2011; consensus statements from the National Institutes of Health; and a Surgeon General report on Bone Health and Osteoporosis (October, 2004). FDA also relied on data from the National Health and Nutrition Examination Survey (2003-2008). Results: FDA's review and analysis of the large body of information described above showed that a significant number of different elements in the NFL and SFL, established in January 1993, were out of date with respect to current nutrition science. Other elements of the label needed updating to present nutrition information in a manner more clear and useful to consumers. Conclusions: FDA made numerous amendments to the requirements for mandatory and voluntary declarations of specific nutrients and amendments addressing different aspects of label formatting and issues related to serving sizes. The final rules published in May of 2016. FDA is drafting numerous guidance documents, in different stages of development, to aid manufacturers in implementing changes in the NFL and SFL. The presentation will provide an overview of the key changes.

Low Saturate High Oleic Canola Oil in Health and Nutrition Xiaolan Luo*, Nisa Tharayil, and Diliara Iassonova, *Cargill, USA*

Nutrition research has been focused on specific food ingredients beneficial to nutrition and health as diet has been linked to various diseases such as diabetes, obesity and cardiovascular disease. Fats and oils are one of the most important components of diet as one-fourth of total daily dietary energy is supplied by these fatty acids. Recently, Cargill scientists have developed a low saturate high oleic canola oil with a high level of mono-unsaturated and moderate level of polyunsaturated fatty acids. The new canola oil has the lowest saturated fat content on the market and can offer a 40% reduction in saturated fat from conventional canola oil. Oxidative stability and performance in food applications of the new canola oil was evaluated by analytical tests such as rancimat and schaal oven tests as well as sensory test in comparison with other specialty and commodity oils. The new canola oil demonstrated superior flavor and high oxidative stability. Its OSI slightly decreased from 18.6 to 18 even after storage for over 3 years at 55F. Food fried in low saturate canola oil showed favorable flavor, texture and high overall liking score while containing significantly reduced saturated fat content. The impact of natural antioxidants on oxidative stability of low saturate canola oil was also investigated. With high oxidative and application performance, low saturate high oleic canola oil offers a healthier choice through reduced saturated fat per serving in labelling of a wide range of foods.

Validation of a HPLC Method for Analysis of Provitamin A Carotenoids (β-carotene, α-carotene and β-cryptoxanthin) Sneh Bhandari*, and Ming Gao,*Merieux NutriSciences, USA*

The 2016 Nutrition labeling regulation by US FDA (FR 33741) replaced the International unit (IU) for label declaration of vitamin A to mcg Retinol Activity Equivalents (RAE). β-carotene, and α-carotene, and β-cryptoxanthin have different RAE values. There is a need for an accurate and precise method for analysis of α-carotene, β-carotene and β-cryptoxanthin in foods for labeling of correct amount of total vitamin A, the current study is an attempt to validate such a method. The carotenoids were extracted using a hot sponification followed by the solvent extraction. The extract was analyzed by a reverse-phase HPLC on a C30 column. The method did provide a good chromatograppy and response of the carotenoids. The NIST SRM 2383 a baby food and BCR 485 vegetable powder reference material were analyzed by the method and the results of β-carotene, α-carotene and β-cryptoxanthin as well as trans-β-carotene were with in the range specified by the respective reference materials for the corresponding carotenoids. The method precision in different food matrices in terms of % RSD values for β-carotene, α-carotene and β-cryptoxanthine were in the range 1.9-5.4%, 3.1-15.1% and 1.6-7.9% respectively. Spike recovery in different matrices of carotenoids was found to be in the range of of 86- 106%. LOD and LOQ of the method in terms of mcg/100g was in the range 1.1-2.2 and 2.1-3.6, respectively. The method was found to be accurate and precise for the analysis of carotenoids in different food matrices. The method can be used for carotenoid analysis for nutrition labeling of vitamin A.

Rheology and Baking Stability of Water in Oil Emulsion Designed as Low saturated Bakery Shortening Fernanda Davoli*[1], Serpil Metin[2], and Paul Smith[3],[1]*Cargill, USA;* [2]*Cargill R&D, USA;* [3]*Cargill Global Foods Research, Belgium*

There is a strong drive for low SAFA shortenings, but development is challenging because of the difficulty in reproducing the "plasticity" of traditional systems with reductions in SAFA. In this study, a two-step approach was taken. First water in oil emulsions were produced and their rheology was manipulated by emulsifier composition (including combinations), in order to find the behavior most resembling a traditional shortening. Then, the water phase was structured as a stable hydrocolloid gel to allow better water management during baking. Emulsion systems (water in oil emulsion) containing 35% saturated fat were prepared using a variety of different emulsifiers and crystallized with careful processing control in a pilot scale SSHE and characterized by rheology and water droplet size. Fat systems with good rheological behavior were selected, then used to further prepare emulsions with alginate to structure the water phase. Application tests were then performed with cookies. Control and manipulation of rheological properties were achieved, showing that the mechanical properties of the emulsion could be manipulated through the choice of emulsifier. Emulsions where the water phase was an alginate gel presented the best application performance in terms of cookie dimensions, suggesting better water control during baking. This research aims to build the foundational knowledge to lead to the development of a low saturated fat system that deliver the expected plasticity of a bakery shortening (responsible for proper dough formation) as well as an emulsion which maintains the final cookie dimensions by managing water loss during the baking process.

Quantification of Furan Fatty Acids by LC-MS/MS and their Identification in New Zealand Marine Oils Matthew R. Miller*[1], Donato

Romanazzi[2], Hajime Uchida[3], Johnathon Puddick[2], Yutaka Itabashi[3], Masashi Hosokawa[4], Toshiyuki Suzuki [3], and Michael Boundy[2], *[1]Cawthron, New Zealand; [2]Cawthron Institute, New Zealand; [3]National Research Institute of Fisheries Science, Japan; [4]Hokkaido University, Japan*

Furan fatty acids (F-acids) are heterocyclic fatty acids with a furan moiety in the acyl chain. They are generally found as minor components in the lipid fraction of marine organisms, plants and mammals. F-acids are potent antioxidants and radical scavengers and are thought to contribute to the health benefits of fish-rich diets. The role and benefits of dietary F acids are yet to be fully understood, however they show promising potential as therapeutic marine drugs for the treatment of inflammatory-disorders. Objectives To develop a rapid quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS) method for un-equivocal identification and quantitation of F-acids in marine oils Methods For the past two years, the Cawthron Institute (NZ) has been engaged in an international research collaboration with the Hokkaido University (Japan) and the National Research Institute of Fisheries Science (NRIFS) of Japan to develop novel methods to identify and quantify F-acids. We have developed LC-MS/MS methods that in positive ESI mode, F-acids gave a prominent [M + H] ion, by which individual F-acids could be detected and identified to a level of 5 ng/mL. Results Our LC-MS/MS method provides selective detection of F-acids in marine oils without the need of lengthy sample processing. Through our research we have also identified a new source of F-acids, a unique New Zealand marine oil with surprisingly high levels of these minor fatty acids, offering new research opportunities in this field.

Physicochemical Properties, Chemical Composition and Risk Assessment of Polycyclic Aromatic Hydrocarbons of Commercial Fragrant Rapeseed Oils Youfeng Zhang*, *School of Food Science and Technology, Jiangnan University, People's Republic of China*

Fragrant rapeseed oil is a type of hot-pressed oil in China. In this work, physicochemical properties, chemical composition, presence and risk assessment of (PAHs) polycyclic aromatic hydrocarbons in 38 representative Chinese fragrant rapeseed oil samples were evaluated for the first time. The acid value (0.64-2.68 mg KOH/g), peroxide value (1.58-4.86 mmol/kg) and color value (R=2.6-5.8 Y=35) were all within codex limits. Thirty-two samples whose erucic acid concentrations exceeded 2%. A well negative linear correlation between oleic acid and erucic acid was shown (R2=0.876), which suggested to be a potential marker for prediction of adulteration. Benzo[a]pyrene and PAH4 (chrysene, benz[a]anthracene, benzo[b]fluroranthene, and benzo[a]pyrene) were nd-6.93 and nd-30.79 µg/kg, respectively. Monte Carlo simulation was applied to deal with the uncertainties in the dietary exposure and risk estimation. The median dietary exposure of BaPeq contents from total PAH4 were found to be 0.0826, 0.0530, 0.3082 and 0.0724 ng·kg-1·d-1 for children, adolescents, adults and seniors of male, respectively, whereas that for the above groups of female were 0.0777, 0.0504, 0.3014 and 0.0659 ng·kg-1·d-1. Results from health risk estimation indicated high potential carcinogenic risk. Fragrant rapeseed oil is still a product subject to contamination by PAHs. Limits for PAH4 of fragrant rapeseed oil should be included in Chinese regulation to improve the safety.

The Role of Fat in Determining the Structural and Textural Properties of Semi-sweet Biscuit
Hasmadi Mamat*, *Universiti Malaysia Sabah, Malaysia*

Fat plays a unique role in many food products due to its hygroscopic nature. In the area of baked goods, biscuits belong to a group of products that contains considerable fat and the overall quality is largely determined by the type of fat used. In this study, the effect of fat type on dough rheology properties and quality of semi-sweet biscuit (rich tea type) was investigated using varies techniques. Four types of fat, namely palm oil, palm olein, palm mid-fraction and butter, which varied in composition and solid fat content, were used to produce semi-sweet biscuits. The role of fat and type of fat were analysed in terms of the texture, appearance and the starch behaviour of the final baked biscuit. Rheological properties of the dough were also compared. Texture profile analysis results showed that the type of oil significantly influenced dough rheological properties. Hardness measurement showed that biscuit produced with higher solid fat oil had higher breaking force, but this was not perceived when tested with a human panel. Gelatinization and pasting results showed that fat type also influenced thermal profiles of starch granules in biscuits with most of the granules retaining their crystallinity. Microscopy observations showed that biscuit produced with palm mid-fraction had an open internal microstructure and heterogeneous air cells as compared to the other samples. As conclusion, fat is an important ingredient in baking products and it plays many roles in providing desirable textural properties of baking products, particularly biscuit.

Effect of Tempering Parameters on the Plasticity and Hardness of Puff Pastry Margarine
Miroslav Buchmet*, *DuPont Nutrition Bioscience, Denmark*

Puff pastry margarine is one of the most challenging products for the margarine industry. There are many demands to good puff pastry margarine; it must be stable, plastic and non- greasy. Plasticity of margarine is determined by fat base and processing. Usually, fat base consists of palm oil and/or its fractions and some liquid oil. Such blends give good plasticity and workability. But to achieve that, it is necessary to temper margarine at proper temperature right after production and before distribution. Results of this study have proven that tempering conditions have a great influence on the margarine quality. Freshly produced samples of full-fat puff pastry margarine were tempered for one week at temperatures of 10, 20, 30°C, and followed by evaluation after one week's and two months' storage at 20°C. Samples were evaluated by hand for plasticity and workability. Hardness of margarine was measured by means of texture analyzer. The samples demonstrated different plasticity and hardness. Hardest and most plastic were samples tempered at 10°C, followed by samples at 20°C. Samples tempered at 30°C were brittle. Observed behavior can be explained by the polymorphic nature of fat. Data can be used as a guideline during margarine production and troubleshooting.

ANA 4a: Trace Contaminants

Chairs: Jessica Beekman, US Food and Drug Administration, USA; and Jan Kuhlmann, SGS Germany GmbH, Germany

Managing Chemical Contaminants in Foods: a Review of Selected Chemicals and Mitigation Strategies Richard Stadler*, *Nestlé Research, Switzerland*

Issues related to foodborne chemical contaminants continue to raise concerns, eroding trust in the integrity of our food supply chains and / or manufacturing practices. Despite significant efforts to provide better visibility / transparency of food origin, as well as to improve agricultural and food processing practices, residual risks may remain that need to be mitigated. In this context, the occurrence in foods of several chemicals have recently raised concern, such as mineral oil hydrocarbons, acrylamide, furan/alkylfurans, chloroesters (e.g. 3-MCPD esters and 2-MCPD esters) and glycidyl esters. The chloroesters and glycidyl esters are formed when vegetable oils and fats are heated, i.e. mainly at the deodorization stage. Notably, some of these compounds (e.g. acrylamide, glycidyl esters) are now regulated in the European Union. In the case of processing contaminants, significant progress has been made over the past decade in understanding their formation and devising mitigation strategies. So-called “Toolboxes” have been established to help manufacturers identify the best approaches to reduce certain contaminants such as MCPD esters / glycidyl esters. The food industry has conducted extensive research into their formation in refined oils, particularly palm oil. Based on extensive research, fats & oils manufacturers and the food industry have developed a catalogue of mitigation strategies, that are being applied to lower the levels of MCPD esters and glycidyl esters to as low as “practically” achievable, whilst maintaining the safety and important organoleptic properties of fats and oils.

Estimated Exposures to 3-MCPD Esters and Glycidyl Esters from U.S. Consumption of Infant Formula Judith Spungen*, *FDA, USA*

3-monochloropropane-1,2-diol (3-MCPD) esters (3-MCPDE) and glycidyl esters (GE) are food contaminants generated during the deodorization of refined edible oils. 3-MCPDE and GE have been found in oils used as ingredients in infant formulas and other foods. 3-MCPDE and GE are of potential toxicological concern because these compounds are metabolized to free 3-MCPD and free glycidol in rodents and may have the same metabolic fate in humans. Free 3-MCPD and free glycidol have been found to cause adverse effects in rodents. Because infant formulas are the sole or primary food source for some infants, dietary exposures to 3-MCPDE and GE from consumption of infant formulas are of particular interest. We estimated 3-MCPDE and GE exposures from consumption of infant formula by infants by 0–6 months of age using US Food and Drug Administration data on 3-MCPDE and GE concentrations (as 3-MCPD and glycidol equivalents, respectively) in a small convenience sample of infant formulas. 3-MCPDE and GE exposures based on mean concentrations in all formulas were estimated at 7–10 and 2 µg/kg bw/day, respectively. Estimated mean exposures from consumption of formulas produced by individual manufacturers ranged from 1 to 14 µg/kg bw/day for 3-MCPDE and from 1 to 3 µg/kg for GE. U.S. infant exposures to 3-MCPDE and GE from consumption of formulas containing palm oil/olein could potentially be reduced if more U.S. manufacturers used oils from non-palm sources or use palm products with lower 3-MCPDE and GE concentrations.

MCPD and glycidyl esters - present and future EU legislation, implementation in German risk management Martin Kaminski*, *Federal Office of Consumer Protection and Food Safety, Germany*

Since 2018 the content of glycidyl esters in oils, fats and infant formula has been regulated in the European Union. EU Regulation 2018/290 is expected to be amended by new maximum limits for bound and free MCPD in different foodstuffs. Current items of discussion in the legislation process are maximum limits for potentially more highly contaminated types of oil as well as the legal assessment of oil mixtures. To monitor the compliance with future legal provisions in practice, legislation has to take into account the current performance of the available analytical methods for MCPD and glycidyl esters. To monitor the compliance of fats, oils and fat-containing compound foodstuffs with legal requirements, high quality analytical methods are needed for various food categories. Well-characterized reference materials are essential to prove and monitor the performance of existing and newly developed methods. To support official food control, the German National Reference Laboratory is building up a proficiency test program and a reference material collection for MCPD and glycidyl esters. A new working group of German expert laboratories was founded with the aim to validate and standardize methods for MCPD and glycidyl esters in compound foodstuffs beyond infant formula.

Opportunities and Drawbacks in the Mitigation of 3-MCPD- and Glycidylesters Nils Hinrichsen*, *Archer Daniels Midland, Co., USA*

Process contaminants like Glycidyl- and 3-MCPD-esters in refined oils and fats constantly gained importance over the past decade. Since glycidylesters are cancerogenic and genotoxic and 3-MCPD-esters are at least also cancerogenic, their mitigation and avoidance is subject of several research projects worldwide. In 2016 the European Union defined legal maximum levels for glycidolesters in food oils and is about to establish also limits for 3-MCPD-esters very soon. Accordingly technologies to produce refined oils and fats with low levels of these process contaminants are of increasing relevance. Possible handles to decrease the levels of 3-MCPD- and Glycidylesters have been published by industrial and scientific associations. Also, the Codex Alimentarius commission is currently working on an updated document. Most frequently used and described mitigation technologies are washing of crude oils, alkaline pretreatment, low-temperature deodorization and post-treatment with acid activated bleaching clays. This presentation discusses the advantages and drawbacks of these technologies and also depicts other less common ideas to mitigate 3-MCPD- and glycidylester levels.

Quantification of MCPDE and GE in Edible Oils and Fats: A High Throughput Method for QC Purposes Ralph P. Zwagerman*[1], and Pierre Overman[2],[1]*Bunge Loders Croklaan, The Netherlands;* [2]*Bunge Loders Croklaan, Netherlands*

As of March 2018, legislation has come into force within the European Union (EU) that places maximum levels on the glycidyl ester (GE) content of vegetable oils intended for use in foodstuffs for the European market. As a result, producers of edible oils are obliged to closely monitor the GE levels in all their products. Furthermore, there are ongoing discussions within the EU to introduce similar legislation, expected by the January 2021, for maximum levels of 3-monochloropropanediol esters (3-MCPDe). Unfortunately, the three available AOCS methods to measure GE are not really suited for a Quality Control laboratory within a production facility, where short

turnaround times are vital. AOCS Cd29c-13 has the highest potential from a turnaround perspective, but remains disputable because it is based on a differential GE measurement. Hence, in 2016 we published an automated procedure in which the shortcomings of the AOCS Cd29c-13 were effectively addressed. Several optimisations were implemented to further reduce turnaround time and improve robustness that resulted in a high throughput method to quantify these process contaminants We present the results of an interlaboratory comparison, which was organised across six laboratories in four countries. Methods involved included AOCS Cd29b-13 as well as the optimised high throughput method, which was carried out both automatically and manually. Based on the results of the interlaboratory comparison, it is concluded that the automated procedure performs slightly better than the manual alternative. Compared to the AOCS Cd29b-13, no significant differences were observed for various types and blends of vegetable oils.

Stability of food contaminants 3-MCPD-, 2-MCPD- and glycidyl fatty acid esters in foods during long-term storage Jan Kuhlmann*, *SGS Germany GmbH, Germany*

3-MCPD, 3-MCPD fatty acid esters (3-MCPDE) and glycidyl fatty acid esters (GE) are heat induced food-borne contaminants that have shown adverse health effects in vivo. Consequently, the European Commission (EC) has released regulation EU 2018-290 on maximum contents of glycidyl fatty acid esters in vegetable oils & fats and infant foods. Presumably, in the first half of 2019, the EC might also regulate 3-MCPD and 3-MCPDE (total 3-MCPD) in specified oils & fats and infant nutrition products. In the last several years, the knowledge about origin, formation, mitigation and analysis of 3-MCPD and glycidyl derivatives has increased significantly. But, very little is known regarding the stability of these analytes during food storage, which becomes relevant when comparing analytical results or preparing reference materials. In an attempt to collect data on analyte stability in foods for which the current regulation applies, commercially available samples were stored for time periods that included the corresponding shelf-life. Different storage temperatures were also chosen to reflect conditions found in supermarkets or analytical laboratories. During the storage-trial, all samples were analyzed periodically for their contents of 3-MCPD and glycidol. Results indicate that free 3-MCPD and 3-MCPDE were stable in all tested food matrices regardless of storage temperature. Contrarily, GE showed matrix and temperature-dependent instabilities that lead to, in some cases, significantly lower bound glycidol levels during food shelf-life. In conclusion, these effects should be considered when analytical results are evaluated, samples are stored for analysis, or in the case of preparing reference materials.

ANA 4b: Authentication of High Value Oils, including Olive Oil, Sensory Evaluation and Correlation with Analytical Results

Chairs: Rodney Mailer, Australian Oils Research, Australia; and Luisito Cercaci, Pompeian, USA

Assessment of Authenticity of Blended Oil by Triacyglycerols and Chemometrics Tools Hong Yang*[1], Wen Ming Cao[2], and Yuan Rong Jiang[2],[1]*Wilmar Biotechnology Research & Development Center (Shanghai) Co.,Ltd , China;* [2]*Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Many oils sold in China and India are a blend of various oils to improve performance, stability and nutritional characteristics, which are required in their respective markets. Quantitative analysis of the proportions of constitutive components is fundamental to the conformity and adulteration checking of edible blended oil products. A Multi Linear Regression (MLR) model with Constrained Linear Least Squares (CLLS) and exhaustion calculation was applied in this study. The source of the varieties in the model is a database (614 pure oils) of triacylglycerols (TAGs) collected by GC-FID and HPLC-RID. There were 20 groups of binary and ternary blended oils consisting of two or three oils out of the five kinds of oils (soybean, corn, peanut, rapeseed, sunflower), which were analyzed and processed separately. Results showed that the method was capable to predict the proportions of constitutive components in the edible blended oils, given that relative errors required less than 20%, the accuracy was 98.2% for the binary system if the proportion of each oil in blended oils was more than 20%, while the accuracy was 84.7% for the ternary system if the proportion of each oil in blended oils was more than 10%. The quantitative method is based on a simple analysis to determine the TAGs composition and thus it is useful for quick segregation and quality control of blended oils in routine analysis.

Flash Gas-Chromatography in tandem with chemometrics: a screening tool to discriminate the olive oil quality Alessandra Bendini*[1], Chiara Cevoli[1], Sara Barbieri[1], Diego L. García González[2], and Tullia GT Gallina Toschi[3],[1]*DISTAL University of Bologna, Italy;* [2]*Instituto de la Grasa (CSIC), Spain;* [3]*Alma Mater Studiorum - University of Bologna, Italy*

According to the IOC documents and the EU Regulations, accredited laboratories performing sensory analysis of virgin olive oils have to apply specific guidelines to work in compliance with the laboratory organization and technical conditions of analysis requirements. These specifications strongly limit the number of samples that can be assessed per day by a panel. The setting up of an instrumental method for rapid screening of quality grades could represent a solution for supporting the sensory panels, reducing their daily work and increasing the efficiency of the controls. This research aims to develop a classification model based on an untargeted elaboration of volatile fraction fingerprints (334 samples analyzed by Flash Gas-Chromatography) in order to predict the commercial category of samples (extra virgin olive oil EVOO, virgin olive oil VOO, lampante olive oil LOO). The raw data related to volatile profiles were considered as independent variables, while the quality grades provided by the sensory assessment were defined using dummy variables. This data matrix was elaborated using a linear technique (PLS1-DA), applying, in sequence, a classification model with two categories (EVOOs vs Non-EVOOs, VOOs vs LOOs). Results from the 334 samples provide satisfactory results in terms of percentages of correctly classified samples (by internal and external validation) confirming the reliability of this approach. This work has been developed in the context of the

project OLEUM "Advanced solutions for assuring authenticity and quality of olive oil at global scale" funded by the European Commission within the Horizon 2020 Programme (2014–2020, GA 635690).

Putting a Gate Around High Quality EVOOs from Specific Origins by ^{1}H NMR Profiling Databases Chiara Roberta Girelli, Laura Del Coco[1], Federica Angilè[2], Francesca Calò[3], Paride Papadia[3], Andrea Biagianti[4], Daniele Barbini[4], and Francesco Paolo Fanizzi*[2],*[1]Department of Biological and Environmental Sciences and Technologies (Di.S.Te.B.A.), University of Salento, , Italy; [2]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali Università del Salento, Italy; [3]Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali Università del Salento 73100 Lecce, Italy; [4]Certified Origins Italia Srl Loc. Madonnino 58100 Grosseto, Italy*

The problem of setting a scientifically sound origin assessment method is a key factor for EVOOs trade and consumer awareness. Analyzing by multivariate statistical analysis (MVA) the ^{1}H NMR-based metabolomic profiles of both laboratory micro-milled and commercial oil samples, specific clustering are usually observed according to EVOOs cultivars and/or geographical origin. Our recent results, in particular on Tuscan PGI [1] and Apulian most popular [2] Italian EVOOs, clearly demonstrate that the observed clustering strongly depends on considered both cultivars and growing areas. This should be necessarily taken into account when building up specific databases aimed to EVOOs origin and authenticity assessment. An essentially flat growing area such as Apulia, characterized by almost homogeneous pedoclimatic conditions, results on a prevalence of cultivar related ^{1}H NMR-based metabolomic profiles. Conversely, the higher variety of different Tuscany "terroirs" requires a much deeper analysis in order to fully understand the ^{1}H NMR profiles of Tuscan PGI EVOOs. Nevertheless, irrespectively of the considered system complexity, ^{1}H NMR-based metabolomic profiles collection represents a powerful tool for EVOOs origin and authenticity assessment. Putting a gate around high quality EVOOs from specific origins by ^{1}H NMR profiling databases could be profitably used to justify the high added value of the product and the sustainability of the related supply chain. [1] Girelli, C.R.; Del Coco, Zelasco, S.; Salimonti, A.; Conforti, F.L.; Biagianti, A.; Barbini, D.; Fanizzi, F.P. Metabolites 2018, 8 (4), 60 [2] Girelli, C.R.; Del Coco, L.; Papadia, P.; De Pascali; S.A., Fanizzi, F.P. PeerJ 2016, 4, e2740.

A New Identity Standard for Olive Oil Refined Gina M. Clapper*[1], Kristie Laurvick[1], and Richard C. Cantrill[2], *[1]USP, USA; [2]Independent Consultant, Canada*

Economically motivated adulteration is an ongoing, global issue. The Food Fraud Database includes more than 200 records of olive oil adulteration with substances including vegetable oils, lower-grade olive oils, chlorophylls and chlorophyllin, and non-food grade oils. As the olive oil industry has expanded in recent decades to new growing regions, current global standards insufficiently address authentic oils from various producing regions, including North America, South America, and Australia. USP was approached by members of the olive oil industry to draft a globally relevant documentary standard to describe authentic olive oil products and to provide related test methods and specifications necessary to properly identify olive oil. This documentary standard is proposed to meet the needs of the growing and expanding olive oil industry and has been developed based on careful consideration of existing international standards for olive oil. All of the proposed specifications included in this FCC Identity Standard are based on refined olive oil. This Identity Standard can be used by industry to

identify Olive Oil, Refined from virgin olive oil, other food oil(s), and to facilitate authentic labeling.

Olive Oil Authentication Using Raman Spectroscopy Combined with Pattern Recognition Analysis Didem P. Aykas*, and Luis E. Rodriguez-Saona, *The Ohio State University, USA*

Extra virgin olive oil (EVOO) is one of the top foods being adulterated with lower quality olive oils (LQOO) or other vegetable oils. The aim of this study was to develop an authentication program for EVOO using Raman spectroscopy signatures combined with pattern recognition analysis. Olive oil samples (n=141) were obtained from the world leading olive oil producing countries. Reference methods were used to evaluate fatty acid profile, free fatty acids (FFA), peroxide value (PV), total polar compounds (TPC), and pyropheophytins (PPP) of samples. Spectra were collected using a portable 1064 nm Raman spectrometer. Prediction models were generated using Soft Independent Model of Class Analogy (SIMCA) and Partial Least Squares Regression (PLSR). The olive oil samples were classified as EVOO, LQOO, and adulterated with vegetable oils, using information from the International Olive Oil research laboratory (Aydin, Turkey), California Olive Oil Council and our reference analysis. SIMCA analysis using Raman spectra showed good sensitivity for the detection of EVOO differentiating it from LQOO and other types of vegetable oils. Furthermore, PLSR regression models developed from the same spectra showed excellent correlation (Rval≥0.93) with reference tests and standard error of cross-validation (SECV) of ~1.0% for major fatty acids, 0.9 meqO2/kg for PV, 0.2% for FFA, 0.4% for TPC, and 1.4% for PPP. The fingerprinting capabilities of portable Raman spectroscopy show potential for detection of EVOO adulteration. This technology can provide industry and regulatory agencies with rapid and specific analysis of EVOO through the use of portable/handheld devices to detect ingredient tampering.

Near-Infrared Spectroscopy as a Rapid Screening Technique to Determine Authenticity and Adulteration of Avocado Oil Ariel Bohman*[1], Kathryn J. Lawson-Wood[2], and Hannah Rance[2], *[1]PerkinElmer, USA; [2]PerkinElmer, United Kingdom*

Avocados, and its cold-pressed oil, have become a more prominent ingredient in cuisines worldwide. The high content of healthy oleic acid in the fruit has been linked to improving heart health and reducing cholesterol. Avocado oil, a high-value product, may be adulterated with lower-value oils to increase profit margins. These adulterant oils may include nut-containing oils which, if incorrectly advertised on the avocado oil packaging, may induce a dangerous allergic reaction for consumers with nut allergies. It is, therefore, important that an accurate and reliable adulteration detection method is available for manufacturers to routinely test their avocado oil. Near-infrared spectroscopy, for detection of avocado oil adulteration, offers many advantages over traditional reference methods, such as GC/MS and HPLC, as it is a rapid, non-destructive technique. In this submission, samples of avocado oil and commonly used adulterants have been analyzed in transmission mode over the near-infrared region spanning from 10,000 to 4,000 cm^{-1}. Many existing targeted approaches for adulterant screening, using Near-infrared spectroscopy, require a quantitative calibration to be developed for each potential adulterant. Alternatively, non-targeted screening approaches such as a SIMCA (Soft Independent Modelling of Class Analogy) model can determine whether a sample has been adulterated but will neither identify nor

quantify the adulterant. Adulterant Screen™, a semi-targeted screening method, combines the advantages of both targeted and non-targeted approaches, allowing easy detection and quantitative estimation of adulteration at relevant levels. This work demonstrates the use of near-infrared spectroscopy, coupled with chemometric methods, for the detection of adulterated avocado oils.

ANA-P: Analytical Poster Session

Chair: Ali Reza Fardin-Kia, US Food and Drug Administration, USA

1. Analysis of Acylglycerols in Edible Oils by Gas Chromatography Using a Unique Stationary Phase Colton Myers[1], Kristi Sellers[1], Jana Rousova[2], Joseph D. Konschnik*[3], Shawn Reese[1], Jaap de Zeeuw[1], and Chris Rattray[1],[1]*RESTEK Corporation, USA;* [2]*Restek, USA;* [3]*RESTEK Corporation, USA*

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 by frauds. By mixing different vegetable oils (e.g. rapeseed, sunflower, etc.) with high quality olive oils, manufactures increase their oil yields and make larger profits on these 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 ration of 1,2 to 1,3-diacylglycerols (DAG). By using a unique gas chromatography (GC) stationary phase without bleed interference and retention time shifting due to phase lost, and is able to resolve TAGs and DAGs, 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 and examination of column bleed at high GC operating temperatures.

2. Analysis of trans-Fatty Acids in Food Products Using Various GC Columns Jana Rousova*[1], Joseph D. Konschnik[2], and Chris English[1],[1]*Restek, USA;* [2]*RESTEK Corporation, USA*

Partially hydrogenated oils, which are the main source of artificial trans-fatty acids (TFAs), have been phased out in Europe and the United States due to concerns of their negative effect on human health. Unfortunately, artificial TFAs remain present in the food industry of the rest of the world. Moreover, certain food product have been exempted from the ban, such as frosting. In order to analyze the presence of TFA in US food products we evaluated several fat-containing products: margarine, shortening, butter flavored popcorn and chocolate frosting. The fatty acids were trans-esterified using sodium methoxide and analyzed on multiple Restek columns, namely Rtx-2330, Rt-2560, and FAMEWAX using GC-FID and GC-MS. With the exception of frosting, none of the studied products contained TFAs. However, there a tradeoff. The elimination of partially hydrogenated oils lead to significant increase of saturated acid content in margarine and shortening as compared to previously reported values by USDA. In terms of column selection, the Rt-2560 was the best choice for the separation of C18:1 isomers extracted from chocolate frosting.

3. Optimizing GC-MS Analysis of 3-MCPD and Glycidyl Esters Jana Rousova*[1], Joseph D. Konschnik[2], and Chris English[1], [1]*Restek, USA;* [2]*RESTEK Corporation, USA*

3-MCPD and glycidyl esters in edible oils are contaminants that are formed through refining processes. Some of these substances have been classified as possible human carcinogens. Methods, which are similar to one another, have been developed by ISO, AOCS, and DGF for analyzing these contaminants. While these method cover extraction and derivatization techniques in detail, very little attention is paid to the GC-MS methods. With emerging automated systems it is important to simplify and speed up the method by optimizing the method parameters as well as the advantages and drawbacks of switching to split injection.

4. Profiling of Sugars in Honey by HILIC-MS

Jinchuan Yang*[1], and Paul Rainville[2],[1]*Waters, USA;* [2]*Waters, USA*

Honey is a popular natural sweetener that is consumed by people either directly or as an ingredient in hundreds of manufactured foods. About 95% of honey dry weight are carbohydrates. Other honey constituents include organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds. Monosaccharides fructose and glucose are the main carbohydrates in honey. The rest of carbohydrates are di- and trisaccharides. There are more than 2 dozens of di- and trisaccharides have been identified (Ref 1). The sugar profile in honey has been used for the determination of type and origin of honey, as well as the detection of adulteration. HPLC techniques, such as HILIC and HPAEC, are commonly used for the sugar analysis. Recently a new HILIC-MS method based on Waters BEH Amide column has been developed for the sugar analysis (Ref 2). It has improved separation resolution for sugars, such as a baseline separation of galactose and glucose. Besides the improved separation, the new method also has used a mass spectrometer, Waters QDa mass detector, to further improve the method selectivity and sensitivity. Here we extend this HILIC-MS approach to honey to explore the capability of the BEH Amide column in the challenging honey sugar profiling. The HILIC-MS method has been modified, and the potential benefits of this HILIC-MS method will be investigated. References: 1) Doner LW: The sugars of honey - a review. J Sci Food Agric 28:443-456, 1977. 2) Mark Benvenuti, Gareth Cleland, Jennifer Burgess, Waters application note p/n 720005767en, Waters Corporation, 2016

5. Thermal Degradation of the Natural Anti-oxidant p-hydroxybenzoic Acid (PHBA) in Macadamia Nut Oil, Olive Oil and Corn Oil

Hardy Z. Castada*[1], Sheryl Barringer[2], Zhaoyu Sun[3], and Xuesong Huang[3],[1]*Department of Food Science and Technology / The Ohio State University, USA;* [2]*Department of Food Science and Technology / The Ohio State University, USA;* [3]*Department of Food Science and Engineering, Jinan University, China*

The structure of phenolic compounds determines their antioxidative property in extending oil shelf-life by preventing lipid oxidation and retarding quality deterioration. Monohydroxy p-hydroxybenzoic acid (PHBA) was recently demonstrated to increase the oxidative stability of oils. However, PHBA undergoes thermal decarboxylation producing phenol and CO2(g). Thus when subjected to high temperature processing, (i.e., frying), natural PHBA in oil undergoes thermal decarboxylation and loses its bioactive antioxidant properties. This study aimed to determine the decarboxylation kinetics of PHBA in macadamia nut oil (MNO), olive and corn oil. PHBA degradation were evaluated at temperatures typical for cooking and frying. PBHA headspace concentration was measured using Selected Ion Flow Tube-Mass Spectrometry. PHBA decarboxylation followed a zero order reaction where degradation is affected by the type of oil matrix. Activation energies (Ea) showed that PHBA is relatively more stable from thermal decarboxylation in MNO (85kJ/mol) than in olive (40kJ/mol) or corn (22kJ/mol) oil. The higher enthalpy of decarboxylation in MNO (82kJ/mol) indicated that PHBA is more inhibited from decomposition as compared to olive (37kJ/mol) or corn oil (19kJ/mol). Moreover, the negative entropy values of PHBA degradation from MNO (-192J•mol-1•K-1), olive (-277J•mol-1•K-1), and corn oil (-325J•mol-1•K-1) indicated that these oils impart some inhibitory properties against PHBA thermal decarboxylation.

6. MCPD Esters and Glycidyl Esters in Infant

Formulas: Current Research at the U.S. Food and Drug Administration Jessica K. Beekman*[1], Michael Granvogl[2], and Shaun MacMahon[1],*[1]US Food and Drug Administration, USA; [2]Technical University of Munich, Germany*

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization step of the refining process, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in refined oils and foods a potential health risk. Dietary exposures to bound 3-MCPD and glycidol from consumption of infant formulas are of particular interest because formulas are the sole or primary food source for some infants. Research studies over the last several years have focused on the analysis of these contaminants in refined vegetable oils and other complex food matrices containing these oils (including infant formula) in an effort to estimate levels of exposure. In addition, EU regulations for bound 3-MCPD and glycidol concentrations in infant formula and edible oils highlight the need for accurate analytical methodologies. Current research at the U.S. Food and Drug Administration (FDA) has focused on developing a method for the extraction and analysis of 3-MCPD and glycidyl esters in infant formulas in order to produce occurrence data for the variety of infant formulas found on the U.S. market and worldwide. A detailed schematic of the extraction methodology and the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of these contaminants will be presented. Additionally, the results of several occurrence studies performed between 2015 and 2019, showing a wide range of 3-MCPD and glycidyl ester concentrations across a variety of infant formulas around the world, will be discussed.

7. Quantitation of Trans-Fatty Acids in Humans: An Assessment of Internal Standards John M. Goodwin*[1], Heather C. Kuiper[2], Emily J. Mueller[2], Samuel P. Caudill[2], and Hubert W. Vesper[2],*[1]CDC, USA; [2]CDC, USA*

Quantitation of trans-fatty acids (TFA) in humans and food by GC is commonly performed using internal standards (IS) to compensate for analyte loss during sample preparation. Fatty acid (FA) analysis by GC-FID typically uses odd-chain FA as internal standards; however, studies have shown that such FA do occur in human samples making them unsuitable for use as IS. Isotopically labeled IS in combination with GC-MS or GC-MS/MS can overcome this limitation. The number of ISs needed to appropriately compensate potential loss for the wide range of FAs occurring in blood is unknown. Using the isotope dilution-GC-MS method developed at CDC, which employs 18 IS, we analyzed human plasma samples and assessed the impact of different combinations of IS on analytical accuracy and precision. Linear mixed model analysis was used to estimate the relative error and bias compared to the CDC method IS, in order to assess how different stable isotope labeled standards affect the analytical performance of FA measurements. All possible subsets of the 18 IS were ranked according to relative error and bias estimates from all 27 analytes, resulting in clear relationships between the number and type of IS used for quantitation and the overall analytical accuracy and precision. When analyzing FAs, no single IS was capable of providing the high level of accuracy and precision achieved with all 18 IS, but a subset of the 18 IS were identified that provide analytical performances similar to those observed with the full set.

8. Separation of Trans Fatty Acids in Human Plasma by Silver Ion High-Performance Liquid

Chromatography and Gas Chromatography-Mass Spectrometry Na Wei*, Sarah Kingsley, Heather C. Kuiper, and Hubert W. Vesper, *CDC, USA*

Trans-fatty acids (TFA) are geometric isomers of naturally occurring cis-fatty acids and can be formed industrially via partial hydrogenation of vegetable oils or naturally in ruminant animals, altering the physical properties and biological effects of the fatty acids (FA). TFA intake has been associated with risk factors for cardiovascular disease. Many positional and geometric FA isomers have been reported in humans, but some may not be fully separated with current GC methods. This could lead to inaccurate results for certain fatty acids. Silver ion chromatography has been widely used in the study of FA in oils and dairy products, but has seen limited application to human blood. We developed a method using silver ion high-performance liquid chromatography coupled to a diode array detector to evaluate the GC method specificity for cis- and trans- isomers in human plasma in conjunction with the CDC isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry method. This approach allows us to fully separate TFA from their cis-fatty acid isomers in biological samples, even in the C18:1 region where cis/trans overlap is common. The chromatographic resolution of four major TFAs, palmitelaidic acid, elaidic acid, trans-vaccenic acid, and linoelaidic acid, from their corresponding cis fatty acid isomers in human plasma was confirmed using this silver ion HPLC approach. Additionally, with this approach we are able to separate and detect over 40 cis- and trans-isomers in human plasma.

9. Monitoring the Oxidative Stability of Olive Oils by Electron Spin Resonance Forced Oxidation Assay Matilde Tura[1], Mara Mandrioli[1], Enrico Valli[2], David Barr[3], Manfred Spraul[3], Agnes Haber[3], Alessandra Bendini[4], and Tullia Gallina Toschi*[4],[1]*Alma Mater Studiorum – Università di Bologna, Italy;* [2]*University of Bologna;* [3]*Bruker, USA;* [4]*DISTAL University of Bologna, Italy*

Oxidative stability of olive oils strictly depends on endogenous factors, such as the fatty acid composition and the quali-quantitative profile in natural antioxidants. Moreover, a number of external factors, including oxygen, temperature, light and eventual presence of metals affects it. Oxidative stability is a parameter linked to the quality and freshness of the olive oils, as it is expected to be higher for products more resistant to oxidation, such as extra virgin olive oils, and to decrease during their shelf-life. In this investigation, the oxidation process of several samples belonging to different categories of olive oils (extra virgin, virgin, lampante and refined) was monitored through Oxidative Stability Index test and Electron Spin Resonance forced oxidation assay. The obtained results by the two analytical approaches were examined, compared and discussed. Peroxides, as primary oxidation molecules, and UV spectrophotometric extinction coefficients were also determined in the examined oils. Electron Spin Resonance can be considered a promising analytical tool to monitor the oxidation of olive oils and particularly to estimate the resistance to oxidation.

10. HPTLC in the Analysis of Lipids Maged Sharaf*, *Camag Scientific, Inc., USA*

Objective This presentation will provide an overview of high-performance thin-layer chromatography (HPTLC) as a tool widely used in the analysis of lipids. Methods Used HPTLC is a widely used, fast and relatively inexpensive technique for the separation of complex mixtures, including lipids. The term lipids encompass a vast class of compounds and was defined by William Christie more than three decades ago as fatty acids and their derivatives, and substances related biosynthetically or

functionally to these compounds. Lipids include fatty acids, cholesterol and esters, steroids, oil-soluble vitamins, glycerides, sphingolipids and glycolipids, phospholipids, and phosphoinositides. Results The unique features, advantages and disadvantages of HPTLC as a tool will be shared along with an assortment of applications in lipid analysis. Conclusions The target of this presentation extends beyond information sharing to an invitation for those interested in collaboration to contact the speaker.

11. Comparison of Fat Content between Home Meal Replacement products and Restaurant Foods in Korea Eunji Choi*[1], Jung Eun Lee[1], Soo Jeong Lee[2], Yejin Song[2], and Byung Hee Kim[3],*[1]Sookmyung Women's University, Republic of Korea; [2]Sookmyung Women's University, South Korea; [3]Sookmyung Women's University, Korea*

The aim of this study was to determine the total, saturated, and trans fat content of home meal replacement (HMR) products currently distributed in Korea and to compare the content levels to those of the corresponding restaurant foods in Korea. Eighteen kinds of HMR samples (n = 79) were collected in 2018 and were categorized to four groups, including three of rice (n = 13), three of porridges (n = 13), six of soups (n = 24), and six of stews (n = 29). Total fats were analyzed using Soxhlet method with diethyl ether extraction but without hydrolysis. Saturated and trans fats were determined from the fatty acid profile of the fat extracts obtained by diethyl ether/petroleum ether extraction after acid hydrolysis. Total, saturated, and trans fat content of restaurant foods was obtained from Korea Food Composition Database. No significant difference was found in total, saturated, and trans fat content between nine kinds of HMR products and restaurant foods. One kind of stews had significantly greater trans fat content than the restaurant food, but its total and saturated fat content was not significantly different from that of the restaurant food. Whereas, the total, saturated, or trans fat content of the eight kinds of HMR products was significantly lower than that of restaurant foods. These results suggest that HMR products of rice, porridge, soup, and stew tend to have a similar or lower content level of total, saturated, or trans fat compared to that of the restaurant foods in Korea.

12. Rapid separation of Fatty Acid Methyl Esters with Agilent DB-FastFAME and the Intuvo 9000 GC System Yun Zou[1], Gustavo Serrano Izaguire*[2], and Phil stremple[3],*[1]Agilent, USA; [2]Agilent, USA; [3]Agilent Technologies, USA*

The analysis of oils, fat, and fat containing food is a common task in governmental, quality control (QC), or contract research organization (CRO) laboratories. The GC analysis of fatty acids as their FAME derivatives is an important tool in the characterization of fats in the determination of total fat and trans-fat content in foods. Many regulatory methods for testing foods such as edible oils require separation of specific cis/trans fatty acid isomers using a capillary column coated with a cyanopropyl stationary phase when determining fatty acid composition. In addition, long GC columns (100 m) and long analysis times (more than 70 minutes) are required to achieve good FAME separations. However, this leads to high analysis costs and low productivity. The new Agilent J&W DB-FastFAME Intuvo GC column is a mid-content cyanopropyl phase specifically engineered for the fast separation of FAME mixtures, including some key C18:1 and C18:2 cis/trans isomers. This research work demonstrates that a 4 minutes analysis time is possible for the separation of a complex FAME mix, using the new DB-FastFAME and the Intuvo 9000 GC System.

13. Discrimination of Korean and Chinese Perilla Seeds by Mineral Analyses in Combination with a Multivariate Statistical Method Jung Eun Lee*[1], Soo Jeong Lee[2], Hyang Sook Chun[3], Sangdoo Ahn[3], and Byung Hee Kim[4],[1]*Sookmyung Women's University, Republic of Korea;* [2]*Sookmyung Women's University, South Korea;* [3]*Chung-Ang University, South Korea;* [4]*Sookmyung Women's University, Korea*
Perilla seed is mostly used to obtain an unrefined oil after roasting in Korea. The Chinese perilla seed and oil products which are intentionally labeled as Korean perilla products frequently appear in the Korean markets, because the retail price of Korean perilla products is 1.4–4.9 times higher than Chinese perilla products. The aim of this study was to discriminate between Korean and Chinese perilla seeds using mineral content data obtained by energy dispersive X-ray fluorescence spectrometer (EDXRF) and inductively coupled plasma-optical emission spectrometer (ICP-OES), in combination with orthogonal partial least squares-discriminant analysis (OPLS-DA). The mineral content data were obtained from 29 samples of Korean perilla seeds and 11 samples of Chinese perilla seeds distributed in Korea. Using EDXRF, 11 species of minerals (Mg, P, S, K, Ca, Mn, Fe, Cu, Zn, Rb, and Sr) were detected in perilla seed samples and their content values were measured. The content values of Ca and Rb were selected as the variables that best discriminate between Korean and Chinese perilla seeds using the S-plot generated by OPLS-DA. A blind trial using 22 samples of perilla seeds demonstrated that Korean perilla seeds with added ≥40 wt% of Chinese perilla seeds could be distinguished by applying the range of the content values of Ca (≥3157.1 mg/kg) and Rb (≤7.6 mg/kg) measured by ICP-OES in the Korean perilla seed samples.

14. A Comparative Study on the Change of Quality, Free Radical and Molecular Structure in Different Edible Oils during Deep-frying by EPR and FTIR Spectroscopy Lirong Xu*[1], Qingzhe Jin[2], and Xingguo Wang[2],[1]*jiangnan university, China;* [2]*Jiangnan University, China*
To compare the change of quality, free radical and molecular structure of different edible oils in the course of frying, palm oil (PO), sunflower oil (SuO), soybean oil (SO) and rapeseed oil (RO) with different fatty acid composition were used. Although the conventional indexes including total polar compounds (TPC), peroxide values (PV) and acid values (AV) were used to evaluate the oxidation of frying oils, the radical and molecular structure changes can be measured with the ESR and FTIR spectroscopy during deep-fat frying. As the TPC, AV and PV were increased, suggesting that lipid oxidation happened during the frying process. With the increase of radical signal intensity, lower oxidation stability was presented in edible oils during frying. The FTIR absorbance of FFA ν(C═O) at 1696 cm−1, –HC=CH-(trans) at 968 cm−1, ROOH at nearly 3471 cm−1 of oil samples was increased during frying indicating the deeper degree of thermal oxidative decomposition. Comparison the TPC, PV, AV, free radical signal intensity and FTIR absorbance intensity over deep-frying process the results showed degree of oxidative stability were PO > SuO > SO > RO. Significant correlations were obtained among conventional methods, ESR and FTIR spectroscopy.

15. High Pressure Preparative HPLC for Prepare Individual Tocopherol and Tocopherolquinone Liyou Zheng*[1], Xingguo Wang[2], and Qingzhe Jin[2],[1]*State Key Laboratory of Food Science and Technology Synergetic Innovation Center of Food Safety and Nutrition School of Food Science and Technology , China;* [2]*Jiangnan University, China*
Objective Since the biological activity of α-tocopherol (α-T) is highest among the four

tocopherols, studies upon this aspect are widely reported. In order to investigate the biological activity of another three tocopherols, this study tried to obtain high purity of these substances and their oxidants, such as tocopherolquinones (TQs) with thin layer chromatography (TLC) and high pressure preparative HPLC. Methods Used Mixed tocopherols were dissolved in methanol, and then applied to the high pressure preparative HPLC (both normal and reverse phase). Concerned with purity and volume mobile phase wasted, the reverse phase system was better for separating the individual T. Detailed information of the reverse preparative HPLC included: mobile phase (methanol: water, 98:2), wave length (295 nm), velocity (1 mL/min), ambient temperatures and C18 columns. After tocopherols obtained, they were mixed with ferric chloride to produce TQs using TLC and preparative HPLC to improve purity of the TQs. Results The purity of δ-, β-+γ-, α-T were 99%, 99%, and 98%, respectively. In addition, yield of the upper three tocopherols were 85.6%, 76.0%, and 60.2%, respectively. And the resulting TQs from the tocopherol isomers after reaction with ferric ions were through TLC and preparative HPLC to achieve the purity over 95%, 92%, and 94% for α-TQ, γ-TQ, and δ-TQ, respectively. Conclusions High purity of tocopherol isomers and their oxidant quinones were obtained with the aid of TLC and preparative HPLC in lab scale. The resulting sunstances seem to be used probably as standards in qualitative and quantitative analysis.

16. Valuable Source of Antioxidants from Agri-industrial Waste Stefano Casiraghi*, *VELP Scientific, Inc., USA*

Objective Agro-industrial by-products have a high environmental impact, but they can represent a rich source of bioactive molecules. In the context of a sustainable economy, a way to reduce waste and provide an additional economic value is the introduction of residues in the productive cycle again, to obtain new resources that can be used as new raw materials. Method Waste from different vegetables, such as onion, artichoke, asparagus, cardoon and grapes were submitted to extraction procedures. The content of the occurring carbohydrates and phenolic compounds was analysed by liquid chromatography. Folin-Ciocalteau assay was used for total phenolic content assessment, and Oxitest reactor to measure the oxidative stability of a model matrix enriched with the extracts. Results Microwave assisted extraction was revealed as the best technique to obtain rich extracts. Analyses performed on samples demonstrated the presence of valuable bioactive substances in all waste materials. Artichokes were found to be rich in polyphenolic compounds, as well as grapes and onion wastes. The main molecules occurring were chlorogenic acid and quercetin. The antioxidant power of the extracts was evaluated by Oxitest, measuring the increment in the oxidative stability of vegetable oil when enriched with even small proportion of extracts. Some extracts also contained fructooligosaccharides and inulins, characterized by prebiotic activity and important technological features. Conclusions Agro-industrial by-products can be considered a valuable source of nutraceutical ingredients that may be used as a potential material for the production of functional foods, or in the cosmetic field.

17. Identification of Off-flavor Compounds in Cereals and Oils by HS-SPME Coupled to GC-MS-O Technique Wang Jing*, *Wilmar, China*

Identification of off-flavor in cereals and oils is a key industrial issue. In this paper, off-flavor compounds were evaluated with the headspace gas analysis using a solid phase microextraction (SPME) coupled with GC-MS-olfactometry.

There are four main sources of off-flavor from cereals and oils: 1. In the process of processing, hexanal and 2,4-decadienal were found to be the main “rancidity” sources through fatty acid degradation during the oxidative process. 2. The pollution caused by the packing materials solvent such as BTEX (Toluene, Xylene) and printing ink (acetic acid, butyl ester, 1-methoxy-2-propyl acetate). 3. The pollution caused by environmental conditions such as storage condition, transport environment or market circulation. For example, phenol, 2,4-dichloro- and phenol, 2,6-dichloro- have been detected in packaging cartons in storehouse which caused by spray pesticide pollution. 4. Artificial additive such as ethyl maltol and 2-acetylthiazole, which caused oil more aromatic also lead to off-flavor in oils.

18. Rapid Prediction of Low (< 1%) trans Fat Content by IR Spectroscopy and Chemometric Analysis: Edible Oils and Fast Food Lipid Extracts Magdi Mossoba*, Samantha Farris, and Sanjeewa R. Karunathilaka, *US Food and Drug Administration, USA*

The United States Food and Drug Administration (FDA) ruled that partially hydrogenated oils (PHO), the major dietary source of industrially produced trans fat (TF), were no longer "generally recognized as safe (GRAS)" for any use in human food. This restriction has been in effect in the US since June 18, 2018 and in Canada since September 17, 2018. Consequently, the objective of this study was to develop a rapid screening procedure using attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy in conjunction with partial least squares regression (PLSR) for the quantitative and accurate prediction of low concentrations of trans fatty acids (TFA) (< 1% of total fatty acids (FA)). Broad-based calibration models were developed for a combined set of samples consisting of edible oils and fast food lipid extracts. Predicted concentrations of TFA in the two matrices showed good correlation with the primary reference data generated by gas chromatography (GC) (R2 > 0.99) and high accuracy as evidenced by low root-mean-square error of cross-validation (RMSECV) values. The lowest TFA concentration determined by GC to be 0.13% of total FA, was accurately predicted by ATR - FTIR / PLSR as 0.18% of total FA. This simple, rapid ATR-FTIR/PLSR methodology has the potential for use as a screening alternative to conventional gas chromatographic methods for predicting the TFA content of edible oils and food lipid extracts for regulatory purposes and quality control of raw material and processed food.

19. Simple Methods for the Determination of PAHs and PAEs in Deodorizer Distillates Obtained from Soybean, Rapeseed, Corn and Rice Bran Oils Longkai Shi*, Qingzhe Jin, and Xingguo Wang,*Jiangnan University, China*
There is a lack of data regarding the contents of polycyclic aromatic hydrocarbons (PAHs) and phthalic acid esters (PAEs) in vegetable oil deodorizer distillate (DD), which is a potential starting material for producing natural tocopherol and phytosterol. In this study, simple GC–MS methods for analysising sixteen PAHs and seven PAEs in the DDs were individually established. The LOQs for PAHs and PAEs were ranging from 0.18 to 0.42 μg/kg and 0.19 to 1.50 μg/kg, respectively. The recoveries for DD samples were in the range of 84.8–115.5% and 84.2–109.3% for PAHs and PAEs, respectively. Furthermore, PAHs and PAEs concentrations in soybean, rapeseed, corn and rice bran oil distillates were evaluated. PAHs were found in all the DD samples and the concentrations of BaP, PAH4 and total PAHs were 0.89–55.58, 8.11–326.07 and 115.77–966.40 μg/kg, respectively. Correspondingly, total PAEs concentrations ranged from 2.45 to 24.52 mg/kg, and the mean value was 7.76 mg/kg. The results illustrated that the contents

of PAHs and PAEs in the DDs were extremely higher than those in the edible oils, thus indicating that specific issues should be considered in the vegetable oil DDs and DD-based products.

20. Objective Evaluation of Crispy and Crunchy Textures of Foods by Acoustic Analysis Tae Sugiyama*[1], Yoshihiro Murano[1], Takashi Toyoshima[1], Kinya Tsuchiya[1], and Fumiaki Satoh[2],[1]*The Nisshin OilliO Group, Ltd., Japan;* [2]*Chiba Institute of Technology, Japan*

Various sensory elements combine in a complex manner to create the deliciousness of foods cooked using edible fats and oils. Textures such as crispness, crunchiness, hardness, softness, and fragility are essential in many foods, especially in deep-fried foods, snacks and baked confectioneries. Each food has a characteristic texture, which is evaluated by sensory tests in general. Although food scientists and engineers have attempted objective evaluations of the characteristic textures of different foods, an optimal method for the texture evaluation is yet to be developed. Here, we hypothesized that texture can be evaluated based on the sound created during mastication. Because individual differences exist in terms of human mastication sounds, we developed a device mechanically capable of crushing foods. By combining this food crushing device with a method to analyze the crushing sounds, we aimed to construct an objective system for texture evaluation that can replace sensory evaluation tests. In this study, we evaluated several foods using the developed food crushing device and acoustic analysis. The use of the device facilitated reproducible mechanical crushing of foods. Then, we analyzed the sounds of foods being crushed to determine their physical quantities, such as sound pressure level, and psychoacoustic evaluation quantities, such as loudness and sharpness. Some acoustic analysis results showed good correlation with the sensory evaluation results of texture. In addition, the acoustic analysis results revealed high within-day and between-day reproducibility. Therefore, we propose that the acoustic analysis results could be used as an index for texture evaluation.

21. Efficient method for a simultaneous determination of monochloropropanediol and glycidol in natural glycerin by GCMS Ruifeng Zhang*, Chuan Zhou[1], Wen Ming Cao[2], and Tiankui Yang,[1]*Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd, China, China;* [2]*Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Glycerin is widely used in the food industry, humectant and pharmaceutical formulations. Monochloropropanediol(MCPD) and glycidol are potential to occur in glycerin refined process. A new method based on isotope dilution and gas chromatography-mass spectrometry for the determination of MCPD and glycidol in glycerin has been developed. It can simultaneously detect three analytes by one pretreatment. The analytes were extracted with diethyl ether, glycidol was converted to 3-bromopropane-1, 2-diol(3-MBPD) by acid sodium bromide solution. MCPD and MBPD derived by phenylboronic acid were determined by GCMS. The present research is effective, short and simple in glycerin matrix. The condition of extraction and glycidol converted to 3-MBPD involved pH and concentration of acid sodium bromide solution were optimized. Limit of detection(LOD), Limit of quantitation(LOQ), linearity(concentration from 0.010 to 1.00 mg/kg) and reproducibility were evaluated in method validation. The linearity showed a regression coefficient of greater than 0.999. It is a reliable method for the quantification of monochloropropanediols and glycidol in free form to supervise glycerin security. The method has been successfully

applied to determine MCPD and glycidol in glycerin samples.

22. Simultaneous determination of bisphenol A, alkylphenol and 2-phenylphenol in edible vegetable oil by solid phase extraction and liquid chromatography with tandem mass spectrometry Mingming zhang*[1], Chuan Zhou[2], Ruifeng Zhang, Wen Ming Cao[3], and Yuan Rong Jiang[3],[1]*Wilmar(Shanghai)Biotechnology Research and Development Center Co. Ltd., China;* [2]*Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd, China, China;* [3]*Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Humans are potentially exposed to several environmental phenols such as bisphenol A (BPA), alkylphenols (APs) and 2-phenylphenol (o-PP) which are industrial chemicals or pesticide residues in food. A simple and quick analysis method was established for the determination of four phenol environmental hormones in edible oil by UPLC–MS/MS and solid phase extraction (SPE). The isotopically labeled standards of related contaminants were used as the isotope dilution standards (IDS) to form the following analyte/surrogate pairings: bisphenol A/D_{16}-bisphenolA, 4-n-nonylphenol/ D_8-4-n-nonylphenol, octylphenol/$^{13}C_6$-4-t-octylphenol, and 2-phenylphenol/D_{16}-bisphenolA. The samples were extracted with n-hexane saturated acetonitrile solution and cleaned by C18 solid phase extraction column, and then analyzed by HPLC–MS/MS in the multiple reaction monitoring (MRM) mode. Under the optimal experimental conditions, the calibration curves of four phenol environmental hormones were linear in the range of 0.5~50 μg/L with correlation coefficients more than 0.9998. The limit of detection and the limit of qualification of the method were 0.1 μg/kg and 0.5 μg/kg, respectively. The mean recovery rate of four environmental hormones were 78.8%~111% combining with the relative standard deviations ranging from 0.936~10.6% (n=6). The method was suitable for the identification and quantification of four phenol environmental hormones in edible oil due to its simplicity and accuracy.

23. Stable isotope dilution assays in quantitation of 4-HNE and 4-HHE in vegetables oils by UPLC/MS/MS Chuan Zhou*[1], Hai Ming Shi[2], Junmei Liang[3], Wen Ming Cao[2], and Yuan Rong Jiang[2],[1]*Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd, China, China;* [2]*Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China;* [3]*Wilmar (Shanghai) Biotechnology Research & Development center Co., Ltd, China*

4-hydroxy-2-trans-hexenal (4-HHE) and 4-hydroxy-2-trans-nonenal (4-HNE) are two α, β-unsaturated lipophilic aldehydes generated in the lipid peroxidation of ω-3 or ω-6 polyunsaturated fatty acids (PUFAs). The two compounds have attracted increasing attention for their potential cytotoxicity and mutagenicity. Due to their unstable properties, it is difficult to establish a reliable analytical method for 4-HNE and 4-HHE. In the present study, a simple extraction with hydrous methanol solution, followed by a derivatization with O-(2, 3, 4, 5, 6-Pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) was developed with a higher recovery of 4-HNE and 4-HHE. A stable isotope dilution assay (SIDAs) was applied to quantitate the concentration of 4-HNE and 4-HHE in vegetable oils and frying oils. Ultra performance liquid chromatography-mass spectrometry analysis was performed in Multiple Reaction Monitoring (MRM) mode. The limit of detection (LOD) and limit of quantification (LOQ) for 4-HNE and 4-HHE were 25 and 80 μg/kg, respectively. Quantitative recoveries ranging from 80 to 120% were obtained by the analysis of spiked oil. The established method is very convenient in the analysis of a variety of vegetable oils or frying

oils within 90 min, without compromising sensitivity and reliability. Its usefulness in the quality assessment of frying oils was extensively proven in a variety of specimens of different botanical origin, for food use, subjected to typical storage or accelerated degradation and oxidization conditions. Its simplicity, rapidity and low cost will enable this method to promote vegetable oils and food manufacturers to effectively control the quality of their products.

24. Analysis of a Multi-class Pesticides Panel in Wine and Olive Oil Extracts by LC-MS/MS Illaria Palini[1], Ed George*[2], Charles T. Yang[3], and Debora D. Addona[4], *[1]ISVEA, Italy; [2]Thermo Fisher , USA; [3]Thermo Fisher, USA; [4]Thermo Fisher, Italy*

Increasing food safety concerns with 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. Tandem quadrupole mass spectrometry offers highly sensitive and selective detection in complex matrices. This poster describes a method for the analysis of multi-class pesticide residues in wine and olive oil using liquid chromatography coupled with triple quadrupole mass spectrometry. A multi-residue method was developed for screening (550+) and quantitation of approximately 300 pesticides in a single 15-minute run with polarity switching. One or two ion ratios were used to confirm each analyte (±30%), plus accuracy of retention time within ±0.1min to show robustness of the method which are required for EU SANTE Guidance 11813_2017. This single method was applied to the analysis of pesticides in wine and olive oil extracts. All pesticides analyzed demonstrated 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 a lipid removal cartridge showed good %Rec at concentration levels of 10ppb and 50ppb between 70-120% which is within the SANTE Guidance. Unknown samples of wine and olive oil were also screened to check for other possible pesticides beyond the required target list to confirm that a single multi-method can rapidly identify additional residues using a triple quadrupole. Furthermore, the method was developed using software with built-in workflows for streamlining method development for routine analysis. The experimental results will be discussed in detail.

25. Investigations in Changing Carrier Gas Helium to Hydrogen for GC-FID Analyses of FAMEs with CLAs Deborah L. Chance*[1], Yiyi Li[1], Raad S. Gitan[1], James K. Waters[1], and Thomas P. Mawhinney[2],*[1]University of Missouri, USA; [2]University of Missouri, USA*

With the helium shortage, this study investigated the effects of using hydrogen as carrier gas for routine GC analysis of fatty acid methyl ester mixtures (FAMEs) and common conjugated linoleic acids (CLAs). GC-FID analyses of FAME mixtures and CLA standards (c9/t11; c9/c11; t9/t11; t10/c12) employed a 30 m mid-polarity capillary column (5%-phenyl 95%-dimethylpolysiloxane). Constant flow carrier gas conditions included: (A) 1ml/min He; (B) 1ml/min H2; and (C) 2.5 ml/min H2. He as carrier gas (A), provided ~20 psi constant inlet head pressure, linear velocity (μ) of ~ 30 cm/sec at 180◦C, and complete baseline resolution of CLAs and 26 bacterial mixture FAMEs. CLAs eluted (RT range ~ 23 min) between C18:0 and C19:0 FAMEs. H2 at 1ml/min (B), yielded inlet ~10 psi, μ ~38 cm/sec; CLA RT shifts to ~ 1.3 min earlier; comparable chromatographic profiles and resolutions for most FAME peaks. CLAs RTs > C18:1 isomers and C18:0 and < C19:0. Early C11:0 and 2-OH C10:0 FAMEs were not well resolved with B. Increasing H2 FR to 2.5 cm/sec

(C) provided ~20 psi at inlet (comparable to A) and μ at 180◦C ~72 cm/sec. This markedly improved C11:0 and 2-OH C10:0 FAMEs' baseline separation (~50% overlap to > 95% resolved). All FAMEs and CLAs were well resolved and a previously undetected peak was evident amongst the CLAs. In conclusion, converting from He to H2 as carrier gas for FAME analyses of these complex mixtures was favorable and made convenient by maintaining consistent inlet pressures between carrier gases.

26. Identification of Major Volatile Components in Olive Oil by SPME-LTM-GC/MS

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Virgin olive oil is a high value oil. In recent years, claims have been made regarding the substitution of olive oil with edible oils of lower commercial value. In general, virgin olive oil is preferably used for its unique taste and aroma for culinary purposes. The volatile aroma components of virgin olive oil are the constituents responsible for its distinctive sensory impression. It has been reported that the chemical structures of these organic components are very diverse. Most of these chemical constituents are classified as alcohols, aldehydes, hydrocarbons and short chain fatty acids. Interestingly, many of these compounds are subject to degradation due to the ripeness of the olive fruit, the processing techniques and the storage conditions of the oil. In this work, the determination of the aroma composition and the level of their oxidation products is investigated as a marker for a low quality or adulterated virgin olive oil. More than twenty virgin olive oils from the US market and twelve authenticated virgin olive oils from California, were analyzed by solid phase microextraction-low thermal mass-gas chromatography/mass spectrometry. Our results confirmed the presence of fourteen major constituents, where 2-Hexenal was the most abundant compound in the market samples and 3-Hexen-1-ol, 1-Hexanol and 3-Hexenyl acetate were the top three components in the authenticated virgin olive oils.

27. Olive Oil Triglycerides Analysis Exploiting Multidimensional Liquid-gas Chromatography Coupled to Isotope Ratio and Quadrupole Mass Spectrometry Simultaneous Detection

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The triglycerides analysis is often used for the detection of the adulteration of olive oil. The profile is characteristic for each kind of oil, as it depends on the fatty acid composition and on the biosynthesis rules, so that it can be used to check for the presence of extraneous oils. However, the quality of extra-virgin olive oil greatly depends also by the plant cultivar and geographical origin. Isotope Ratio Mass Spectrometry (IRMS) is commonly recognized to be able to provide information about the geographical, chemical, and biological origins of substances on the bases of isotopic abundances of the elements that comprise the material. Commonly a separation is performed prior to isotope ratio analysis using LC or GC techniques. However, for highly complex samples a single chromatographic step could not be effective for the complete purification of target components. Multidimensional chromatographic approaches could enhance the purification power of specific fractions before the detection. LC coupled to GC is a powerful technique when a pre-separation step is required aiming to remove non-volatile components or/and to reduce the complexity of a sample. Applied to olive oil triglycerides, the RP-LC step provides the pre-separation of the fraction based on the separation number (SN) depending on the total carbon number subtracting two for each double bond. The

resulting fractions show a greatly reduced complexity avoiding coelution problems respect to direct GC analysis. The present work deals with the development of an LC-GC-MS/IRMS prototype characterized by the improved resolution of the heart-cut mode. Two different chromatographic mechanisms were employed with simultaneous qMS and IRMS detection. After the LC pre-separation, fast GC was applied for the separation of each fraction transferred before the IRMS/qMS step providing quali/quantitative and isotopic ratio information (δ13C). Different origin and cultivar olive oils were analyzed and the results are here presented.

28. Reliable identification of intact lipids by high efficiency liquid chromatography techniques and a novel linear retention index database Danilo Sciarrone*, *Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Italy*

Reliable Identification of Intact Lipids by High Efficiency Liquid Chromatography Techniques and a Novel Linear Retention Index Database. Francesca Rigano1, Danilo Sciarrone2*, Marianna Oteri2, Paola Dugo1,2,3, and Luigi Mondello1,2,3, 1Chromaleont s.r.l., Italy, 2Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Italy, 3Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Italy
Lipidomics is the metabolomics branch that studies lipids within a living system, aiming to determine specific markers of pathologies or inflammations. Gas chromatography (GC) methods, which focus on typical ratio between specific components like the n-6:n-3 polyunsaturated fatty acids, are widely accepted within the scientific community. Recently, the lipidomics approach opened new insight toward the determination of holistic lipid profiles. In fact, monitoring intact lipids can reflect more deeply the dysregulation of lipid metabolism in response to exogenous stimuli and provide elucidations on the perturbation of essential metabolic processes in which each species is involved. Ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) represents the most employed technique in lipidomics. Nevertheless, achieving a fast, exhaustive and reliable identification is still a challenge, due to the not repeatable and poorly informative nature of atmospheric pressure ionization (API) MS techniques, normally hyphenated to LC, that avoid the building and the widespread use of LC-MS databases. In the present research, a novel linear retention index (LRI) system is proposed as alternative identification method, to be applied as stand-alone tool, as well as in combination with MS data. For such a purpose, the chromatographic resolution acquires primary importance, since the identification will be mostly based on the elution properties. After the building of a database containing more than 200 lipids, different biological and food samples were identified only on the basis of LRIs with a tolerance of ±15 LRI units with respect to the tabulated values, leading to an automatic and correct peak assignment for the majority of analytes.

29. Development of a fully automatic analytical method for the evaluation of fatty acids in human blood spotted on DBS paper by gas chromatography Giuseppe Micalizzi[1], Danilo Sciarrone[1], Luigi Mondello [1,2,3], [1]Dipartimento di Scienze Chimiche, Biologiche, Farmaceutiche ed Ambientali, University of Messina, Italy, [2]Chromaleont s.r.l., Messina, Italy, [3]Unit of Food Science and Nutrition, Department of Medicine, University Campus Bio-Medico of Rome, Italy

The complete elucidation of fatty acid methyl esters (FAMEs) in human blood is a useful and routine tool to understand health

state of a patient. In fact, the lipidomic investigation offers necessary information for the prevention and the care of the individual considering the fundamental functions that the fatty acids perform in organism. The goal of this research work has been the development of a simply and versatile analytical strategy for the complete characterization of fatty acids profile of whole blood. Developed method uses a direct derivatization procedure that not requires the long and laborious steps of liquid-liquid extraction. Sodium methoxyde (MeONa) in methanol and boron trifluoride in methanol (BF3) have been used as derivatizing agents. The efficacy of developed protocol has been tested evaluating the fatty acid methyl esters profile of NIST SRM-1950, "Metabolites in human plasma", by using GC-MS and GC-FID instruments. An innovative bio-sampling method, namely Dried Blood Spot (DBS), has been introduced in the protocol. It not requires health personnel and medical facilities; compared to the conventional bio-sample collection techniques, DBS are less expensive in term of time and money. AOC-6000 SHIMADZU preparative station has been used to perform totally automatic sample preparation. The robotic instrument is equipped with different syringes for the addition of the derivatizing agents, vortex and oven (hot derivatization) to convert the circulating lipid in FAMEs. For fatty acid analysis is sufficient a single blood drop (ca. 50 μL) by punching fingertips dried on DBS support. This is the first quantitative approach for the global FAMEs characterization of SRM-1950 spotted on DBS paper. This analytical strategy may facilitate studies devoted to large-scale lipid investigation.

30. Evolution of the Orbitide Gene Family in Cultivated Flax (Linum usitatissimum L.) Ziliang Song*[1], Timothy Sharbel[2], and Martin Reaney[2],[1]*University of Saskatchewan, Canada;* [2]*1Department of Plant Sciences, University of Saskatchewan, Canada*

Flax (Linum usitatissimum L.) produces non-polar cyclic peptides (orbitides) mainly in the seed, conferring bitter flavor to the seed oil. With known biological activity such as immunosuppressive and anticancer effects, the flaxseed orbitides are attractive genetic engineering targets for pharmaceutical production. However, only 28 variant forms of orbitides for which the linear precursors are encoded by 4 genes have been discovered in flax thus far by a bottom-up chemical approach. High sequence diversity of these orbitides potentially implicates a much larger and more diverse gene family. Using the characteristic flanking residues and the repetitive pattern of previously known gene sequences as probes, genome mining has identified 41 loci encoding 413 putative orbitide domains in the genome of flax cultivar "CDC Bethune". These loci display consistent gene architecture, consisting of a variable repeat-containing region flanked by an upstream leader sequence and a downstream follower sequence. Phylogenetic analysis of the flanking regions has clustered the 41 loci into multiple gene subfamilies of which the paralogues were the result of gene duplication. In contrast to the highly conserved flanking regions, variation in the sequence and number of repeat units within the orbitide-encoding region suggests independent evolution of the repeats after gene duplication. Each repeat motif was shown to encode a non-polar segment (orbitide) followed by a polar segment (spacer). The variability and periodicity of repeats represent a complex model of gene evolution. Understanding the gene family from an evolutionary perspective will lay a foundation for functional studies and utilization of orbitides across species.

31. Classification of EVOO varieties; quantitation of blend ratios and detection of adulteration using A-TEEM, a novel

spectroscopic technique Karoly Cstorday*, Horiba Scientific, Knightsbridge Road Piscataway NJ 08854.

Extra Virgin Olive oils and vegetable oils were classified using A-TEEM spectroscopy based on fluorescence excitation and emission matrices. The obtained spectroscopic fingerprints allowed the clear distinction between all the oils, and specifically the varietals of Extra Virgin Olive oils, as well as the lot-to lot differences within the same product of a well-known brand. Quantitation of blends of single varietals as well as of EVOO's with refined oil of the same brand is shown to be possible with high precision.