ANA 1a: Spectroscopic, Spectrometric and Chemometric Methods for Lipid Analysis

Chairs: *Sanjeewa Karunathilaka, US Food and Drug Administration, USA; and Bernd W.K. Diehl, Spectral Service AG, Germany*

Portable Raman Spectroscopy and Chemometric Methods for the Analysis of Marine Oil Dietary Supplements Betsy J. Yakes*, Sanjeewa R. Karunathilaka, Kyungeun Lee, Lea Brückner, and Magdi Mossoba, *US Food and Drug Administration, USA*

Marine oil supplements containing long-chain omega-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are the most commonly used nonvitamin, nonmineral dietary supplements in United States due to their potential health benefits. While established methods such GC-FID excel at analysis of omega-3 PUFAs, there is a need for rapid and accurate screening of these omega-3 PUFA products to ensure both quality and accuracy of label declaration. As such, Raman spectroscopy using portable instruments followed by chemometric analysis was performed to understand the potential for rapid, on-site evaluation of these supplements for fatty acid content. For this study, 109 marine oils were procured, and spectra acquired for each neat (underivatized) oil on three Raman devices: (1) a portable Raman spectrometer with a unique laser setup and data processing to decrease fluorescence interference, (2) a handheld Raman analyzer with onboard chemometrics for immediate pass/fail triaging of complex samples, and (3) a smart-phone sized Raman spectrometer containing orbital raster scanning that allows for larger sampling areas. For each instrument, a broad-based calibration library comprised of a wide variety of marine oils was made using partial least-squares regression (PLSR) to predict fatty acid composition. The current study evaluates the capabilities of each Raman spectrometer for the simple, rapid analysis of marine oil products for potential use in verifying label declarations and quality control during dietary supplement production.

Vibrational Spectroscopy and Chemometric Procedures for the Rapid Assessment of Olive Oil Authenticity Magdi Mossoba*, Sanjeewa R. Karunathilaka, Cynthia Srigley, Kyungeun Lee, Lea Brückner, and Betsy J. Yakes, *US Food and Drug Administration, USA*

FDA is mandated with protection of the US public against intentional adulteration of foods for economically motivated gain, including having jurisdiction over deceptive label declarations found with adulterated extra virgin olive oil (EVOO). In April 2016, a US Congressional Committee expressed concerns related to reports of the high prevalence of imported olive oil sold in the US that is adulterated or mislabeled. Such fraudulence reportedly includes the mixing of EVOO with seed oils, which could adversely impact the health of consumers who are allergic to seed oil. This issue underscores the urgent need for more rigorous analytical methodologies, including untargeted rapid screening tools, for detecting such fraudulence. We have applied rapid vibrational spectroscopy and chemometric procedures to the screening of 72 retail commercial products labeled extra virgin olive oil for the classification, and/or prediction of volatile content, fatty acid composition, and the type and

quantity of a refined edible oil potentially mixed with authentic EVOO. The current spectroscopic/chemometric predictions will be compared to those based on the International Olive Council (IOC) official method COI/T.20/Doc. No 20/Rev. 3 (2010) for the determination of olive oil purity; this method is based on the targeted determination of the absolute difference between the experimental HPLC values for TAGs with equivalent carbon number 42 (ECN42_HPLC) and the theoretical value for TAGs with an equivalent carbon number of 42 (ECN 42_theoretical) calculated from GC-based fatty acid composition.

Automated Multicomponent Phospholipid Analysis Using ^{31}p NMR Spectroscopy: Example of Vegetable Lecithin and Krill Oil Bernd W.K. Diehl*, and Yulia B. Monakhova, *Spectral Service AG, Germany*

Nuclear magnetic resonance spectroscopy (NMR) is widely applied in the field of metabolomics due to its quantitative nature and the reproducibility of data generated. However; one of the main challenges in routine analysis by NMR is to obtain valuable information from large datasets of raw data in a high throughput, automatic, and reproducible way. In this study, a method to automatically annotate and quantify 12 phospholipids (PLs) in vegetable lecithin (soy, sunflower, raps) and krill oil is introduced. Automated routines were written in MATLAB environment for quantification of phosphatidylcholine (PC), phosphatidylinositol (PI), lyso-phosphatidylcholine (LPC), phosphatidylserine (PS), phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and lyso-phosphatidylethanolamine (LPE) in lecithin and of PC, PC-ether, LPC, PE, APE, LPE in krill oil matrix. The routine includes NMR spectra import, extraction of data points, peaking of minima and maxima in the data, integration, quantitation against internal standard, reporting of results as Word file and their importing in our internal database. Our extensive studies on a representative set of more than 1000 lecithin (soy, raps, sunflower) and krill samples showed that the automated routine can automatically and accurately calculate the concentrations of all PLs. No systematic or proportional differences between automated and manual evaluation were detected. The developed program produces accurate results with the advantage of being fully automated and requires less than 5 seconds for each analysis. This tool is already used in high-throughput PL analysis of krill and lecithin and will be adjusted to other matrices (egg, milk, chocolate, etc.) as well.

Analysis and Detection of Olive Oil Adulteration using Fourier Transform Near-Infrared Spectroscopy Ariel Bohman*[1], Kathryn J. Lawson-Wood[2], and Robert Packer[1], *[1]PerkinElmer, USA; [2]PerkinElmer, United Kingdom*

Olive oil has seen a large increase in market demand due to its linkage with lowering one's risk of heart disease associated with its high monounsaturated fat content. As the demand and value of olive oil increases, less reputable producers look for ways to cut costs and increase profit margins resulting in the substitution or dilution of olive oil with less expensive edible oils. Economically motivated adulteration is the intentional addition of lower value substances to a product to increase company profit margins. The prevalence of adulterated olive oil has been

increasing with a reported 80% of Italian olive oil being considered fraudulent. Near-infrared spectroscopy, for detection of olive oil adulteration, offers many advantages over traditional reference methods, such as GC/MS and HPLC, as it is a rapid, non-destructive technique. Samples of olive oil and commonly used adulterants have been analyzed in transmission mode over the near-infrared region spanning from 14,000 to 4,000 cm^{-1}. Near-infrared spectroscopy coupled with chemometric analysis methods, such as soft independent modelling of class analogy (SIMCA), is capable of successfully distinguishing between olive oil and edible oils commonly used in the adulteration of olive oil. The objective of this work is to demonstrate the utility of near-infrared spectroscopy coupled with chemometric methods in the detection of adulterated olive oils. Analysis of transmission spectra of pure and adulterated olive oil samples will be presented to highlight the benefits of near-infrared spectroscopy as a rapid, non-destructive alternative to traditional screening methods for the detection of olive oil adulteration.

ANA 1b: Lipidomic Analysis

Chairs: *Francesca Giuffrida, Nestec SA, Switzerland; and J. David Pinkston, Kellogg Co., USA*

Lipidomic Profiling–An Integral Technology for Research and Development Elizaveta Freinkman*, *Metabolon, Inc., USA*

Lipids are a diverse class of metabolites that serve many biological functions such as energy storage, structural components of cell membranes, and signaling. Accurate measurements of lipids are essential for biomarker discovery and for clarifying biological questions. This presentation will cover Metabolon's different offerings pertaining to lipid research, and these include the complex lipid panel, sebum lipid panel, stratum corneum lipid panel, and the global metabolomic profiling. The applications of lipidomic profiling in research will be exemplified by case studies. Surveyor, the complex lipid data visualization and analysis software tool will be briefly discussed as well.

Non-targeted Analysis for Quality and Authenticity Determination of Olive Oil. James A. Donarski[1], Victoria Bailey-Horne[1], Enrico Valli[2], Diego L. García González[3], and Tullia G.T. Gallina Toschi[4], *[1]Fera Science Ltd., UK; [2]University of Bologna; [3]Instituto de la Grasa (CSIC), Spain; [4]Alma Mater Studiorum—University of Bologna, Italy*

Europe is currently the largest producer of olive oil accounting for more than 70% of the world's production. Non-EU countries are expanding their domestic production and increasing the competitiveness of the global olive oil market. The high price of olive oil, the distinctive sensory profile and its reputation as a healthy source of dietary fats makes olive oil a target for adulteration by illegal blending with other vegetable oils and deliberate mislabelling. The lack of efficient and harmonised analytical methods for detecting olive oil fraud has led to significant weaknesses that are exploited by counterfeiters. As a result, olive oil adulteration for the purpose of financial gain has become one of the biggest sources of agricultural fraud in the EU. OLEUM is a collaborative 4 years EU project (September 2016-2020) with 20 partners from 15 countries. The project has 3 strategic objectives i) develop new and/or improved analytical methods, ii) create the OLEUM Databank and iii) establish the OLEUM Network. In this context, Fera has developed analytical methods to evaluate the quality and authenticity for a large data set of olive oils. The quality will be determined through volatile analysis with a focus on the relevant sensory defects (fusty, muddy-sediment, etc.) by a thermal desorption unit gas chromatography time of flight mass spectrometer. The authenticity analysis will be based on phenolic compounds determined by liquid chromatography time of flight mass spectrometry (LC-TOF-MS) as a fingerprint profile. This presentation will give an overview of these developed non-targeted analytical methods, the related preliminary results and statistical data analysis.

Supercritical Chromatography in Lipidomics Applications: "Finally Ready for Prime Time?"

Paolo Lecchi[1], Yao Lu[1], Erwin Kaal[2], Rob Van der Hoeven[2], and Dominik Burger[1], *[1]DSM Nutritional Products, USA; [2]DSM Food Specialties, The Netherlands*

Supercritical Fluid Chromatography (SFC) has all the premises for being an ideal component of analytical methods aim at characterizing complex lipid matrices. For instance, SFC has an operating range that suits the polarities of most of the lipid structures and it allows faster separations with lower operating costs. Moreover, SFC has been successfully interfaced to a variety of detectors, such as mass spectrometers equipped with ion sources operating at atmospheric pressure. Despite these positive aspects, SFC is still not frequently used in lipidomics applications. We will present insights and considerations on the benefits and pitfalls encountered when implementing analytical methods that include SFC for the analysis of lipid extracts. This presentation will also include a direct comparison between SFC-MS and other workflows for lipidomics, and will emphasize the pros and cons of using SFC separation in MS-based analytical platforms for the comprehensive analysis of lipids.

A Rapid Non-destructive Method for Determining Quality Parameters of Edible Oils

Kathryn J. Lawson-Wood*[1], Ariel Bohman[2], and Robert Packer[2], *[1]PerkinElmer, United Kingdom; [2]PerkinElmer, USA*

Edible oils are an integral part of human diets; being using in virtually all types of culinary practices. The global market for edible oils is expected to exceed 200 million metric tons by 2020, thus simple analysis of oil quality is essential to maintain process efficiency. Various parameters are used to assess edible oil quality, including iodine value (IV), free fatty acid (FFA) content, and numerous other parameters, as demonstrated in this submission. Traditional methods of analysis are typically carried out using standardized methods approved by the American Oil Chemists' Society (AOCS) and/or the Germany Society for Fat Science (DGF). However, these methods usually determine only one particular parameter and analysis is often time-consuming. Additionally, sample preparation can be extensive, with hazardous solvents and reagents being required. Near-Infrared (NIR) spectroscopy is an already widely used technique in the food industry for quantitative analysis of nutritional and quality parameters. Chemometric techniques, such as partial least squares (PLS) used in conjunction with NIR spectroscopy, allow multivariate analysis of samples by building a library of standards of pre-determined property values. In this submission, NIR spectroscopy with PLS calibrations for the determination of quality parameters in various edible oils is demonstrated. Oil samples are simply pipetted into a fixed pathlength glass vial and measured in the transmission sample compartment of the spectrometer. Analysis is rapid, non-destructive, simple to undertake and allows multiple variables to be determined instantaneously.

ANA 2a: Analysis of Fats and Oils Applying Advanced Lipid Analysis Techniques

Chairs: *William C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA; and Walter Vetter, University of Hohenheim, Germany*

Use of Countercurrent Chromatography (CCC) for the Preparative Isolation of Lipid Compounds Walter Vetter*, Marco Müller, Katharina Wasmer, Andrea Goncalves Peca, and Medisa Muric, *University of Hohenheim, Germany*

Objective. Countercurrent chromatography (CCC) is a preparative, all-liquid based chromatographic method predominantly used for the isolation of natural compounds. Separation is obtained by means of two immiscible liquids, i.e. the solvent system, inside a hollow tube. The solvent system is permanently mixed and de-mixed inside the so-called CCC centrifuge which substitutes the column of an otherwise similar periphery of CCC and HPLC. Development of suitable solvent systems is the key-factor for successful CCC applications. Limitations in the use of CCC exist especially in the nonpolar range of lipid compounds. Methods Used. During the last years we have developed solvent systems (e.g. the BTF system) and used new or rare CCC modes (heart-cut-2D-CCC, recycling mode, co-current mode, multiple injection mode) for the isolation of different lipid compounds. Results. Typically, ~1 g lipid sample was injected and ca. 5–100 mg of the target compound were isolated per run (run time ~1–3 h), in dependence of its contribution to the sample and the required purity. Recent successful applications (isolation of tocotrienols, tocomonoenols, amyrins, furan fatty acids, etc.) will be shown with focus on the proper selection of starting materials, solvent systems and special CCC modes. As a further benefit, CCC fractionation allows to detect minor lipid compounds which are usually overlooked. Conclusion. CCC can be successfully used for the isolation of valuable lipid compounds in the medium mg-range (per run). Isolated lipid compounds can be used to investigate their biological activity or as reference standards for quantitative analyses.

Investigation of Olive Oil Substitution with Other Edible Oils by Ultra High-Performance Liquid Chromatography Separation of Triglycerides Pierluigi Delmonte* and Andrea Milani, *US Food and Drug Administration, USA*

In recent years several claims have been made regarding the substitution in the marketplace of olive oil with edible oils of lower commercial value. Olive oil, produced from the pulp of the fruit, is characterized by a distinctive triglycerides (TAG) composition compared to seed oils. The difference between the theoretical and experimentally determined content in TAG with equivalent carbon number (ECN) 42 is the most widely applied parameter to detect this economic adulteration. The incomplete TAG separation provided by traditional high-performance liquid chromatography (HPLC) conditions applied to the determination of the delta-ECN 42 value limits the ability of detecting sophisticated frauds. In this work the TAG separation is achieved by utilizing multiple ultra-high-performance liquid chromatography (UPLC) separation columns in series, obtaining the separation of almost all major TAGs contained in olive oil. Identification of individual TAGs separated by UPLC is achieved by LC-GC in offline mode. The detailed TAG compositions of

available reference olive oils and potential seed oils adulterants allow the determination of more sophisticated parameters beyond the delta-ECN 42 for detecting the partial or complete substitution of olive oil with other edible oils.

Comprehensive Dual Liquid Chromatography with Quadruple Mass Spectrometry, LC2MS4, for *Jacaranda Mimosifolia* Triacylglycerols

William C. Byrdwell*, *USDA, ARS, BHNRC, FCMDL, USA*

Online comprehensive two-dimensional liquid chromatography has been combined with quadruple parallel mass spectrometry for LC1MS2 x LC1MS2 = LC2MS4 experiments. Two mass spectrometers are used for qualitative and quantitative analysis during non-aqueous reversed-phase liquid chromatography (NARP-LC) as the first dimension, in addition to a UV detector, fluorescence detector (FLD), evaporative light-scattering detector (ELSD), and corona charged aerosol detector (CAD). High-resolution accurate-mass (HRAM) electrospray ionization mass spectrometry (ESI-MS) on an Orbitrap instrument was used in parallel with atmospheric pressure chemical ionization (APCI) MS on a tandem sector quadrupole (TSQ) instrument in the first dimension. Two additional mass spectrometers plus UV detection are used for qualitative analysis of the second dimension, which employs a lab-made silver-ion column for UHPLC. Atmospheric pressure photoionization (APPI) TSQ MS and ESI-MS were used in parallel for the second dimension. In the first dimension, diacylglycerols (DAGs) and triacylglycerols (TAGs) in *Jacaranda mimosifolia* (blue jacaranda) are reported as relative percentage composition, while alpha-tocopherols and gamma-tocopherol are quantified by calibration lines, using d_6-alpha-tocopherol as the internal standard. In the second dimension, TAG isomers are separated according to double bond-containing fatty acids in different positions on the glycerol backbone (i.e., regioisomers).

Development of Lipidomics-based Reference Materials and Reference Data for Oils

John Bowden*, *National Institute of Standards and Technology, USA*

With a growing number of new lipidomic users, instrumentation, and applications, efforts aimed to dissect the finer details of lipid measurement and improve community-wide harmonization are imperative. This presentation will highlight recent efforts by the National Institute of Standards and Technology (NIST) to improve lipidomic measurement by promoting the creation and implementation of oil-based reference materials and reference data. Initial efforts have focused on human health and consumer-based reference materials. Using Standard Reference Material (SRM) 2378 (serum from donors who took fish or flaxseed oil supplements), an interlaboratory comparison exercise for lipidomics was executed, resulting in determining the extent of agreement present in current lipidomic measurement within the community, the formation of consensus means with associated uncertainties for lipids present, and the identification of challenges associated with current lipid measurement. The presentation will also highlight new efforts to provide reference materials and reference data for the analysis of olive, argan, and krill oil. The utility of high resolution mass spectrometry-based lipidomics will be addressed for the characterization of oils, in concert with the

formation and community-wide application of oil-based "sample-method-data" products.

A Rapid and Efficient Method for Lipid Separation with Supercritical Fluid Chromatography Coupled with High Resolution Mass Spectrometry. Sheher Moshin, Agilent Technologies, USA

A method was developed for rapid separation of a broad range of lipids based on Supercritical Fluid Chromatography (SFC) and Electrospray Ionization (ESI) Quadrupole Time of Flight Mass Spectrometry. The method allows separation of a broad range of lipid classes in less than 15 minutes. This approach offers distinct advantages of comparative UHPLC methods in terms of speed and resolution.

Results will be shown for optimization of separation, acquisition and detection parameters. Several columns including the new Hilic Zwetterion column, mobile phases, mobile phase additives, flow rates and column temperatures have been evaluated for best inter/intra class lipid separation. Data acquisition parameters with data dependent acquisition of lipid samples have been evaluated with the goal of maximizing the number of detectable lipids.

Results will be presented for biological samples. The entire workflow including data analysis with feature extraction, spectral identification with Simlipid and statistical analysis with Agilent Mass Profiler Professional will be shown.

The Hybrid Search: A New Mass Spectral Library Search Approach for Compound Classification. Arun S. Moorthy[1], Brian T. Cooper[2], William E. Wallace[1], and Stephen E. Stein[1], *[1]National Institute of Standards and Technology, USA; [2]University of North Carolina at Charlotte, USA*

Compound identification is a key task across numerous chemical application areas, including but not limited to food, pharmaceutical, petroleum and lipidomics. Mass spectral library searching has become one of the primary analytical techniques employed in compound identification. The limitation of most library search algorithms is the requirement that the mass spectral database contain a representative spectrum of the query compound (analyte). This limitation is particularly restrictive when novel molecules are synthesized (or discovered in the case of lipidomics) more rapidly than they can be added to mass spectral libraries. In this presentation, we describe the new "Hybrid Search" method that combines fragment-ion and neutral-loss matching when computing similarity match factors. In doing so, the search algorithm generates high scores for compounds that differ by a single chemical modification yet share similar fragmentation behavior (chemical *cognates*). Accordingly, if an analyte is NOT contained within the library, it can still be correctly classified given that its chemical cognates are contained in the library. Following a detailed description of the algorithm, numerous example applications are presented, including the identification of glycerophospholipids.

ANA 2b: Olive Oil, including Sensory Analysis

Chairs: Selina C. Wang, University of California-Davis, Olive Center, USA; and Susan Seegers, Bunge North America, USA

Contribution of Flavor Compounds to Explain New Sensory Defects in Virgin Olive Oil: The Example of "Frostbitten Olives" Diego L. García González[1], Inmaculada Romero[1], Ramón Aparicio-Ruiz[1], Noelia Tena[1], Ana Lobo[1], María Teresa Morales[2], and Aparicio Ramón[1], *[1]Instituto de la Grasa (CSIC), Spain; [2]University of Seville, Spain*

The aroma of virgin olive oil determines, together with other parameters, the quality designation and therefore its price in the market. The strict control of virgin olive oil by means of the organoleptic evaluation has contributed to the fact that the quality of this product on the market has reached high standards. The current knowledge on volatile compounds and sensory analysis leads to new opportunities to improve the performance of panel tests and to understand why some sensory defects are sometimes present and the technological reasons for them. An extensive research has been carried out on the characterization of those volatiles responsible for the main sensory defects (rancid, fusty, mustiness-humidity, winey-vinegary). Other sensory defects, which were less usual only a few decades ago, are becoming much more common today due to a change in the weather pattern in the last few years. One of them is the off-flavor called "frostbitten olives" produced as a consequence of freeze injuries in olives. In this study we address the analysis of the volatile composition of virgin olive oils characterized with this defect. The volatile information allowed characterizing grouping these oils into two types, one of them characterized with "soapy" and "strawberry-like" perceptions, and the other type characterized with "wood" and "humidity" descriptors. Different volatile compounds explained these differences.

The Profitable Relation between Sensory and Analytics in Virgin Olive Oil Quality Detection. Tullia Gallina Toschi[1], Sara Barbieri[1], Chiara Cevoli[1], Ole Winkelmann[2], Karolina Brkić Bubola[3], Florence Lacoste[4], Milena Bučar-Miklavčič[5], Ummuhan Tibet[6], Ramón Aparicio-Ruiz[7], Diego L. García González[7], and Alessandra Bendini[1], *[1]DISTAL University of Bologna, Italy; [2]Eurofins Analytik GmbH, Germany; [3]Institute of Agriculture and Tourism, Croatia; [4]Institut des Corps Gras, France; [5]Science and Research Centre Koper, Slovenia; [6]Ulusal Zeytin ve Zeytinyağı Konseyi, Turkey; [7]Instituto de la Grasa (CSIC), Spain*

To meet the expectations of the consumers and preserve the image of virgin olive oils (VOOs), it is necessary to guarantee its quality and authenticity. Despite many methods to detect VOO fraud and assess its quality are available at international level, the need of supporting the sensory analysis was reported in the call H2020-SFS-14a-2014 and is one of the main objective of the OLEUM project. Specifically, the setting up of rapid instrumental methods could represent a solution for achieving a preliminary screening of a high number of samples and/or for supporting the sensory panels in the discrimination of boundaries samples. In this research, a set of 180 VOOs, sampled during the first year of the OLEUM project, was sensory assessed (EU Reg. 1227/2016). The sensory

evaluation was performed by 6 sensory panels, having different nationality and experience. The headspace volatile profiles of this set, analyzed by many chromatographic techniques, were also analyzed by flash gas chromatography electronic nose (FGC E-nose). The chromatograms were elaborated by multivariate statistical analysis to develop a classification model able to quickly discriminate between "extra virgin" and "no extra virgin" olive oils. This approach could reduce the number of samples to be assessed by a sensory panel and decrease the number of cases giving rise to an uncertain or debated classification. This work was 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, G.A no. 635690).

Deep Insight Into the Minor Fraction of Virgin Olive Oil by Using New LC-MS and GC-MS Multi-class Methodologies: Application to Discriminate Samples from Different Protected Designations of Origin Alegria Carrasco-Pancorbo[1], Lucía Olmo-García[1], Juan J. Polari[2], Xueqi Li[3], Aadil Bajoub[1], Karin Wendt[4], Nikolas Kessler[4], Carsten Baessmann[4], Alberto Fernández-Gutiérrez[1], Selina C. Wang[2], [3], and Alegría Carrasco-Pancorbo[1], [1]Dept. of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain; [2]Dept. of Food Science and Technology, University of California Davis, USA; [3]Olive Center, University of California Davis, USA; [4]Bruker Daltonik GmbH, Germany

The emergence of multi-class analytical methodologies might greatly increase throughput and reduce cost, while avoiding the complexity and redundancy of single-chemical class determinations. Thus, in this study, LC-MS and GC-MS platforms were used to develop two new methodologies capable of carrying out the simultaneous determination of more than 40 compounds belonging to different VOO minor chemical classes within a single run. A non-selective and highly efficient liquid-liquid extraction protocol was optimized for VOO minor components isolation. The separation and detection conditions were adjusted to determine phenolic and triterpenic compounds, free fatty acids and tocopherols by LC-MS, plus sterols and hydrocarbons by GC-MS. A comparative assessment of both methods in terms of analytical performance, easiness, cost, and adequacy to the analysis of each class was performed. The new methods were then applied to study 126 oil samples from six different Mediterranean PDOs (Meknès and Ouazzane (Morocco), Priego de Córdoba and Baena (Spain), Kalamata (Greece) and Toscana (Italy)). Data treatment implied the selection of molecular features, bucketing, filtering, scaling and normalization; these steps as well as the application of principal components analysis and partial least square-discriminant analysis to the LC-MS and GC-MS data were done by using MetaboScape® software. Noticeable discrimination among the six evaluated PDOs was achieved taking into account the data coming from both platforms. The contribution of a few thousand molecular features to the statistical models was evaluated in depth and several compounds such as elenolic acid, acetoxypinoresinol, oleuropein and ligstroside aglycones, and some other tentatively identified substances were pointed out as possible PDOs distinctive markers.

^{1}H NMR–metabolic Profiles of Monocultivar EVOOs for PDO, PGI and 100% Italian Blend Production Assessment Chiara Roberta Girelli, Laura Del Coco[1], Federica Angilè[2], 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*

Mediterranean food tradition is based on three basic essentials: wheat, olives and grapes. Nevertheless, olive oil is the central element for its increasing consumption around the world. The need of a scientific tool to assess EVOOs geographical origin represents a hot topic issue, since the EU Regulation 182 of 6 March 2009 (on the compulsory labeling of EVOOs with the geographical origin of the olives) still lacks an official validation methodology. The same is for the EU Regulation 1151/2012, which seeks to enhance Europe's agricultural quality policy by increasing the coherence of various high-quality products, such as EVOOs, marketed under a PDO and PGI labels. We are currently involved in several NMR-based metabolomics and chemometric studies to assess cultivar composition and geographical origin of extra virgin olive oils, in particular for PDO, PGI and 100% Italian blend EVOOs production (mainly from Apulia and Tuscany regions) [1]. A large-scale analysis of 100% Italian blend and monocultivar oils from specific geographical areas was performed by ^{1}H NMR spectroscopy together with multivariate statistical analyses. Our results confirmed the usefulness of suitable monocultivar EVOO samples, possibly genetically certified, for the construction of ^{1}H NMR metabolic profiles databases aimed to cultivar and/or geographical origin assessment. [1] Del Coco L., Mondelli D., Mezzapesa G.N., Miano T., De Pascali S.A., Girelli C.R., Fanizzi F.P. (2016) J Am Oil Chem Soc 93: 373-381. Girelli, C.R., Del Coco, L., Fanizzi F.P. (2016) Eur J Lipid Sci Tech 118: 1380–1388. Girelli C.R., Del Coco L., Fanizzi F.P. (2017) Sustainability 9: 1471 and references therein

"Musty", "Fusty, Muddy Sediment", and "Rancid" Off-flavors in olive Oils are Well-known: but what is Behind on a Molecular Level? Michael Granvogl*, Anja Neugebauer, and Peter Schieberle, *Technical University of Munich, Germany*

Extra virgin olive oils (EVOO) are very popular edible oils because of the valuable nutrients and flavors. The aroma of a food is one of the most important criteria for consumers' buying behavior. Thus, a detailed knowledge of the respective key odorants is of high interest and, even more important, of the molecules causing distinct off-flavors, known as "musty", "fusty, muddy sediment", and "rancid". The molecular sensory science concept was used to clarify the formation of these off-flavors in EVOOs using a positive control showing the desired sensory attributes and oils eliciting the abovementioned sensory defects. This methodology is based on comparative aroma extract dilution analysis via gas chromatography-olfactometry, followed by identification experiments via gas chromatography-mass spectrometry. The most potent aroma compounds are quantitated by stable isotope dilution assays and odor activity values (ratio of concentration to respective odor threshold) are calculated. Finally, aroma simulation experiments are performed to validate the analytical data. In addition, the identified key odorants were quantitated in

further EVOOs and in olive oils with one of the analyzed off-flavors to put the obtained results on a broader basis, e.g., by principal component analysis. Main differences were obtained for esters, acids, and phenolic compounds. Their formation, caused for example by microorganisms via the so-called Ehrlich pathway, will be discussed in the lecture. In summary, the “musty”, “fusty, muddy sediment”, and the “rancid” off-flavors in EVOOs were characterized for the first time on a molecular level using the combination of an analytical-instrumental, sensorial, and statistical approach.

ANA 2c / LOQ 2a: Evaluation and Prediction of Oxidative Stability and Shelf-life

Chairs: Min Hu DuPont Nutrition & Health USA; and Rick Della Porta Pepsico / Frito-Lay USA

The Combination of High Oleic Oils and Natural Antioxidants as a Powerful Tool for Shelf Life Extension Susan Knowlton*, *DuPont Company, Pioneer, USA*

High oleic oils from soybean, canola, and sunflower have made significant nutritional and stability improvements in food manufacturing as they replace both partially hydrogenated and conventional (high polyunsaturated), commodity oils. Despite their natural stability resulting from a low polyunsaturated fatty acid content, some manufacturers require further improvements in shelf life stability as they replace synthetic antioxidants in their formulations. Traditional antioxidants like TBHQ, BHA, BHT, and others have fallen into disfavor as consumers are demanding shorter, simpler, and more 'natural' ingredients on food packaging labels. The combination of high oleic oils with natural antioxidants is a valuable tool to meet these demands. Data showing the extension of shelf life achieved with these combinations will be presented.

Chickpea Germination Improves the Antioxidative Activity of its Soluble Phenolic Compounds. Minwei Xu*, and Bingcan Chen, *North Dakota State University, USA*

Soluble bound phenolic compounds (SBPCs) extracted from germinated chickpea (*Cicer aretinium* L.) were fractionated using SEC-HPLC coupled with auto fraction collector regarding to the molecular weight variation. Three fractions categorized by molecular weight were collected at the optimum germination time (6 days). *In vitro* assay, including total phenolic content (TPC), 2', 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability and oxygen radical absorbance capacity (ORAC), and striped soybean oil-in-water emulsion system were employed to evaluate the antioxidative activity of each fraction. LC-QTOF-MS was employed for identifying the structure change of soluble bound moieties which can explain the variation of the antioxidative activity. Molecular weight of SBPCs tended to increase during chickpea germination. Higher molecular weight soluble phenolic compounds had greater antioxidative activity in emulsion system. Some of the structures of SBPCs were identified by LC-QTOF-MS, which may be related to the difference of antioxidative activity in each fraction. The findings can be applied to exploit antioxidants with strong antioxidative efficacy in food systems.

Antioxidant Activities of Sugars and Protein in Low Moisture Cracker System Thanh P. Vu*, Lili He, D. Julian McClements, and Eric A. Decker, *University of Massachusetts Amherst, USA*

In American diet, low moisture foods, such as crackers, are a top saturated fat contributor exposing a potential risk of coronary heart disease for American consumers. Replacing saturated fat by unsaturated fat could improve the nutritional properties of crackers, yet this would require antioxidant strategies to prevent lipid oxidation and maintain the shelf life of the product. In this study, reducing sugars were found to increase oxidative stability in crackers. Glucose, maltose and maltodextrin at the same dextrose equivalence resulted in increased hydroperoxide (30, 48, and 48 days, respectively)

and hexanal lag phases (60, 81, and 78 days, respectively), compared to control cracker (9 day hydroperoxide lag phase and 33 day hexanal lag phase). The antioxidant activity of maltose was effected by water activity (aw) and concentrations. At aw values of 0.22 and 0.68, maltose increased hydroperoxide and hexanal lag phases, compared to its activity at aw 0.05. For example, crackers with 5.4% maltose at aw = 0.22 and 0.68 had 63 day and 186 day hexanal lag phases, respectively, compared to 48 days at aw = 0.05. Gluten addition from 2.5 to 10%, at aw = 0.05, did not affect oxidative stability, but at aw = 0.68 it increased hexanal lag phases. Casein only slightly increased hexanal lag phase at 7.5 and 10%, aw = 0.22. The ability of reducing sugars with low sweetness (e.g. maltose) to inhibit lipid oxidation in model crackers suggests that they could be an effective antioxidant strategy.

Oxidative Stability of Margarines, Shortenings and Spreads Min Hu*, *DuPont Nutrition & Health, USA*

Oxidative stability and shelf life of food emulsions like mayonnaise and salad dressing have been widely studied. However, the oxidative stability of W/O food emulsions such as margarines, shortenings and spreads have not been thoroughly studied yet, and the researches on this area have started drawing a great deal of attention in both industry and academia. Usually, a bulk oil or fat may be more oxidatively stable than an O/W emulsion containing the bulk oil or fat. How about the case when comparing an O/W food emulsion with a W/O food emulsion? Prooxidant transition metal ions like ferrous iron have a big impact on oxidative stability of O/W food emulsions such as mayonnaise and salad dressing. What is the case when a W/O emulsion like a 40% fat spreads contains transition metal ions? An antioxidant working well in a O/W food emulsion would work efficiently in an W/O emulsion? The presentation will highlight different evaluation methods being used to assess the oxidative stability of butter fat, spreads and margarines, investigate the impacts of different antioxidant blends on the oxidative stability of spreads with varying fat contents, and study how chelators could impact the oxidative stability of different spreads containing varying levels of fat.

Shelf-life Extension of Meat and Meat Products by Using Natural Antioxidants Henna FS Lu*, *Kalsec Europe Ltd, UK*

Recently, the use of natural antioxidants has gained attention globally as the result of rising consumer demand for clean label solutions. Due to the presence of unsaturated fat in membrane phospholipids, lipid oxidation occurs in meat during processing and storage. Oxidative stability of various meat and meat products including ground beef, British sausage and chicken fillets were conducted. Meat and meat products were prepared with and without inclusion of antioxidants, followed by storage at different temperature and packaging condition. The stability was monitored through measurements such as visual observation, color measurement, sensory evaluation and secondary volatile oxidation profile by GC-MS (SPME). The effect of synthetic antioxidants such as BHA, BHT, Sodium Metabisulphite and ascorbic acids versus natural antioxidants such as rosemary, green tea, vinegar and acerola on the oxidative stability of meat and meat products were investigated. Results showed that inclusion of natural antioxidants significantly improved the shelf life of meat products. A better

color protection, better sensory attributes and a lower level of oxidation compounds were observed in products with antioxidants added. In addition, different secondary volatile oxidation profiles were obtained for different meat products. In short, natural antioxidant showed a similar performance as compared to that of synthetic antioxidant and therefore provide an alternative to replace synthetic antioxidant. The finding of these studies provide valuable information and new insights to meat suppliers to improve the shelf life of their meat and meat products.

Antioxidant Testing – An Application Review.
Rick Della Porta*, *Pepsico/Frito-Lay, USA*

A review of the practices and applications of testing antioxidants on edible oils. The challenge between academic research and practical application is critical for getting a true understanding of how and antioxidant may be used. The test protocols and analysis methods are reviewed in general terms in hope of making a better connection between researchers and end-users.

ANA 2d/LOQ 2b: Sensory Analytics and Analytical Methods for Assessing Lipid Oxidation and Shelf-life

Chairs: Jian Kong, Abbott Nutrition, USA; and Richard Della Porta, Frito-Lay, USA

Antioxidant Efficacy and Impact of Storage Conditions Marie Shen[1], Lan Ban[1], and Chandra Ankolekar*[2], *[1]Kemin Food Technologies, USA; [2]Kemin Industries Inc., USA*

Oxidative stability of oils can be measured by accelerated studies such as OSI, Schaal oven, or by monitoring real time chemical and sensory changes at ambient condition. Most of the literature have depended on accelerated conditions for evaluating antioxidant efficacy for the relative ease and quick results. However, debates over the reliability and accuracy still exist. And it is not clear whether complex mixture as plant extracts behave the same way as synthetic antioxidants. We hope to answer these questions with full analyses of free radicals, peroxides, secondary oxidative byproducts and active compound degradation in the treated oils that are stored in both accelerated and ambient conditions. In this study, common ingredients including TBHQ, rosemary extract (RE), mixed tocopherols (MT) and oil soluble green tea extract (OSGT) were tested. Comparing one-year ambient storage and 30-day accelerated storage, it is showed that although the absolute values for each test parameter were not the same, the trends in antioxidant efficacy were every similar. For example, OSGT showed strong dose response in both conditions and performed better than RE and MT. However, the relative degrees of improvement varied for RE and OSGT, that under ambient storage, their relative performances were better. This improvement positively correlated with the improved stability of the active compounds under ambient storage condition. Based on this study, we confirmed that accelerated conditions can serve as quick reference tools but it is needed to establish ambient models that both the activity and stability of an active compound would serve as independent parameters.

Sensory Directed Chemical Analysis of Oxidized Marine Oils Roy D. Desrochers*, *Tufts University Sensory and Science Center, USA*

Abstract not available.

Developing a Sensory Oxidation Quality Scale Monica L. Godbout*, *Abbott Nutrition, USA*

Abstract not available.

Assessing Virgin Olive Oil Stability and Shelf Life at Moderate Conditions by FTIR Spectroscopy Endowed with a Mesh Cell Accessory Noelia Tena[1], Ramón Aparicio-Ruiz[1], Ana Lobo[2], María Teresa Morales[3], Aparicio Ramón[2], and Diego L. García González*[1], *[1]Instituto de la Grasa (CSIC), Spain; [2]Instituto de la Grasa (CSIC); [3]University of Seville, Spain*

Virgin olive oil (VOO) stability is one of the major topics today because producers are demanding more effective methods to guarantee that oils from extra virgin category remains in this high-quality category during all the shelf life. However, moderate conditions of light and temperature are sometimes enough to produce a quality loss in a few months. The current methods (e.g., Rancimat) apply high temperatures and, therefore, their results are hardly correlated with the real conditions of

storage. In this context, mesh cell has been proposed as a rapid tool designed to monitor chemical changes that occur as a consequence of oxidation at moderate conditions by Fourier transform infrared (FTIR) spectroscopy. In order to evaluate this approach, monocultivar VOOs have been stored in mesh cells under different temperatures (at 23, 35, 65 °C) and different light intensities (400, 1000, 7000 lx) simulating the real conditions during storage and transport. The oil stability of the samples determined by using this accessory has been compared with the oil stability determined with Rancimat. The FTIR spectra revealed a remarkable increase of hydroperoxides and the subsequent formation of secondary oxidation products (e.g., alcohols). The fact that this method measures the stability of the oil from a multi-factor perspective (temperature and light) and includes several chemical species (primary and secondary oxidation products) makes the results more reliable to optimize VOO handling (e.g., packaging and storage temperature) according to the real conditions of storage.

ANA 3: General Analytical

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

Rapid Identification and Relative Quantification of the Phospholipid Composition in Commercial Lecithins by ^{31}P-NMR Ying Yang*, Richard Hiserodt, and Jing Li, *International Flavors & Fragrances Inc., USA*

^{31}P NMR analysis of samples prepared in a sodium cholate detergent system was used as a method for the identification and quantification of enzymatic hydrolysis products of lecithin. To precisely characterize all of the hydrolysis products from commercial lecithin, a series of enzymatic reactions of each phospholipid with phospholipase PLA1 were conducted and monitored by ^{31}P NMR at different times. Twenty six phosphorus-containing hydrolysis products from six classes of phospholipids (PC, PI, PS, PE, PG, PA) were found and determined by ^{31}P NMR measurement. The impact of pH on the chemical shift values for these hydrolysis products was observed and reported. To our best knowledge, this is the first report of 31P NMR chemical shift values for entire lyso-phospholipids hydrolyzed from 6 classes of phospholipids. Rapid and routine analysis of phospholipid composition in commercial lecithins by ^{31}P NMR was achieved without the need of phospholipid standards.

Applications for the LC-GC Technique in Routine Fat and Oil Analysis Torben Küchler*, *Eurofins Analytik GmbH, Germany*

A standardized and robust sample preparation is the basic requirement for a reliable analytical method. Many sample preparation methods in the fat and oil analysis depend on the selective separation of lipid classes by polarity with column chromatography or liquid-liquid extraction. To avoid this time consuming manual operation step, an alternative is the selective separation of target analytes via HPLC. Combined with an on-line coupled GC system, rapid and robust methods can be achieved. The presentation shows an overview about different applications for the evaluation of quality and authenticity of fats and oils with the LC-GC technique: Distribution and content of sterols for the judgement of identity and authenticity, detection of steradienes for the proof of a heat treatment of virgin oils, content of fatty acid ethyl esters in olive oil as a marker of poor raw material quality and determination of PAH as an important contaminant with a special LC-LC-GC application. An outlook is given for possible future applications.

Overcoming Issues and Challenges in the Analyses of Tocols in Oils Mei Han Ng* and Ahmad Kushairi Din, *Malaysian Palm Oil Board, Malaysia*

Accuracy and reliability of analyses for tocols (tocopherols, tocomonoenol and tocotrienols) have always been a cause for concern as the tocols are highly potent antioxidative compounds and thus, are easily susceptible to oxidation or degradation. The scarcity of tocotrienols and tocomononeol standards also contributed to the difficulty in analyses and calibrations. Official AOCS method recommended the use of α-tocopherol as reference in the absence of tocotrienols standards, which is a common practice. However, this is often not accepted by

industry players due to the question on the different response of the individual tocopherols and tocotrienols in UV spectroscopy, resulting in discrepancy in analyses. In addition, the concentration of tocomonoenol is often not reported although its presence is significant in palm oil. A chromatographic method that is able to address all the concerns faced in the analyses of tocols need to be developed. This paper reports on the development of a comprehensive and reliable method for the HPLC analyses of tocopherols, tocomonoenol and tocotrienols in oil. Limit of detection, limit of quantitation, linear range of analyses as well as the calibration of all the individual tocols, including tocomonoenol, in the absence of authentic standards are taken into consideration. Crosscheck of the method developed with various laboratories showed that the method is accurate with good reproducibility. This method may be adopted as the official method for the analyses of tocols in the future.

Tocopheryl Esters—Analysis of Novel Vitamin E Conjugates in Vegetable Foods: Occurrence, Concentrations and Digestibility Walter Vetter*, Stephanie Krauß, and Vanessa Darwisch, *University of Hohenheim, Germany*

Objective: Vitamin E comprises a group of mainly four tocopherols (alpha-, beta-, gamma- and delta-T) along with four tocotrienols and a few minor compounds. Tocopherols are typically considered to occur in their free form in foods. Recently, we noted the presence of tocopheryl esters with different fatty acids in red and yellow bell pepper. In this study, we screened further vegetable foods and present a method for the quantification of tocopheryl esters in foods.

Methods Used: Extracted lipids were fractionated by SPE into (i) hydrocarbons, (ii) tocopheryl esters and (iii) free tocopherols. The separated tocopheryl ester fraction was analyzed by GC/MS in intact form and after saponification and silylation. Reference standards of alpha- and gamma-tocopheryl esters were synthesized for quantitation. In vitro incubation experiments were performed with artificial digestion juices.

Results: Tocopheryl esters were detected and quantified in four matrices (bell pepper, chili pepper, cucumber, walnuts). Both the tocopherol pattern and the absolute amounts were varied. In all four matrices, the concentration of the tocopheryl esters was higher than the corresponding concentration of the free tocopherols. Subsequent incubation with artificial digestion juices showed that tocopheryl esters were not cleaved into free tocopherols and fatty acids. Hence, tocopheryl esters do not directly contribute to the tocopherol activity of the food samples.

Conclusions: The bioactivity and possible contribution of tocopheryl esters to the vitamin E activity needs to be assessed given the comparably high abundance in the tested food matrices. More data is required on the role of tocopheryl esters in foods.

A Method for Detection of Partially Hydrogenated Oils (PHO) in Food Matrices Containing Vegetable Oils Sneh Bhandari[1], Ming Gao[1], and Pierluigi Delmonte[2], *[1]Merieux Nutrisciences, USA; [2]US Food and Drug Administration, USA*

Foods on sale in the U.S. are being reformulated to minimize the content of partially hydrogenated oils (PHO) among their ingredients. Recent determinations and regulations regarding PHOs in foods indicate the necessity of distinguishing between the trans fatty acids (TFA)

produced during oil refining and partial hydrogenation. While most TFAs occurring in PHOs are also present in refined oils, their distribution pattern and concentrations are different. In this study we propose a methodology for detecting the occurrence, in foods, of TFAs originating from partial hydrogenation, including in the presence of TFAs from other sources. The determination of the presence of PHOs is based on the evaluation of the content of mono-unsaturated TFAs, and other selected fatty acids. Food samples were prepared for gas chromatographic analysis according to AOCS Official Method Ce1k-09. The gas chromatographic quantification of fatty acids, including individual *trans*-18:1 positional isomers, was achieved with an SLB-IL111 (200 m x 0.25 mm x 0.20 µm) capillary column applying the method developed by Delmonte et al (2011). The accuracy of the method was tested by analyzing various vegetable oils spiked with different quantities of PHOs. The method could accurately detect PHO in 96–97% of spiked samples containing at least 0.5% TFAs (% of total fat). The method was applied to the analysis of about 100 commercial food samples, part of which reported PHOs among their ingredients.

References: Pierluigi Delmonte, Ali-Reza Fardin Kia, John K.G. Kramer, Magdi M. Mossoba, Len Sidisky, Jeanne I. Rader (2011). Journal of Chromatography A, 1218: 545–554

Supplementation Studies Involving Natural trans Fatty Acids: Real Technical Challenges, Actual Solutions Etienne Guillocheau*, Daniel Catheline, Philippe Legrand, and Vincent Rioux, *Agrocampus-Ouest, France*

Objectives and hypothesis: Growing evidence suggests that natural trans fatty acids should be distinguished from industrial ones, regarding their impact on human health. One trans isomer at a time should therefore be considered in nutritional studies. However, a high amount combined with an excellent purity of the corresponding isomer remains a challenge. This study aimed at providing means to overcome such issues.

Methods Used: The purification of several grams of trans-palmitoleic acid (C16:1 n-7 trans, TPA) was considered in this study, starting from the cis-palmitoleic acid (C16:1 n-7 cis, CPA). Fatty acids were used as ethyl esters. Several sources of CPA were evaluated. The following means were critically assessed: Flash HPLC, urea complexation, and crystallization in solvent. Within each step, the purity was checked by GC-MS, and the best means was chosen.

Results: Macadamia nut was deemed as a reliable source of CPA, the other cis-C16:1 isomers being present in low amounts. Purification of CPA was best carried out using Flash HPLC. The isomerization of CPA to TPA was easily done. Removing as much CPA as possible from the CPA-TPA mix was best performed by crystallization in solvent, ensuring a 99%-purity of the final TPA product.

Conclusions: Complementary means were used to get high amounts of highly purified-TPA. Not only ethyl esters were suitable for purification purposes, but they made the purity control by GC-MS easy. Provided the availability of the

corresponding cis-isomer, our purification strategy can be applied to any trans monounsaturated fatty acid.

Determination of sn2-position Fatty Acid in Long-chain Triglycerides(LCTs) and Medium- and Long-chain Triglycerides(MLCTs) with Enzymatic Alcoholysis by GC-FID Wei Ting Ting, Wen Ming Cao, and Yuan Rong Jiang, *Wilmar (Shanghai) Biotechnology Research & Development Center Co., Ltd., China*

A new sn2-position fatty acid composition analysis method was established in this research. The method was not only used in plant oils with long-chain triglycerides (LCTs), such as sunflower seed oil, rapeseed oil, but also in medium- and long- chain triglycerides (MLCTs). 2-monoaclyglycerols (2-MAGs) were achieved from enzymatic alcoholysis of triglycerides and analyzed by GC-FID after derivation. 2-MAGs as detected targets were proved to be appeared and retention time was confirmed, and 1(3)-MAG was absent by GC-QTOF. The condition test was developed in this research and the experiment condition, such as enzyme amount, reaction temperature, reaction time and derivative reagent volume were optimized by single factor test. 2-C8-MAG, 2-C10-MAG, 2-C12-MAG, 2-C14-MAG, 2-C16-MAG and 2-C18-MAG were determinated in MLCT by GC-FID. The results of long chain triglycerides compared with porcine pancreatic lipase method were in satisfactory. This method was competitive to analyze triglycerides contained medium chain fatty acids which could not be analyzed by porcine pancreatic lipase method, and it provided shorter pretreat time and better repeatability due to detected targets changed and SPE or TLC separation removed.

Normal Phase UV Compatible HPLC Separation of Hydroxylated and Non-hydroxylated Lipids for Metabolic Flux Analysis Hari Kiran Kotapati* and Philip D. Bates, *The University of Southern Mississippi, USA*

The major objective is to develop a normal phase HPLC method to completely separate hydroxylated and non-hydroxylated triacylglycerols (TAGs) and diacylglycerols (DAGs) classes for online liquid scintillation counting within 14C based metabolic flux experiments. The secondary objective was to have a solvent system compatible with detection of lipids at 210 nm to track lipid mass with a non-destructive and commonly available detector for use with fraction collecting. Different stationary phases, for normal phase HPLC, including silica and modified silica supports were examined for our studies during the course of method development. PVA coated silica support provided excellent separations between the hydroxylated TAG classes from non-hydroxylated DAGs that were very difficult to separate via previously reported chromatographic techniques for hydroxylated neutral lipids. This normal phase HPLC analysis technique was combined with β-ram detector to perform continuous flow liquid scintillation counting, which is important for rapid and efficient quantification of labeled lipids from radioactive based enzyme assays or in vivo metabolic flux experiments. We have successfully performed chromatographic separations of non-labeled individual lipid classes by UV detection, and radioactive lipids extracted from [14C]acetate in vivo metabolic labeling experiments in developing Arabidopsis thaliana seeds. In this study, the samples collected from

different time points during the course of a continuous 14C metabolic labeling experiment were analyzed and the biosynthetic precursor-product relationships of major lipid classes were identified.

Mitigating the Deteriorating Effect of Biofuel in Engine Oil Jerome D.A. Kpan*[1], and Juergen Krahl[2], *[1]Technology Transfer Automotive Centre of Coburg University of Applied Sciences and Arts, Germany, Germany; [2]Coburg University of Applied Sciences and Arts; Ostwestfalen-Lippe University of Applied Sciences, Germany*

This study presents the feasibility of using the process of adsorption in mitigating the negative impact of the use of biodiesel on the crankcase oil. This is done by suppressing the buildup and/or selectively removing oligomers formed in the oil as a result of the degradation of biodiesel. Biodiesel blended with neat base oil (20% biogenic fuel and 80% neat base oil) were thermo oxidatively aged. The separation of the oligomers was carried out through fixed bed columns made of Magnesium aluminum hydroxycarbonate and 1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene in the ratio of 1: 2. An amount of 10g of the combined adsorbent was used per 1L of oil. The Magnesium aluminum hydroxycarbonate compound was placed on top of 1,3,5-trimethyl-2,4,6-tris(3,5-di-tert-butyl-4-hydroxybenzyl)benzene in the column. 100 ml of the oil sample run through the adsorbent bed at a temperature of 130°C at a time. Also the combined adsorbents were also added to the sample and aged. The acid number and peroxide value were determined. Size exclusion chromatography was used to analyse the oligomers. The FTIR analysis showed that adsorption suppressed about 80% formation of acidic products. This is collaborated with the size exclusion chromatography and the total acid number. This study has shown that adsorption is a potential useful tool for the suppression of oligomers formation. Therefore, if biodiesel will be a significant fuel of the future then preventing its role in short oil change interval by the process of adsorption is a great achievement.

ANA 3.1/PCP 3a: Bioprocessing for New/Value-added Protein Utilization: Digestibiity Issues/Analytical Measurements

Chairs: Sneh Bhandari, Merieux Nutrisciences, USA; Buddhi Lamsal, Iowa State University, USA; and Bishnu Karki, South Dakota State University, USA

Matrix Effect on the *in vitro* Immunodetection of Food Allergens. Qinchun Rao, Xingyi Jiang, and Behnam Keshavarz, *Florida State University, USA*

To protect the public health, the U.S. food manufacturers have been required to label food allergens or ingredients derived from eight major allergenic foods since 2006. Currently, the presence of misbranding and/or undeclared food allergenic residues is the No. 1 cause of food recalls in the US. In order to (1) fight food fraud, (2) better comply with the food regulations, (3) decrease the food recalls economic loss to the food industry, and (4) reduce the risk of food allergy, it is necessary to develop reliable and robust in vitro detection methods to prevent the occurrence of undeclared allergenic residues in foods. As the major fish allergen, parvalbumins (PV) from mullet and salmon in two sample models were used to elaborate the relationship between matrix effect, extractability of PVs, and their thermostability during in vitro immunodetection. Matrix-induced thermal instability of PV was mainly due to physical (hydrophobic effect) and chemical (thiol-disulfide interchange) interactions. Our results illustrate that the addition of sodium dodecyl sulfate (SDS, surfactant), β-mercaptoethanol (reducing agent) or ethylenediaminetetraacetic acid (EDTA, metal chelator) during sample preparation could not only increase the extractability of PV but also enhance its immunodetection using two PV-specific monoclonal antibodies. Our findings demonstrate an overdose on EDTA made PV monomer Ca2+-free and led it undetectable by PARV19. Overall, it is never enough to emphasize that matrix effect on target analyte quantification is unignorable during food allergen detection because any false negative assay outcomes may induce potential or severe life-threatening allergic reactions in consumers.

Protein Quality Evaluation in Protein Enhanced Formulations Including Those Based on Oilseed Based Proteins Sneh Bhandari*, *Merieux Nutrisciences, USA*

There's growing evidence that high-protein food choices do play a role in health and more consumers are looking for high quality proteins from varied sources including those from plants. Oilseeds protein are becoming a newly recognized source of dietary proteins particularly to meet growing needs of large segments of word population. More and more new protein products are becoming available from different oilseeds for opportunity to incorporate in a broader variety of foods to make nutritionally enhanced products. The accurate assessments of protein quantity and quality in newly available sources of the dietary proteins and the formulations and product based on those has is important. This evaluation has acquired additional importance now because of current new trends of the development of protein enhanced products. Protein quality needs to be evaluated to determine its percent daily value for nutritional labeling in US and can be done by the use of PDCAAS in food meant for ages >1 year. Formulation of a nutritionally incomplete protein

with an ingredient containing complementary protein can result in a product with complete protein with improved PDCAAS value. FAO has proposed a new protein quality measure digestible indispensable amino acid score; DIAAS which is yet to receive a wider acceptance by regulatory agencies.

Simultaneous Quantification of Hydrolysis Degree, Protein and Mean Weight of Peptides Released during Enzymatic Proteolysis. Sophie Beaubier[1], Irina Ioannou[1], Xavier Framboisier[2], Olivier Galet[3], and Romain Kapel[2], *[1]LRGP—UMR CNRS 7274, France; [2]Reaction and Process Engineering Laboratory UMR-7274, France; [3]Avril Group, France*

Enzymatic proteolysis is an industrial process used in a wide range of applications (improvement of functionalities, nutrition, bioactive peptides production…). Study of this process consists in kinetic follow-up of the protein conversion rate, the hydrolysates size and the hydrolysis degree. To determine these 3 parameters, three different analysis are required which can have drawbacks particularly for vegetable protein hydrolysis. The communication presents an original methodology to quantify simultaneously these three criteria by size-exclusion chromatography (SE-HPLC). The approach is based on absorbance profiles and the estimation of molar extinction coefficient of each point of this one from the mixture aminogram of hydrolysates. Peak area of protein eluted into column dead volume informs on protein conversion rate and the peptide signal permits to determine size and DH of hydrolysates. As a first step, the approach was tested on the hydrolysis of animal and vegetable proteins with Alcalase 2.4L. A corrective factor was determined for each substrate from the linear correlation between the DH value obtained with the methodology and TNBS method, used as reference method. Then experimental validation tests realized with others enzymes were analyzed by SE-HPLC and TNBS and pH-Stat methods. Good quantification of DH value was observed (90% of validation tests) compared to TNBS method. The developed methodology is a powerful tool for monitoring enzymatic proteolysis both for animal and vegetable proteins while minimizing time. Moreover, it could be used for functionalities, digestibility or bioactivities analysis of produced hydrolysates.

Nutritional Evaluation of Modified Carinata Meals in Finfish. Tom Kasiga and Michael Brown, *Dept. of Natural Resource Management, South Dakota State University, USA*

The recommended inclusion of carinata Brassica carinata meal (CM) in animal feeds is currently ≤10%. However, CM use in fish feeds has not been tested but will likely be limited by high concentrations of fiber, glucosinolates (GLS) and sinapine. GLS and sinapine tolerance in Hybrid Striped Bass Morone chrysops ♀ X M. saxatilis ♂ (HSB) was tested using incremental amounts of cold-pressed carinata meal (CPCM). Inclusion of >2.71 µmoles of GLS and >0.31 mg of sinapine/g (>10% CPCM) of diet reduced feed intake, resulting in reduced fish growth. To reduce antinutrients, we processed CM by aerobic conversion (AC) using fungi ssp. followed by a single wash to produce aerobically converted carinata meal (ACCM). In a Rainbow Trout Oncorhynchus mykiss (RBT) trial, we replaced up to 75% of FM in a low animal protein (20%) diet containing FM as the only animal protein source. Replacements ≥ 50% FM (≥10% ACCM) reduced fish growth. Due to low

utilization of ACCM by RBT, we used low (20%) but similar animal protein contents (10% FM and 10% poultry by-product meal) and included up to 30% ACCM or 30% double-washed carinata meal (DWCM) in HSB diets. Growth of HSB fed the FM reference diet was similar to that of HSB fed 30% ACCM or 30% WCM. Thus, ACCM can replace more FM in diets but in combination with animal meals (≥20%). Because ACCM was low in GLS and sinapine but high in fiber, current research is focused on reducing fiber in ACCM.

ANA 4: Trace Contaminants, including Processing Contaminants

Chairs: Jessica K. Beekman, US Food and Drug Administration, USA; and Mark W. Collison, Archer Daniels Midland Co., USA

Comparison of Analytical Methodologies for the Analysis of Bound MCPD and Glycidol in Edible Oils and Infant Formula Jessica K. Leigh*[1], Kaitlin Grassi[2], Shaun MacMahon[1], Jan Kuhlmann[3], Adam Becalski[4], Greg Jaudzems[5], and Fabien Robert[5], *[1]US Food and Drug Administration, USA; [2]U.S. Food and Drug Administration, United States; [3]SGS Germany GmbH, Germany; [4]Health Canada, Canada; [5]Nestle Quality Assurance Center, United States*

Process-induced chemical contaminants 3-monochloro-1,2-propanediol (3-MCPD) esters, 2-monochloro-1,3-propanediol (2-MCPD) esters, and glycidyl esters are formed in edible vegetable oils during the deodorization step of the refining process. As they are considered potentially carcinogenic and/or genotoxic, their presence in refined oils and foods may pose a potential health risk. For this reason, research efforts over the last several years have focused on developing methodology for the extraction and quantitation of these contaminants in oils, infant formula, and other complex food matrices in an effort to estimate levels of exposure and monitor possible mitigation approaches from adjusting processing parameters. In addition, proposed EU regulations for free and bound 3-MPCD and glycidol concentrations in infant formula and edible oils highlight the need for accurate analytical methodologies. Currently, there is no standard infant formula reference material containing known MCPD and glycidyl ester concentrations; therefore, verifying the accuracy of analytical methods has been difficult. The work discussed in this presentation compares a number of analytical methodologies for the analysis of bound 3-MCPD and glycidol in an attempt to confirm method performance. Analytical methodologies, including those used by the U.S. Food and Drug Administration, Health Canada, SGS Germany, and Nestle, for the quantitative analysis of bound 3-MPCD and glycidol in various samples of oils and infant formulas will be compared. In addition, data will be presented comparing the results of the use of direct and indirect detection methods with the infant formula extraction procedure developed by the FDA. Finally, a summary of the quantitative results obtained using each analytical method will be presented.

Detection Limits and Challenges in Low Level Analysis of MCPD and Glycidol using AOCS Method Cd 29c-13. Mark W. Collison and Kevin Adlaf, *Archer Daniels Midland Co., USA*

AOCS Method Cd 29c-13, "2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by GC/MS (Difference Method)", is widely used in industry because of its rapid analysis time and ability to be automated. Proposed MCPD and glycidol limits in infant formula are far lower than those proposed for oil consumed by adults. The current presentation centers on questions of whether indirect GCMS methods are sufficiently sensitive for analysis of oils intended for or extracted from infant formula, especially AOCS Cd 29c-13, and discusses some of the challenges to achieving very low limits of detection with indirect GCMS methods.

Recent Status of EU-regulation on 3-MCPD and Glycidol in Oils/Fats, Infant Formulae and Analytical Solutions Available Jan Kuhlmann*, *SGS Germany GmbH, Germany*

Glycidyl fatty acid esters (GE; "bound glycidol") as well as 3-Mono-chloro-propane-diol esters (MCPD-E, "bound 3-MCPD") are known to be undesired food borne contaminants that frequently occur together in refined edible oils and fats and foods made from these. Free MCPD might be generated more frequently in compound foods in course of heat treatment. It might also migrate from diverse contact materials into foods. 3-MCPD and glycidol are classified as carcinogens (category 2B and 2A respectively) Recently the European Committee (EC) is working on regulations for the maximum levels of free and bound 3-MCPD as well as glycidyl fatty acid esters in powdered and liquid infant formulae and related products as well as in oils and fats intended for direct human consumption or use as an ingredient in food. The draft regulation focuses as well on vegetable oils and fats destined for the production of baby food and processed cereal-based food. Accordingly, there is an obvious need for analytical methods that meet recent and future regulation. In this regard, a guideline seems to be helpful as during the last 10 years, when the occurrence of significant amounts of MCPD and glycidyl fatty acid esters in various food matrices was reported, an extraordinary broad variety of analytical methods for the determination of these compounds have been published and some also validated. This presentation intends to focus on the status quo of the European regulations on 3-MCPD and glycidol derivatives and the availability of analytical methods therefore.

Modern Analytical Tools in MCPD and Glycidol Analysis: Research and Routine Analysis Perspectives Katerina Mastovska*[1], Vojtech Hrbek[2], Beverly Belkova[2], Barbara A. Mitchell[3], Urairat Koesukwiwat[4], and Jana Hajslova[2], *[1]Covance Food Solutions, United States; [2]University of Chemistry and Technology, Czech Republic; [3]Covance Labs, Inc., USA; [4]Covance Food Solutions, Singapore*

Traditionally, the analysis of monochloropropanediols (MCPD) in foods has been focused on the determination of free 3-MCPD in a limited number of target, mostly hydrolyzed products using derivatization followed by GC-MS analysis. The discovery of MCPD esters (re)ignited the interest in the toxicity, formation, occurrence, potential mitigation, and naturally also analysis of free and bound forms of 3-MCPD and related compounds (2-MCPD and glycidol). From the analytical perspective, this brought significant challenges in terms of the myriad of target analytes (various esters), broader scope of matrices and concentration levels of interest. There are two basic analytical approaches to the MCPD/glycidol analysis: direct determination of all free and bound forms or indirect analysis after conversion (hydrolysis) to MCPD/glycidol free forms. The indirect (total) analysis is suitable for high-throughput routine analysis and compliance testing. Similarly to the traditional analysis, the free forms undergo derivatization followed by GC-MS or GC-MS/MS analysis, the latter providing more selectivity for low-level analysis of highly complex matrices, such as infant formula. The direct analysis is more suitable for research applications because it can provide information about the composition and relative abundances of the various ester forms in the

investigated samples. LC-MS/MS has been mostly employed for this purpose but there are other techniques that can provide more information without the need for analyte-specific method conditions, such as non-targeted, high-resolution MS. Also, the LC separation could be replaced by supercritical fluid chromatography (SFC), which offers different separation selectivity and some unique possibilities for the analysis of MCPD/glycidol esters.

Toxicity Evaluation of 2-MCPD and Estimation of Intestinal Absorption of the Monoesters.
Yomi Watanabe[1], Naoki Kaze[2], Kaeko Murota[3], Hirofumi Sato[4], Yuri Osafune[5], and Araki Masuyama[5], *[1]Osaka Research Institute of Industrial Science and Technology, Japan; [2]Ueda Oils & Fats MFG., Japan; [3]Kindai University, Japan; [4]Osaka Municipal Technical Research Institute, Japan; [5]Osaka Institute of Technology, Japan*

Monochloropropane diol (MCPD), first reported in vegetable protein acid-hydrolysate, has 2 isomers. The major one, 3-MCPD, is categorized as group 2B; substances that are possibly carcinogenic to humans by International Agency for Research on Cancer. More toxicological evaluation and estimation on intestinal behavior are required for the other isomer, 2-MCPD. We have previously reported that 2-MCPD is estimated to be absorbed in intestine in a similar manner to 3-MCPD when evaluated *in vitro* using intestinal epithelial cell membrane model. The two detected similarly in blood serum after their administration to rat duodenum, and was estimated to reach to hepar *in vivo*. When 2-MCPD monoester was administrated to rat duodenum, free MCPD was detected in blood serum in a similar manner to that from 3-MCPD monoester, as well. The toxicity of 2-MCPD was then evaluated using human hepatic cell line, Hep-G2. LD_{50} of 2-MCPD was several times higher than 3-MCPD, whereas 10^2 times higher than glycidol, when evaluated the cytotoxicity by measuring rates of the living and dead cells by Cell counting kit-8 and Cytotoxicity LDH Assay Kit-WST (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Cytotoxicity of 2-MCPD was thus estimated to be the lowest among the three substances, which was consistent to our previous observation using Chinese hamster V76 cells.

The Importance of Aligning Analytical Limits with Health-based Guidance Values: Process-formed Compounds Case Study
Paul R. Hanlon*, *Abbott Nutrition, USA*

Exposure to substances created by food processing has been a part of the human experience since the advent of cooking with fire. While human exposure to these substances has always been a part of our history, it is only through the development of analytical methods that we have become aware of the extent of our exposure to these substances. The continued development of sophisticated methodology has created a critical need for an aligned approach between the risk assessment and analytical method development for these substances. Both of these processes feed into the development of regulatory limits for these substances, however, the direct interaction between risk assessment and analytical method development is often not considered. Misalignment of these processes can lead to confusion about the relevance of detection of these substances at concentrations below the health-based guidance values (HBGVs). For example, detection of substances at trace levels has led to hazard-based evaluations where

any level of exposure is assumed to be dangerous. Creating a model where fit for purpose methods are developed aligning limits of detection with HBGVs has the opportunity to improve risk management activities by focusing activities on the areas that have the highest priority for consumer safety. This is especially important for substances like process-formed compounds where the diversity of substances results in an increased opportunity for sensitive analytical methods to detect "new" compounds.

Healthy but also Flavorful Food: Mitigation Strategies for Food-borne Toxicants Combined with Sensory Properties Accepted by Consumers Michael Granvogl*, *Technical University of Munich, Germany*

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

MOSH/MOAH and Plasticizers: Status quo of Analysis and Activities of the Authorities in the EU Jan Kuhlmann*, *SGS Germany GmbH, Germany*

Mineral Oil Saturated Hydrocarbons (MOSH) together with Mineral Oil Aromatic Hydrocarbons (MOAH) and plasticisers are complex groups of undesired minor components in foods. Due to their lipophilic nature and ubiquitous distribution they do occur in a broad variety of foods and also frequently in edible oils and fats. NGO's and Authorities as for instance the EFSA CONTAM panel started to focus on these new contaminants since toxicological studies raised indications for the possibility of undesired health impacts on consumers. MOSH/MOAH as well as plasticisers represent groups of related single components. As these groups might contain more than hundred or in case of MOSH and MOAH even thousands of individual components risk assessment and analysis are challenging and compromises have to be taken. This presentation tries to identify the recent opinion of the authorities in the EU, which action might be taken in future and which analytical solutions are available so far.

Immuno Magnetic Solid Phase Extraction Combined with Cleanup to Determine Aflatoxin B1 in Vegetable Oils Hongshun Yang* and Xi Yu, *National University of Singapore, Singapore*

Aflatoxin B1 (AFB1) contamination is a serious problem in edible oil industry. It causes severe health threat and economic loss. In this study, a novel approach, low temperature cleanup (LTC) combined with immuno magnetic solid phase extraction (IMSPE), was proposed to quantify AFB1 in vegetable oils. LTC assisted in freezing out the interference from oil matrix while IMSPE helped preconcentrate the targeted analyte. In order to carry out the IMSPE, anti-AFB1 monocolonal antibody functionalized magnetic nanoparticles were first synthesized. Extraction of oil samples was then carried out followed by fluorometric quantitative analysis. The proposed method showed satisfactory efficiency and reproducibility with recovery rates in the range of 79.6%-117.9% and relative standard deviation below 11.48%. Correlation of the method was satisfactory as well with linear correlation coefficient being 0.9941. The method was applied to analyze five different kinds of vegetable oils from Singapore local supermarket including canola, peanut, soybean, corn, and olive oil. No AFB1 was detected in any of the mentioned oil samples. Overall, the proposed method is accurate, sensitive, and reliable. It is convenient and promising in detecting trace chemical contaminants in oils.

Effect of the Composition and Structure of Excipient Emulsion on the Bioaccessibility of Pesticide Residue in Agricultural Products Ruojie Zhang[1] *(Honored Student Award Winner; Lipid Chemistry and Nutrition Award Winner;)* D. Julian McClements[1], Lili He[1], Zipei Zhang[1], Wenhao Wu[2], Yeonhwa Park[3], and Baoshan Xing[2], *[1]University of Massachusetts Amherst, USA; [2]Stockbridge School of Agriculture, University of Massachusetts Amherst, USA; [3]Dept. of Food Science, University of Massachusetts Amherst, USA*

Oil-in-water emulsions, such as dressings, dips, sauces, and creams, are commonly co-ingested with fruits and vegetables. Previous studies have shown that co-ingestion of emulsions with fruits and vegetables may substantially increase the oral bioavailability of lipophilic nutraceuticals present within these natural products. However, many natural products also contain potentially detrimental bioactive agents that may be introduced during crop production and storage, such as pesticides. The purpose of the current study was therefore to examine the potential impact of co-ingestion of emulsions with natural produce on the bioaccessibility of a hydrophobic pesticide. In current study, the influence of co-ingestion of food emulsions with tomatoes on the bioaccessibility of a model pesticide (chlorpyrifos) was studied. The results indicated that the bioaccessibility of chlorpyrifos (a highly lipophilic pesticide) was shown to depend on the

type and amount of co-ingested lipids in a model food emulsion. Pesticide bioaccessibility increased as the lipid content of the emulsions increased and was higher when the lipid phase consisted of long chain rather than medium chain triacylglycerols. However, the bioaccessibility did not depend strongly on lipid droplet size, suggesting that the total quantity of mixed micelles formed from lipid digestion was more important than the rate of their formation. Overall, these results suggest that emulsions might increase the bioavailability of undesirable pesticides, which may increase their risks to human health. Moreover, the results suggest that food effects should be taken into account when assessing the potential toxicity of pesticides.

ANA 5: Marine Oils and Other Products

Chairs: Cynthia Srigley USA; Adam Ismail USA

Oxidative Status and Nutrient Label Claim Accuracy of the Top 50 Selling Omega-3 Products in the US Adam Ismail*, *Global Organization for EPA and DHA Omega-3s, USA*

Abstract not available.

Sensory Vocabulary for Marine Omega-3 Oils Wenche Emblem Larssen*[1], E. Monteleone[2], M. Hersleth[3], [1]Møreforsking, Norway. [2]Dept. of Agricultural Biotechnology, University of Florence, Italy, [3]Nofima, Norway

Even though the Omega-3 industry has strict restrictions concerning the chemical quality of marine oil, they lack a defined methodology and a vocabulary for evaluating the sensory quality. This study was conducted to identify the sensory descriptors of marine oils and organize them in a sensory wheel and vocabulary for use as a tool in quality assessment. Oil-samples from six of the largest producers of omega-3 products in Norway were collected and analyzed both sensory, by six industry expert panels and one trained sensory panel, and chemically. Through a series of language sessions, a total of 184 aroma (odor by nose), flavor, taste and mouthfeel descriptors were generated. A sensory wheel based on 60 selected descriptors grouped together in 21 defined categories was created to form a graphical presentation of the sensory vocabulary. In addition, a selection of the oil samples was also evaluated by a trained sensory panel using descriptive analysis and correlations between sensory and chemical analysis was studied. This showed a positive correlation between primary and secondary oxidation products and sensory properties such as rancidity, chemical flavour and process flavour and a negative correlation between primary oxidation products and acidic. This research is a first step towards the broader objective of standardizing the sensory terminology related to marine oils.

An Examination of Marine and Vegetable Oil Oxidation Data from a Multi-Year, Third-Party Database Anna A. De Boer[1], Adam Ismail[2], Keri Marshall[3], Gerard Bannenberg[2], Kevin L. Yan[1], and William J. Rowe[1], *[1]Nutrasource, Canada; [2]Global Organization for EPA and DHA Omega-3s, USA; [3]DSM Nutritional Products, USA*

Fish oil dietary supplements remain popular sources of eicosapentaenoic (EPA) and docosahexaenoic (DHA) omega-3 fatty acids. However, some studies have suggested that commercially available fish oil supplements are excessively oxidized, impacting oil quality and safety. Thus, we assessed oxidation data from a third-party database of 1900+ globally-sourced fish oil samples, including peroxide values (PV), p-anisidine values (p-AV), TOTOX, and acid values (AcV). Additionally, we compared fish oils to krill, algal, sunflower, and extra-virgin olive oil. Fish oil products were predominantly compliant with voluntary industry limits: for PV, 13.9% exceeded 5 mEq O_2/kg (2.2% exceeded 10); for AcV 2.1% exceeded 3 mg KOH/g, while for p-AV in unflavored oils, 6.1% exceeded 20, (3.8% exceeded 30), and 8.8% exceeded TOTOX limits (26). Further, the fish oil median PV was similar to algal and sunflower oil, 4.8-fold greater than krill oil, and 5.2-fold less than extra-virgin olive oil. The fish oil median p-AV differed non-significantly between oils. Finally, the fish oil

median AcV was similar to algal and extra-virgin olive oil, was 3.4-fold greater than sunflower oil, and 11.9-fold less than krill oil. This research has provided new insight that retail fish oil products predominantly meet regulatory guidelines, and are comparable in oxidative status to other commercially available dietary oils.

Chemical Changes During the Acute Oxidations of Fish Oils Austin S. Phung[1], Selina C. Wang[1], Adam Ismail[2], Gerard Bannenberg[2], and Ameer Taha[3], *[1]University of California-Davis, Olive Center, USA; [2]Global Organization for EPA and DHA Omega-3s, USA; [2]University of California, Davis, USA*

Fish oil is one of the most popular dietary supplements sold on the market. However, it oxidizes more rapidly due to the presence of polyunsaturated fatty acids (PUFAs). We measured the oxidative status of two fish oils, hoki and anchovy, at varying time points over a 30-day period. To do this, we replicated the oxidative conditions used in a previously published article that oxidized hoki oil with bubbling oxygen for 30 days under standard fluorescent lights at room temperature. In addition, two other oxidation methods were evaluated – heating the oils in an oven at 50°C with or without bubbling oxygen. Chemical measurements such as peroxide and p-anisidine values were used to assess the oxidation and develop oxidation curves of both oils over the 30-day period. There are two main objectives of this study: (1) to establish a more standardized fish oil oxidation protocol; (2) to estimate the oxidation status of fish oil products on the market by fitting on our oxidation curves. Preliminary results for anchovy oil suggest that thermal conditions produce higher p-anisidine values compared to bubbling oxygen after 30 days. However, for hoki oil, the thermal conditions produce p-anisidine values similar to the bubbling oxygen method. This suggests that the two oils do not follow the same trend for oxidizing rate with the three oxidation methods.

Compositional Analysis of Algal Biomass, an Emphasis of Unique Contribution of Algal Lipids Lieve Laurens*, *National Renewable Energy Laboratory, USA*

Algal lipid yields are unmatched by any terrestrial feedstock, which makes their use ideal for renewable fuels, chemicals and food and feed ingredients. Using algae as fuel and bioproduct precursor feedstocks has proven challenging due to the lack of complete characterization of the biomass and lipids. There remain many lipid components unidentified or difficult to study. This presentation will cover the state of technology of algal biomass characterization to date and the current challenges moving forward. As a case study, the utilization of ultra-high-resolution, fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the identification and quantification of novel components will be discussed. Using a combination of Kendrick mass sorting and elemental characterization of molecular ions, series of compounds can be identified and help with the elucidation of unknown components in algal lipids. The superior mass accuracy and resolving power of the FT-ICR-MS in combination with existing, more traditional, chromatography and mass spectrometry tools has proven to be necessary for creating a reference library of novel compounds found in algae. The data collected for a number of different species illustrates a dynamic lipid profile and class distribution as the

cells are transitioning from early to late stage of cultivation.

Sterol Fingerprinting in Algae, a New Method for a New Feedstock Stefanie Van Wychen, and Lieve Laurens*, *National Renewable Energy Laboratory, USA*

Improving biomass production and utilization is imperative for commercializing a future algae-based biorefinery. Sterols are high-value products that can be isolated from algal biomass and have not been studied before in the context of a conversion pathway to biofuels and bioproducts and provide value as either nutraceuticals or as feedstocks for novel surfactant production. Mass spectral analysis indicates that there are 11 sterol compounds in Chlorella and 16 in Scenedesmus. The compounds we identified fall within two major classes: 28-carbon 4-desmethylsterols and 29-carbon 4-desmethylsterols. Within the 28-carbon group, the spectra either more closely resemble campesterol or ergosterol. Within the 29-carbon group, the spectra could be grouped into compounds with a MW of 484 (such as stigmasterol, fucosterol, Δ5-avenasterol) and those with a molecular weight of 486 (such as sitosterol or Δ7-stigmastenol). We present data on the development and thorough validation of a new, and much simplified, method based on direct acid transesterification for sterol characterization, derived from either steryl esters or glycosides in algae and highlight the growth phase specific accumulation of this class of compounds.

Analysis of Omega-3 Polyunsaturated Fatty Acids (PUFA) in Phospholipid Oils: A Design of Experiment Approach for Method Optimization Cynthia Srigley and Isa C. Orr-Tokle, *US Food and Drug Administration, USA*

Phospholipid oil dietary supplements, such as those containing krill or herring roe oils, have become increasingly popular alternatives to fish oil supplements due to the enhanced bioavailability of omega-3 polyunsaturated fatty acids (PUFA) that the phospholipid structure reportedly provides. Conventional methods for analyzing fish oils, which typically contain fatty acids in triglyceride or ethyl ester forms, have proven ineffective when used on the phospholipid oil matrix. Therefore, the objective of this study was to optimize a method for accurately quantifying fatty acids in phospholipid oils. Six different analytical methods were initially evaluated for their effectiveness in converting the fatty acids of a krill oil test material into fatty acids methyl esters for subsequent analysis by gas chromatography with flame ionization detection. The three methods which yielded the greatest amounts of total fatty acids were taken for further testing, upon which Official Method Ce 2c-11 of the American Oil Chemists' Society (AOCS) was found to produce the highest yield. A design of experiment approach was then performed to evaluate the effects of strength of reagents, presence and type of antioxidant, inclusion of acid hydrolysis procedure, and duration and temperature of heating reactions. Results indicated that AOCS Official Method Ce 2c-11 is the optimal procedure for accurately

quantifying fatty acids in phospholipid oil. This official method was then applied to the analysis of commercial phospholipid oil dietary supplements to evaluate their fatty acid compositions and to measure their eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) content.

Evaluation of an Ultra Inert WAX-phase Column for the Analysis of Fatty Acids and FAMEs

Gustavo Serrano Izaguire, Allen Vickers, Yun Zou, and Daron Decker, *Agilent, USA*

GC Columns with Polyethylene Glycol (PEG) stationary phases are commonly used for analyzing compounds with polar functional groups, and are well suited for food, flavor, and fragrances applications. A current challenge with traditional PEG phases, however, is the need to incorporate functional groups, such as nitroterephthalic acid, to separate challenging analytes like acidic organic compounds. These modifications, unfortunately, reduces column lifetime, maximum operating temperatures and are prone to react with some active analytes. Continuing with our recent advances in Ultra Inert (UI) technology, we are introducing a new PEG UI phase, specifically designed for the analysis of fatty acids in free and ester forms. This new GC Column (DB-FATWAX Ultra Inert), delivers superior inertness, better long-term thermal stability and greater sensitivity than any other traditional WAX column. The excellent peak shapes obtained for acidic compounds, eliminate the need to use two separate GC columns for the analysis of free fatty acids (FFAs) and their esters in the same sample. In this work, we present a variety of applications on the analysis of free fatty acids, fatty acid methyl esters (FAMEs) and fatty acid ethyl esters (FAEEs) using DB-FATWAX Ultra Inert; including determination of volatile FFAs from dairy products, analysis of Omega 3 and Omega 6 per AOCS Ce 1b-89, and FFAs and FAMEs in complex mixtures with other organic acids.

Trans-fat Determination by Gas Chromatography Vacuum Ultraviolet Detection

Jonathan Smuts*[1] and Barbara A. Mitchell[2], *[1]VUV Analytics, USA; [2]Covance Labs, Inc., USA*

Vacuum ultraviolet detection for gas chromatography (GC-VUV) has been applied to the analysis of fatty acid methyl esters (FAME). Here we specifically apply the technology for the quantitative determination of trans-fat in various food samples. Two GC columns are explored, 100% cyanopropyl and a PEG-based column. Linearity is demonstrated and then used to calculate relative response factors. These are then applied to a variety of food samples: fish oil, biscuit, baby formula, gummy vitamin and butter. Finally, quantitative GC/VUV results are compared to GC/FID.

ANA-P: Analytical Poster Session

1. **Purification of Native Cyanogenic Glycosides from Flaxseed** Veronique J. Barthet* and Tao Fan, *Canadian Grain Commission, Canada*

The four cyanogenic glycosides (linustatin, neolinustatin, linamarin, and lotaustralin) were purified from intact mature and germinated flaxseeds after extraction followed by a preparative HPLC step. The confirmation of the identification of the purified compound was done by GC-MS. A single preparation experiment generated from 3 to 14 mg of these cyanogenic glycosides. Individual cyanogenic compound was obtained in a pure and native form allowing the increase of analytical method accuracy, reliability, and sensitivity.

2. **Crystalline Pattern of Phytosterols in High Oleic Sunflower Oil for Food Applications** Mayanny G. Silva, Valéria S. Santos, Lisandro P. Cardoso, Maria Helena A. Santana, and Ana Paula B. Ribeiro, *University of Campinas, Brazil*

The incorporation of phytosterols in foods has been receiving an increasing attention, because they are considered functional compounds. The bioactivity of phytosterols resides in the fact that they have molecular structures very similar to cholesterol. Thus, a competitive absorption mechanism in the intestine is established between them, reducing blood cholesterol levels. The objective of this study was to evaluate the crystalline pattern of the analytical grade stigmasterol (SS) (99% purity) comparing to a commercial food grade free phytosterol mixture (FPM), as well as to investigate the crystalline behavior of FPM into high oleic sunflower oil (HOSO). The phytosterol profile of the FPM was characterized by AOCS method Ch 6-91. The powder X-ray diffraction (XRD) measurements were carried out for SS, FPM and FPM:HOSO blends at 0, 20, 50 and 100% FPM concentrations. The XRD analysis was performed at room temperature in a Philips PW 1710 diffractometer (PANalytical, Almelo, Netherlands), using Bragg–Brentano geometry (θ:2θ). The FPM profile presented 44.05% β-sitosterol, 26.77% stigmasterol, 23.56% campesterol, and 5.62% corresponded to minority compounds. The SS, FPM and FPM:HOSO blends showed similar XRD profiles, with very small differences in peaks displacement. The degree of crystallinity of the blends decreased with the increase of HOSO. In this way, the crystalline behaviour of phytosterols was not changed when it was applied as a food grade mixture in a lipid system (FPM:HOSO), even if it is compared to a pure phytosterol (SS).

3. **Thermal Properties and Solid Profiles of Hardfats-Soybean Oil Blends for Formulation of Lipid Carriers** Mayanny G. Silva and Ana Paula B. Ribeiro, *University of Campinas, Brazil*

Soybean oil (SO) is a widely available low cost raw material with high nutritional value. To increase its functionality for the formulation of different types of lipid carriers, its technological properties can be adjusted with the addition of hardfats, which are low-cost industrial products resulting from the total catalytic hydrogenation of liquid oils. The present work evaluated binary blends (50:50 w/w) of hardfats from palm kernel (PKO), palm (PO), soybean (SO), microalgae (MO) and crambe oils (CR) with SO, in relation to thermal behavior and solid fat content profiles

(SFC). Thermal analysis of the samples was performed by differential scanning calorimetry according to the AOCS method Cj 1–94. The SFC was determined by nuclear magnetic resonance, according to the AOCS official method Cd 16b-93; direct method, measurements in series, of 10 to 70ºC with interval of 5ºC. Melting point (TM) was obtained from the SFC data, considered as the temperature corresponding to a solids content of 4 g/100 g. At 25ºC, all blends showed a SFC between 17.8 and 51.4 g/100 g. These values decreased with the increase in temperature in a non-linear fashion until the complete melting between 33.7 and 67ºC. All blends showed large crystallization peaks, indicating diversity of the triacylglycerol composition. Multiple melting peaks were also observed, indicating recrystallization in more stable polymorphic forms. Blends of hardfats and liquid oils, therefor, stand out as a potential and low-cost option for the formulation of lipid carriers due to the possibility of wide variation in their physical properties.

4. **Comparative Recovery Analysis of Conjugated Linoleic Acids (CLA) Following Different Methylation Protocols** Yiyi Li, Raad S. Gitan, Deborah L. Chance, James K. Waters, and Thomas P. Mawhinney, *University of Missouri, USA*

Conjugated linoleic acids (CLAs) include a group of positional isomers of octadecadienoic acids (18:2) that are naturally occurring polyunsaturated fatty acids, synthesized in the rumen of animals, found in related meat and dairy products, and enhanced in grass-fed animals. Early in our investigations of these products, it became apparent that, depending on the methylation method employed, the quantitative analysis of the methyl ester of each CLA (FAME-CLA) was variably underestimated. To address this recovery concern of the major CLAs found in our samples, three different methylation procedures were evaluated with each of four CLAs (c9/t11, c9/c11, t9/t11, and t10/c12) as their methyl ester standards (>96%, Matreya). Treatments in methanol were: (A) sodium hydroxide/boron trifluoride (NaOH-BF3, 100^{o}C, AOCS, Ce 2-66), (B) 4% sulfuric acid (H2SO4, 80^{o}C, AOAC 965.49), and (C) 4% methanesulfonic acid (MSA, 60^{o}C, 2h). An Agilent GC equipped with a Supelco SP2560 column (100M x 0.25mm x 0.20µm film) was used for FAME analysis. Results show that the recovery of each FAME-CLA was significantly higher ($p<0.001$) when treated with MSA than with either H2SO4 or BF3. For (A) and (B), recovery of c9/c11 FAME treated with H2SO4 was significantly greater than with NaOH-BF3 ($p<0.001$), whereas, recoveries of c9/t11 and t10/c12 FAMEs were significantly greater with NaOH-BF3 than with H2SO4 ($p<0.001$). No significant difference was observed for t9/t11 FAME with either (A) or (B) treatment. The recovery data suggest that more accurate determination, via greater recoveries, of FAME-CLAs can be achieved with the use of 4% MSA in methanol.

5. **1H-NMR Measurement of Polar Phenolic Compounds: Reliable Determination of the Geographical Origin of Olive Oils** Torben Küchler* and Ole Winkelmann, *Eurofins Analytik GmbH, Germany*

The mislabelling of olive oil with respect to its geographical origin is a frequently encountered fraud. According to European Regulation (EU) No 29/2012 it is mandatory to declare the

geographical origin of an olive oil on the bottle, but so far no generally accepted analytical method exists to verify this labelling. Among the three main producing countries in the Mediterranean area, namely Italy, Greece and Spain, customers particularly value Italian olive oil. Presumably, olive oil from Greece and Spain is mislabelled and sold as Italian olive oil on a regular basis, as Italian olive oil is traded at a higher price. The 1H-NMR analysis of olive oil after dilution in CDCl3 has been exhaustively described in many scientific studies related to geographical origin already. Due to the inherently low sensitivity of NMR, this approach provides mainly information about the fatty acid profile and little information about minor components present in the sample like polyphenols. In our experience, this data can only be used to discriminate olive oil on a regional, but not on a country level. By employing a simple extraction with acetonitrile, a drastic enrichment of polyphenols and other polar compounds can be achieved. 1H-NMR analysis of these extracts can be used to classify olive oils according to geographical origin on the country level with striking success. Employing a database of about 1000 reference samples that were collected during the last five years, we are able to verify the geographical origin of olive oils from Italy, Greece and Spain with a high rate of success (> 95%).

6. **Using GC-MS and Helium to Resolve Positional Isomers of trans-C16:1 and trans-C18:1 Fatty Acids** Etienne Guillocheau*, Daniel Catheline, Philippe Legrand, and Vincent Rioux, *Agrocampus-Ouest, France*

Objective and hypothesis: Separation and analysis of positional isomers of trans-C16:1 and trans-C18:1 fatty acids usually rely on gas chromatography (GC), with flame-ionization detector (FID) and hydrogen as gas vector. Because hydrogen results in good peak resolution, it is unclear whether the use of mass spectroscopy (MS) and helium as gas vector would yield worse outcomes. Therefore, this study aimed at showing the abilities of GC-MS in combination with helium when dealing with positional isomers of trans-C16:1 and trans-C18:1 fatty acids.

Methods used: GC-MS in combination with helium was used. Both partially hydrogenated fish oil (PHFO) and dairy fat, derivatized as fatty acid methyl esters (FAME), were considered because of differences of trans-C16:1 and trans-C18:1 isomer distribution. To rule out the effect of stationary phase, two different GC columns were used. Optimized conditions determined in each case.

Results: Using optimized conditions, GC-MS and helium led to good resolution of trans-C16:1 and trans-C18:1 positional isomers, in respect with what can be obtained with hydrogen and FID. This held true for both dairy products and PHFO for which differences in profiles were clearly highlighted. Both GC columns provided similar resolutions.

Conclusion: GC-MS in combination with helium is suitable for analysis of positional isomers of trans-C16:1 and trans-C18:1 fatty acids.

7. **A Microscopy Study of the Structure of Njangsa and Other Selected Seeds: Method Development A Microscopy Study of the Structure of Njangsa and Other Selected Seeds: Method Development.** Benjamain M. Bougouneau[1], Michael Moore[2], Samuel A.

Besong[1], and Alberta N A Aryee[1], *[1]College of Ag. & Related Sciences, Department of Human Ecology, Delaware State University, USA; [2]Optical Center for Applied Research, Department of Physics and Engineering, Delaware State University, USA*

Njangsa seed contain about twice as much oil as soybean (45—67%), and high amount of the conjugated linoleic acid: alpha eleostearic acid. Black-eyed peas, and bush and Kent mango kernels are valued for their nutritional quality and potential antioxidant properties, respectively. Very little is known of their microstructure. Microscopy studies may reveal structure, packing, and composition of the cotyledon, which may provide information on the seed's susceptibility to enzymatic attack, stability under various processing conditions and pre-extraction techniques for efficient component recovery. After using a series of unsuccessful sectioning techniques, black-eyed pea, mango kernels, and Njangsa seeds were soaked in springwater to soften the embryonic tissue, fixed in 4% paraformaldehyde and vacuumed for 24 h. The fixed seeds were cut into 30-40 micron sections using a tissue chopper and placed on a small shallow dish with Calcofluor white (a stain that labels cellulose). The fluorescence created by the Calcofour white stain was animated under a 405-laser line on the confocal microscope, and starch granules were strongly polarized. DiOC-18(3) stain marked proteins and lipoproteins under a 488-laser line on the confocal microscope. Various features and cellular constituents, mostly small spherical protein, and oil bodies, and lipoprotein interspersed within the cytoplasmic network were visualized. Swelling and fixing seed tissue prior to thin sectioning is a feasible method for preserving the lipids in seed tissues. DiOC is a viable stain for identifying total lipids in hydrated fixed plant seeds, while confocal microscopy is a viable modality for localizing macro-molecules in hydrated seed sections. Conventional paraffin embedding techniques for processing seeds would strip away lipids, our method preserve lipids for microscopy analysis. This new method require minimal sample preparation protocols and analysis can be performed on a time scale of seconds.

8. **The LC-UV Analysis of 16 Cannabinoids of Interest in Commercially Available CBD Oils.**

Joseph D. Konschnik, Justin A. Steimling, and Ashlee M. Reese, *Restek Corporation, USA*

More than 100 cannabinoids have been isolated from cannabis in addition to the five most commonly tested: THC, THCA, CBD, CBDA, and CBN. While methods have been published that show the separation of these major cannabinoids, many do not take into account the possibility of interference from other cannabinoids that may be present. This is most problematic in concentrates where minor cannabinoids can be enriched to detectable levels that were not observed in the flower. Additionally, some terpenes have been shown to absorb UV light at 228 nm, the wavelength cannabinoids are typically detected, which can result in an additional source of interference. In this study, the LC-UV separation of 16 cannabinoids of interest was performed while monitoring for the potential impact from minor cannabinoids and terpenes on reported potency values. The method is applied to commercially available CBD oils that have recently become suspect due to inaccurate label claims.

9. **Rapid Measuring and Modelling Total Polar Compounds in Frying Oils using a Flash Gas Chromatography Electronic Nose** Lirong Xu[1], Li Xu[2], Qingzhe Jin[3], and Xingguo Wang[3], *[1]Jiangnan University, China; [2]School of Food Science and Technology, Jiangnan University, China; [3]Jiangnan University, China*

A flash gas chromatography electronic nose (FGC E-nose) was used for rapid measuring total polar compounds (TPC) in frying oil samples. TPC of frying oil samples was measured by the AOCS official method for reference. Qualitative discrimination between frying oil samples with different frying time was conducted using the FGC E-nose in combination with discriminant function analysis (DFA). The different TPC values in the samples were calculated by partial least squares regression (PLS) model from FGC E-nose. The DFA result indicated that the FGC E-nose could be used for differentiation of frying oil samples with different frying time. The results showed a good linear relationship (R2=0.98; SD=1.433) between TPC in oil samples measured by AOCS method and that estimated by PLS model, indicating that the proposed approach can be used as an alternative to AOCS method as an innovative tool for rapid measurement of TPC in frying oils.

10. **Electron Paramagnetic Resonance Spectroscopy Study of Milk Fat Globule Membrane Dynamics during Simulated Digestion** Maha Alshehab, Madhu S. Budamagunta, John C. Voss, and Nitin Nitin, *University of California, Davis, USA*

Lipid digestion is an interfacial process involving interactions of enzymes and bile salts with the lipid body interface. Milk Fat Globules (MFGs) with their unique interfacial structure and membrane composition are a key nutritional source for mammalian infants, however, there is a limited understanding of the dynamics of fat digestion in these structures. In this study, we have developed an Electron Paramagnetic Resonance (EPR) spectroscopy approach to evaluate real time dynamics of MFGs interfacial structure during simulated intestinal digestion. To measure these dynamics, MFGs were labeled with spin probes, partitioning of EPR probes into MFG membrane was validated using saturation-recovery measurements to estimate the depth parameter (Φ). After validation, the selected spin probe was used to evaluate MFG membrane fluidity as a measure of the interface's integrity in the presence of bile salts and pancreatic lipase. Independently, bile salts were found to have a rigidifying effect on the spin probed monolayer of the membrane, while pancreatic lipase resulted in an increase in membrane fluidity. Collectively, the effect of lipase appears to be diminished in the presence of bile salts. These results indicate the efficacy of EPR in providing an insight into small time scale molecular dynamics of phospholipid interfaces in MFGs. Understanding interfacial dynamics of naturally occurring complex structures can significantly aid in understanding the role of interfacial composition and structural complexity in delivery of nutrients during digestion.

11. **Infrared Spectroscopy and PLS Procedures for the Rapid Prediction of EPA and DHA Contents in Marine Oil Dietary Supplements** Sanjeewa R. Karunathilaka, Cynthia Srigley, Betsy J. Yakes, Sung Hwan Choi, Lea Brückne[1], and Magdi Mossoba[1], *[1]US Food and Drug Administration, USA*

Long chain omega-3 (n-3) polyunsaturated fatty acids are predominantly found in marine-

derived foods and dietary supplements, including fish oil (FO) supplements. Due to the potential health benefits, FO dietary supplements are now the third most commonly used supplements next to multivitamin-mineral and calcium-containing supplements. The high demand for these FO dietary supplements requires strict process control and quality assurance measures to monitor FA concentrations, which in turn need simple, fast, and accurate analytical techniques to verify product compositions. Infrared spectroscopy (IR) is a highly desirable analytical technique for process control and monitoring applications, due to its simplicity, limited sample pre-treatment requirement, and rapid measurement time. Two IR spectroscopic methods including Mid-Infrared spectroscopy (MIR) and Near Infrared spectroscopy (NIR) combined with chemometrics were evaluated to rapidly predict the FA contents, especially eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) of FO supplements purchased from US-based online retailers. A broad-based single calibration set that consisted of representative samples from a variety of marine oil sources was used to develop partial least squares regression (PLSR) calibration models. This simple, fast, and nondestructive quantitative method has the potential to be used for the rapid screening of marine oil products for verifying label declarations and also in quality control and monitoring during production.

12. **Applying High Speed Gas Chromatography for the Speciation of Fats in Foods and Edible Oils.** Joseph D. Konschnik, Colton Myers, Kristi Sellers, and Scott Adams, *RESTEK Corporation, USA*

The United States Food and Drug Administration (FDA) requires disclosure of trans fat content of conventional foods and dietary supplements. Sample preparation for the most common methods involves derivatization of the hydrolyzed free fatty acids, converting them over as methyl esters (FAMEs) followed by high resolution GC-FID analysis. Overall fatty acid composition can be determined with Polyethylene Glycol (PEG) capillary columns where double bonds of unsaturated fatty acids are mainly of cis configuration. Separation and differentiation of cis / trans structures require long length, highly polar columns containing biscyanopropyl stationary phases. GC methods are quite slow; AOAC 996.06 requires over an hour of GC run time per sample not including oven cool down. This work examines FAMES using a high-cyano containing capillary column by AOCS Method Ce-1j-07, AOAC Method 996.06, as well as other applications applying the use of shortened narrow bore columns, high carrier gas flows, and fast oven temperature programming as routes to reduce GC run times. The trade-off between separation performance and analysis time is explored with the conclusion that relatively complex mixtures of FAMES can be separated with greatly reduced analysis time.

13. **Buffer Optimization for Accelerated SDS Depletion by Transmembrane Electrophoresis in Top-down Proteomic Workflows.** Subin R.C.K. Rajendran[1], Khaldun Al Azzam[2], Nicole Unterlander[1], and Alan Doucette[1], *[1]Dept. of Chemistry, Dalhousie University, Canada; [2]Al-Ghad International College for Applied Medical Sciences, Saudi Arabia*

Top-down proteomics entails the analysis of intact proteins by mass spectrometry (MS). Detergents such as sodium dodecyl sulfate (SDS)

play essential roles in sample processing (eg protein isolation/extraction, cell lysis, maintaining solubility) ahead of MS. Unfortunately, SDS is detrimental to MS. While several strategies are available for SDS depletion, they suffer multiple concerns including incomplete SDS removal, inadvertent protein loss, or involving multiple complex and/or time-consuming steps. Recently, our group introduced an automated device for SDS depletion termed transmembrane electrophoresis (TME) (Kachuk, J. Proteome Res., 2016, 2634). With TME, SDS migrates under influence of an electric field as the protein remains confined in a sample cell behind semipermeable membranes. We achieve high protein purity (>99%) and high yield (>95%) in a relatively short (60 min) time frame. The present study aims to improve SDS depletion through examination of the influence of sample composition, ultrafiltration membrane, TME buffer properties (pKa, conductivity) and applied electric field on TME. Tris-tricine buffer system allows application of higher currents with reduced Joule heating, resulting in faster SDS depletion. TME therefore presents a rapid, single-step and robust tool for preprocessing samples ahead of proteomic analysis.

14. **Isolation and Identification of Stearidonic Acid Geometric Isomers.** Pierluigi Delmonte, Andrea Milani, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, USA

Seed oils rich in 18:4n-3 (Stearidonic acid, SDA) are marketed as a more sustainable, cost effective alternative source of omega-3 fatty acids compared to marine oils. The refining of these oils is expected to cause the geometric isomerization of the highly unsaturated 18:4n-3 to produce trans fatty acids (tFA). To date, there is no study describing the identification and gas chromatographic separation of the 16 geometric isomers of 18:4n-3. In this study we have isolated all the 18:4n-3 geometric isomers by silver ion HPLC (Ag^+-HPLC), using up to 6 chromatographic columns in series. Each geometric isomer was partially reduced with hydrazine to determine the geometric configuration of its double bonds. We have determined the gas chromatographic elution order of all 18:4n-3 geometric isomers utilizing a 100 m x 0.25 mm 100% poly(biscyanopropyl siloxane) capillary column maintained at the elution temperature of 180°C, and have investigated their occurrence in Ahiflower oil. The 4 mono-trans 18:4n-3 isomers produced during the refining of Ahiflower oil were chromatographically resolved from each other, but c6,t9,c12,c15-18:4 co-eluted with the tetra-cis isomer. These two fatty acids were resolved by reducing the separation temperature to 150°C, but this change produced tetra-cis 18:4n-3 to co-elute with t6,c9,c12,c15-18:4. Combining the results from two isothermal separations (180°C, 150°C) was necessary to quantify the 4 mono-trans 18:4n-3 fatty acids in Ahiflower oil.

15. **A Method for Analyzing TAGs Composition of Human Milk Fat using UPC2-Q-TOF-MS.** Xinghe Zhang[1] and Guanjun Tao[2], *[1]School of Food Science and Technology, Jiangnan University, China; [2]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, China*

Triacylglycerols (TAGs) of human milk fat (HMF) is one of the most complex lipids in nature. It's difficult to identify their categories and determine their content. We analyzed TAGs

in HMF using Ultra Performance Convergence Chromatography (UPC2) coupled with quadruple time-of-flight mass spectrometry (Q-TOF-MS). UPC2 separates TAGs with highest efficiency. While SURVEY method was used to collect MS/MS spectrum signal automatically by selective bombardment of specific parent ions, when MS spectrometry was carried on. Through this manner, different TAGs sharing the same retention time and mass charge ratio (m/z) was identified. The result indicated that 93 TAGs types of human milk were identified. The USU type of TAGs (unsaturated fatty acid at sn-1,3 and saturated fatty acid at sn-2 position) accounted for about 48.62%. There were 24 types of TAGs show content more than 1%. OPL (15.58±0.04%) was the predominant TAG in China human milk, followed by OPO (13.43±0.05%), OOL (8.30±0.01%), OLL (6.14±0.02%). And the results show significant difference between individuals. Some oil and fats, such as refined Bashar fish oil, lard, walnut oil, olive oil, peanut oil and soybean oil were analyzed by this method, showed that this method is suit for separating and analyzing TAGs from different sources.

16. **The Rapid Analysis of Terpenes in Cannabis.** Ron R. Honnold, *Agilent, USA*

There is a growing need for the analysis of terpenes in cannabis and cannabinoid concentrates and we are collaborating with major laboratories to rapidly develop high-end instrument configurations and methodologies that are robust, accurate and precise while still allowing for easy to implement workflows in high productivity laboratories. These methods must assure safety and quality and the list of terpenes that are to be analyzed is continuously being increased by state and local governing bodies. A simple configuration for this analysis connects headspace sampling techniques with GC or GC-MS systems. This presentation will discuss this system and methodologies for the rapid and robust analysis of terpenes in cannabis and cannabinoid samples. By optimizing flows and temperatures in both the GC and Headspace system and changing to better columns the methods are faster and more selective.

17. **HPTLC with Tandem MS and HR-MS for Structural Identification in Lipidomic and Other Complex Lipid Samples.** Vicente L. Cebolla[1], María P. Lapieza[2], Luis Membrado[1], Maria Savirón[3], Jesus Orduna[4], and Judith Nichols*[5], *[1]Instituto de Carboquimica/CSIC; [2]Instituto de Carboquimica/CSIC, Spain; [3]CEQMA/CSIC, Spain; [4]ICMA / CSIC; [5]CAMAG Scientific, Inc., USA*

We report that high-performance thin-layer chromatography (HPTLC), a popular technique for providing lipid-class separations, can also be useful for direct, rapid, and relevant structural identification of individual molecular species within each lipid class. After HPTLC separation of lipid mixtures using gradient-based AMD2, the zones of interest on the plate can be selectively on-line transferred to an ESI-MS instrument via an elution-based interface. MS/MS (MSn, using an ion-trap instrument) and HR-MS spectra (QToF) can be obtained because the respective sodium adducts were fragmented in the positive ion mode, and sodium remained the charge of their fragment ions. Likewise, semiquantitative / comparative profiles of individual molecular species in a lipid class can be obtained by ESI-MS, as:

i. ionization efficiencies are similar for molecular species belonging to a given lipid class;

ii. the development solvent is evaporated before detection;
iii. the interface elution solvent has a constant composition and, in this way, similar ionization efficiencies are obtained for the individual lipids in a class. This work focuses on profiling and identification of neutral lipids (NLs), sphingolipids (SLs) and phospholipids (PLs), in three analytical cases:
    1) mono (MGs) and diacylglycerides (DGs) (in positive ion mode) and fatty acids (FAs) (in negative ion mode) as impurities in a fatty acid methyl ester (FAME)-based biodiesel sample;
    2) molecular species of neutral sphingolipids (SL), such as sphingomyelins (SMs) and globotriaosylceramides (Gb3), in human plasma;
    3) phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and phosphatidyglicerols (PGs) associated to membrane proteins of photosynthetic purple bacteria.

18. **Identification of Degradation Products after Subcritical Water Hydrolysis of Hemp Oil using GC-MS and FTIR-ATR.** Andres F. Aldana Rico[1], Ruben O. Morawicki[1], Jerry W. King[2], Rohana Liyanage[2], Chris Mazzanti[1], Marco E. Sanjuan Mejia[1], and Antonio J. Bula Silvera[1], *[1]Universidad del Norte, Colombia; [2]Critical Fluid Symposia, USA*

When water reaches the subcritical region, changes in the dielectric constant and ionic product improve its hydrolysis capabilities of non-polar compounds such as lipids. However, if the applied temperatures or exposure times are excessive, molecule decomposition due to thermal degradation can occur. This research aims to identify byproduct of thermal decomposition species of hemp seed oil hydrolyzed under subcritical water conditions using FTIR-ATR and GC-MS analytical techniques. The experimental set up consisted of two syringe pumps that injected controlled amounts of degassed water and hemp seed oil through a stainless-steel coil reactor pressurized by a manual backpressure regulator and maintained at specific temperatures inside a temperature-controlled oven. After cooling in an ice bath, samples were collected from the reactor exit stream, centrifuged, and the oil layers used for analyses. FTIR-ATR analysis was done using 100 repetitive scans to determine the absorbance readings over 14 specific wavenumbers looking for probable different chemical functionalities. GC-MS analysis was performed to identify remaining fatty acids and their decomposition products. FTIR-ATR analysis showed that elevation of reaction temperatures favored the formation of aldehydes and fatty acid dimers as judged by the intensity of absorbances at 1700 and 900 cm (-1) wavenumbers respectively, which significantly increased above 200°C. This was confirmed by the GC-MS analysis that based on the retention time correlations and spectra identified the probable presence of long-chain aldehydes, such as cis,cis,cis-7, [1]0, [1]3-hexadecatrienal, 9, [1]2, [1]5-octadecatrienal, and polymerization products like dimers of palmitic (C16:0) and linoleic (C18:2) acids.

19. **Analysis of Heavy Metal Concentrations and Human Exposure from Hemp Oils and Hemp Products.** Patricia Atkins and Sean Curran, *SPEX CertiPrep, USA*

The cannabis industry has flooded the market with new products in need of analysis with few regulations to govern the industry. Recently, concerns have arisen about safety of this, unregulated market. Sadly, a potentially

significant group of contaminants has been largely ignored: toxic metals. Federally legal or quasi-legal hemp products (hempseed oil, hemp extracts, CBD oil & extracts), those without THC, are widely available on the market. Such products are also used as base oil for the addition of cannabis & cannabinoid extracts. However, due to a ban on cultivation in the US, virtually all the hemp used is imported from other countries. Studies of other consumable commodities (spices, teas, grains, etc.) exported from around the world have reported widespread heavy metal contamination. Cannabis plants (hemp & recreational varieties) are bio-accumulators of heavy metals. In the processing of agricultural products, a large amount of plant material is consumed to extract concentrates and oils, thereby increasing the risk of heavy metal contamination. The scope of this study was twofold; firstly, to analyze various legal hemp products currently on the market and, secondly, to use these as a model for methods development for testing of restricted products. Samples were digested using microwave digestion and analyzed by ICP-OES and ICP-MS to determine the elemental composition of these products and the concentration of potentially toxic heavy metals. A large number of the tested hemp products detected heavy metal contamination.

20. **Fatty Acid Analysis with Applied Retention Time Locking.** Barbara A. Mitchell, Scott Wejrowski*, Youa Herr, and Thomas Vennard, *Covance Labs, Inc., USA*

For labeling and health concerns, the analysis of food products for saturated fat, trans fat, and total fatty acids is now routine. However, when dealing with a wide variety of matrices, the chromatography can be complex. Due to the uniqueness of each new column and their use for extended periods of time, minor differences in the chromatography can occur. Specifically, there can be fluctuations in elution order and resolution. These potential issues can affect efficiency in obtaining results. To improve productivity and quality, we designed a practice to maintain similar retention times across GC systems using a feature called retention time locking. Because fatty acids profiles contain multiple peaks and often have temperature ramps, perfect alignment of retention times is difficult. Analysis of the retention time profiles for 15 columns of varying time of use showed that each column had a unique pattern. However the flow rates required to match target retention times leveled off after the elution of 9c, 12c, 18:1 linoleic methyl ester. Taking advantage of this tendency, a simple formula to update column flow rates was created using only two marker peaks. Whether a lab contains one GC used for many different assays or many GC's dedicated to fatty acid analysis, this best practice allows the analyst to quickly adjust column flow rates to achieve uniform retention times after maintenance or installation of a new column.

21. **Analysis of Vitamin D and Previtamin D in Food Products.** Jinchuan Yang, *Waters, USA*

Vitamin Ds are essential human nutrients that are responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. The two most common forms of vitamin D in the diet are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin Ds can thermally interchanged to previtamin D forms. The previtamin Ds are also bioactive, and should be counted as part of the

vitamin D content. Recently, the FDA required that vitamin D content must be declared on all food labels (1). The analyses of vitamin D in foods often measure the main forms (D2 and D3) only. The previtamin D forms are often not measured. This could cause some error in the vitamin D content. Recently, we developed a LC-MS based method that can measure the vitamin Ds and the previtamin Ds. (2) This method has been applied to a number of foods. The performance of the analysis method will be discussed. Reference: 1) Food labeling: Revision of the nutrition and supplement facts labels, US Federal Register Vol. 81, No. 103, May 27, 2016 2) J. Yang, G. Cleland, K. Organtini, Analysis of vitamin D and previtamin D in food products, Waters app note 720006064en, July 2017.

22. **Unique GC Column Selectivity for Time and Cost-efficient Separation of Complex cis/trans Fatty Acid Methyl Esters in Food.** Ramkumar Dhandapani, *Phenomenex, USA*

Testing of Fatty Acid Methyl Esters (FAMEs) not only allows for authentication of oil products, but also serves as an indicator of any adulteration. Complex FAMEs including cis/trans fatty acids, however, have historically been challenging to separate in a reasonable period of time. Commercially available cyano phase GC columns traditionally have had analysis times of nearly an hour and utilized column lengths up to 100 meters, which presents a costly, time-consuming analysis process. In this study, we present a unique cyano-based GC stationary phase, Zebron ZB-FAME, which is optimized to improve performance for complex FAMEs analysis. Samples of commercially available canola oil, coconut oil, olive oil, and walnut oil were derivatized using a BF3-methanol reagent to convert the fatty acids to FAMEs. These derivatized oils were then analyzed on several on commercially available cyano phases, and evaluated for time- and cost-efficiency. The unique selectivity of the ZB-FAME column presented in this work provided shorter run times using a cost-effective 30-meter length, while also providing complete resolution of a 37-component FAMEs mixture. In addition to these optimizations, we discuss the possibilities of post-run bake out and column lifetime improvement offered by the column's high upper temperature limit.

23. **New Method for Fast and Straightforward Determination of Oxidation Stability of Fats and Oils.** 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. An exemplary shelf-life investigation of pure and encapsulated flavor oils is presented showing the impact of the optimum encapsulating system. The significantly reduced measurement time and a high repeatability of the method represent the major advantages of our method, allowing for quick and direct measurement of the oxidation stability for research, process and test bench control.

24. **FET Analysis of Solvents in Cannabis Oil: Adapting to Changing Regulations.** Amanda Rigdon[1], Anne Jurek[2], Julie Kowalski[3], and Frank Dorman[4], *[1]Emerald Scientific, USA; [2]EST Analytical, USA; [3]Trace Analytics, USA; [4]Pennsylvania State University, USA*

Cannabis is a large and growing market, legal in 28 states with $6B in revenue in 2017. A growing product in the industry is cannabis concentrates. In addition to simple vaporization by the end user, cannabis concentrates may be used to prepare alternate dosage forms such as tinctures, topical salves, and transdermal patches. Like all pharmaceutical products potentially harmful residual solvents from the manufacturing process must be minimized and monitored. While dissolution methods for residual solvents analyses exist (e.g., USP), complex matrices such as cannabis concentrates usually require matrix-matched standards to ensure accurate quantification. Through the use of full evaporation technique-headspace gas chromatography, accurate quantification for residual solvents can be achieved in concentrates without the need for matrix-matched standards. Established in 2013, the industry-standard FET method was developed for compatibility of existing regulations at the time, which had most solvent limits set at < 100 ppm. Current regulations now require a quantification range of 1–5000 ppm. Multiple Headspace Extraction (MHE) experiments were conducted on hemp oil, and matrix interferences were evaluated over a range of concentrations. Linearity was evaluated using multiple split ratios in order to cover the large concentration range required for analysis. Storage and handling stability of high-level standards was performed. Matrix interferences were determined to be minimal. Linearity was acceptable over a range of 10–5000ppm, using FID detection and 1–5000ppm using MS detection. Solvent standard stability is acceptable over several uses.

25. **Fast Simultaneous Determination of Capsaicin, Dihydrocapsaicin and Nonivamide for Adulteration in Edible and Crude Vegetable Oils Coupled with UPLC-MS/MS.** Chuan Zhou, Dianping Ma, Wen Ming Cao, Hai Ming Shi, and Yuan Rong Jiang, *Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd., China, China*

Capsaicinoids are pungent components in hot peppers, which have been detected in waste cooking oil. However, trace analysis of capsaicinoids in edible and crude vegetable oils is a challenging task due to the complex matrix. In this study, a simple liquid-liquid extraction and solid phase extraction (SPE) coupled with RP-UPLC-ESI-MS/MS method was developed for the quantification of capsaicinoids in edible and crude vegetable oils to screen the adulteration with waste cooking oil. This method was used to simultaneously determine 3 capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) with capsaicin-d_3, and

dihydrocapsaicin-d_3 as internal standards. This allows the complete analysis of a sample with only an hour of time, even with sample pretreatment and chromatography. The linear range of 3 capsaicinoids ranged between 0.5 and 40 µg/kg. The limit of detection (LOD) and limit of quantification (LOQ) for capsaicinoids were calculated as 0.15 and 0.5 µg/kg, respectively. Quantitative recoveries ranging from 92.9% to 105% were obtained by the analysis of spiked oil. The relative standard deviations were less than 5% (n=6). The established method could potentially overcome the interference of triacylglycerols and fatty acids in edible and crude vegetable oils and have been successfully applied to analyze real oil samples. This method provides a rapid and reliable method for the detection of adulteration with waste cooking oils in vegetable oils.

26. **Determination Polycyclic Aromatic Hydrocarbons in Tocopherol and Ether Compound by Gas Chromatography Tandem Mass Spectral.** Tong Li, Ruifeng Zhang, Chuan Zhou, Hong Yang, Wen Ming Cao, and Yuan Rong Jiang, *Wilmar Biotechnology Research & Development Center (Shanghai) Co., Ltd., China*

The novel methods have been developed and confirm for determination of four polycyclic aromatic hydrocarbon (Chrysene, benzi(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene) in tocopherol products, including mixed tocopherol, tocopheryl acetate, and tocopheryl succinate. For mixed tocopherol and tocopheryl acetate, they were extracted by dimethylsulfoxide-methanol solution. About tocopheryl succinate, it was purified by Molecularly Imprinted Polymers after saponification. The methods have been applied and validated in GC-QQQ with MRM mode. The limit of quantitation was decreased to 1.09-3.31 µg/kg and recovery range from 73%-123%. Compared with previous methods, it has simple pretreatment process and stable performance that relative standard deviation was controlled under 3%. The methods on the four PAHs can be satisfied almost of tocopherol products quality control requirements.

27. **A Primary Animal Fat Adulteration Application: Determination Branched Chain Fatty Acid in Beef and Mutton Tallow with GC-Q-TOF & GC-FID and Evaluation.** Tong Li, Peijin Tong, Hong Yang, Wen Ming Cao, and Yuan Rong Jiang, *Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Branched chain fatty acid (BCFA) is characterized fatty acid in ruminant animal oil, special for beef and mutton tallow. Methyl-12-methyltetradecanoate, methyl-13-methyltetradecanoate, methyl-14-methylhexadecanoate, and methyl-15-methylhexadecanoate are typical branched chain fatty acid and widely distributes in beef mutton tallow. it ultilize Methyl undecanoate as internal standard and was methyl-esterified with KOH-menthol solution, and qualitied by GC-Q-TOF, and then, determined by GC-FID. Meanwhile, the method has been compared three common GC column and evaluated separation of branched-chain fatty acid, including SP-2560, from SUPELCO, HP-88 and CP-Sil 88, from Agilent. As the result, SP-2560 has the best performance in separation BCFA. This method preform well linearity and range from 5 mg/kg to 100 mg/kg, and detected limitation was from 1–1.5 mg/kg; quantity limitation range from 1.5–4 mg/kg. In beef tallow-palm oil binary blending system, using the signal BCFA as index to calculate

adulteration proportion, when ratio of beef tallow is more than 70%, the relative error can be lower than 10%.

28. **A Novel Method for Quantitative Analysis of Blend Oil Based on GC-FID and NPDA.**
Peijin Tong, Hong Yang, Wei Ting Ting, Tong Li, Wen Ming Cao, and Yuan Rong Jiang, *Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Edible blend oil, which is mixed with a variety of oils according to the physiological metabolic requirements, makes up for the deficiency of nutritional function structure of single species edible fat, as well as improving the stability and functional performance. Qualitative analysis of edible blend oil is still a big challenge, not to mention quantitative analysis. A novel method for simultaneously identification and quantification of edible blend oil based on novel none preconditions database algorithm(NPDA) was developed. The database of triacylglycerols (TAGs) collected by GC-FID contains five common oil species (614 samples in total). 20 groups of binary and ternary blend oils were analyzed and processed separately, showing a robust result with the accuracy of 88.14%. 15 binary and 10 ternary blind samples prepared were analyzed by the developed method, and the accuracy was 92% with the relative errors less than 20%. This method is based on a simple analysis to determine the composition of TAGs with the most prominent advantage of quantifying the contents of different type of oils in edible blend oil without knowing the composition of the oil species in advance, showing a bright future in quick segregation and quality control of edible blend oil in routine analysis.

29. **Extending GC Column and Detector Lifetime Using Cartridge Style Gas Management Filters**
Ramkumar Dhandapani*, *Phenomenex, USA*

Gas chromatographic separation relays on purity of carrier and detector gases. Impure gases can detector stabilization issues, baseline disturbance, peak tailing due to exposed active surface and column bleed. To prevent these troubleshooting issues from happening, cartridge style gas filters and gas traps were designed which were easy to install and easy to replace. Unlike traditional style gas traps, these filters provided a sensitive color indicator that changes color sharply indicating the saturation of cartridge. To prove the significance of carrier gas purity, experiments were performed in gas chromatography with and without gas filters and the results showed raised base line and excessive column bleed. Hence, the use of cartridge-based gas management is proven to be essential part of gas chromatography analysis to extend GC column and GC detector's life time.

30. **Research on Nutrient Components of Camellia Oil and Olive Oil in China**
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China is the earliest country in the world cultivating Camellia oleifera with a cultivating history of 2000-3000 years. In 1960s, Olea europea was imported and cultivated firstly in China. The oil nutrient components of Camellia oleifera and Olea europea cultivated in China including fatty acid, triglyceride, tocopherol, squalene and sterol were investigated. The main fatty acids in Camellia oleifera seed oil (camellia oil) were oleic (74.31%–83.65%), palmitic (7.12%–9.57%), and linoleic acid (5.43%–9.83%). The percentage of mono-unsaturated fatty acid

in camellia oil was 77.50%–84.44%, while 8.67%–12.04%, 5.58%–10.24% of saturated and poly-unsaturated fatty acid. The major triglycerides were OOO, SLO and LOO, of which OOO content was the highest (81.23%). α-Tocopherol was the main composition of total tocopherol and presented a content of 90% (116–749mg/kg). Squalene in camellia oil was 21–336 mg/kg. Sterol was 1099-2298mg/kg, of which β-sitosterol and Δ7-stigmastenol were the main composition. Oleic (65.85%-80.12%) and linoleic acid (2.55%–17.22%) were the main fatty acids in Olea europea oil (olive oil). Triglycerides in olive oil mainly included OOL, POL+SLL, OOO, SOL, POO, SOO, and POS+SSL. OOO and SOL were 52.77%–72.19%, 24.47%–40.18%, respectively. The α-tocopherol content was 109.38–310.50 mg/kg and 2.43–33.98 mg/kg of γ-tocopherol. Squalene in olive oil was 1913.04–7042.63 mg/kg. Sterol was above 1000 mg/kg in olive oil, of which β-sitosterol with 84.52%–87.08% and Δ5-avenasterol with 6.09%–8.23% were the main component.