ANA 1: Advanced and Rapid Techniques in Lipid Analysis

Chairs: William Byrdwell, USDA, ARS, BHNRC, FCMDL, USA; *and Bernd W.K. Diehl,* Spectral *Service AG, Germany*

A Routine, Rapid Analytical Method for Identifying and Quantifying Triacylglycerol Mixtures Sarah E. Mayfield*[1], Rohana Liyanage[2], Jackson O. Lay[2], Andrew Proctor[1], and Koen Dewettinck[3],[1]*Dept. of Food Science, University of Arkansas, USA;* [2]*University of Arkansas, USA;* [3]*University of Gent, Belgium*

Edible oils are often described in terms of fatty acid composition, but their nutritional and sensory properties can differ based on how they are arranged in TAGs. Therefore, it would be helpful to have a routine, rapid analytical method for identifying and quantifying TAGs in many edible oils. The use of chromatographic separation prior to ionization and MS detection greatly simplifies the identification and quantification of TAGs, but previous LC-MS methods have either required long gradient run times or multiple product ion scans and thus were inappropriate for rapid, routine analyses. Therefore, the objective of this study was to develop an LC-MS method for the routine identification and quantification of TAGs in edible oils that involved rapid, effective chromatographic separation and MS detection, leading to direct and reliable data analysis. Various edible vegetable oil TAGs were separated using two C18 columns in series with an isocratic gradient and ionized and detected by ESI-MSn. The chromatographic conditions achieved sufficient TAGs resolution with a run time of 40 minutes, simplifying analysis of mass spectral data with a significantly shorter total analysis time than previous methods. TAGs were identified based on m/z values and fragmentation patterns and relative quantification was done using chromatogram peak areas. This method rapidly provided accurate TAGs profiles and produced high precision among replicates with direct and reliable data analysis, and therefore has the potential for routine analyses of multiple samples.

Analysis of Vitamin D in Food Jinchuan Yang*, Kari Organtini, and Gareth Cleland, *Waters, USA*

Vitamin Ds are essential human nutrients 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). There is a concern that the intake of vitamin D among some American population groups are inadequate. Recently, the FDA required that vitamin D content must be declared on all food labels(1). The analysis of vitamin D in food often involves saponification, extraction, clean-up, and HPLC. One of the challenges in vitamin D analysis is the tedious sample preparation and the interference from the sample matrix. Recent developments in vitamin D analysis include the use of a derivatization agent, 4-phenyl-1,2,4-triazole-3,5-dione, or PTAD, to simplify the sample prep, to reduce the interference and increase sensitivity for vitamin D analysis. In this study, the use of PTAD for the vitamin D analysis by UPLC-MS is investigated. Another technique, UltraPerformance Convergence ChromatographyTM (UPC2), will also be explored.

Comprehensive Two-dimensional Liquid Chromatography with Quadruple Parallel Mass Spectrometry, LC1MS2 x LC1MS2 = LC2MS4 William C. Byrdwell*, *USDA, ARS, BHNRC, FCMDL, USA*

Comprehensive two-dimensional liquid chromatography typically uses two LC systems with different stationary phases that employ orthogonal

retention mechanisms (often silver-ion chromatography followed by reversed-phase (RP) chromatography) to produce a more thorough separation than is possible by one technique alone. Detection is usually done by UV detection and mass spectrometry at the outlet of the second-dimension column, which requires numerous 2nd-D samplings across the 1st--D peak to recreate the complete profile in the 1st dimension, to avoid "under-sampling" that leads to poor chromatographic profiles. We have employed non-aqueous reversed-phase (NARP) HPLC as the first-dimension separation coupled to a lab-made ultra-high-performance liquid chromatography (UHPLC) silver-ion column, made by a new technique, for silver-ion UHPLC as the second dimension separation. A wireless communication contact closure system has been used to coordinate all instruments and detectors via remote wireless communication. Dual parallel mass spectrometers (LC1MS2) plus UV, fluorescence, corona CAD, and ELSD detection methods are used to monitor the first dimension, coupled with dual parallel mass spectrometers (LC1MS2) plus UV detection methods used to monitor the second dimension, for eight detectors overall constituting comprehensive two-dimensional chromatography with quadruple parallel mass spectrometry (LC2MS4).

Differential Ion Mobility Spectrometry Dramatically Improves the Specificity of Untargeted Lipidomics Analysis by "Shotgun" Analytical Approaches Paul RS Baker*, *SCIEX, USA*

The importance lipid mediators play in host immune inflammatory responses has translated into efforts to identify and quantify lipid molecular species and explore their relationships to human disease. Lipid identification starts with global lipid profiling strategies using electrospray ionization mass spectrometry (ESI-MS/MS). The complex data arrays generated during sample analysis can then be processed by lipid identification software and principal component analysis to generate candidate lipid biomarkers, which can then be validated by targeted quantitative analysis. Despite this seemingly straight-forward means to lipid biomarker discovery, the actual process is quite challenging due to the high number of lipid isobars and isomers that interfere with qualitative and quantitative analysis. Recently, Differential ion Mobility Spectrometry (DMS) coupled to mass spectrometry has been shown to be very effective at resolving complex mixtures of lipid isomers without the need for extensive chromatography. In this report, DMS was used in tandem with a TripleTOF® instrument to generate untargeted lipidomics data via infusion in the MSMSALL scan mode (a universal "shotgun lipidomics" approach). The DMS enabled class isolation during analysis that removed isobaric interference during MS/MS experiments and provided clear identification and quantitation of the molecular species within each lipid class. Additionally, it was observed that different adducts within the same lipid class (e.g., chlorine Vs. acetate adducts of PC) can also be resolved, effectively solving a particularly challenging problem in infusion-based lipidomics. In summary, the combination of DMS with the MS/MSALL workflow provides a powerful means to improve the specificity of untargeted lipidomics analysis and increase confidence in lipid identification and quantification.

The Thermo Q Exactive LC-MS instrument combined with LipidSearch Software: Application of a Chromatography-based Workflow to Analysis of Lipid Extracts from Seeds and Plants Daniel J. Gachotte[1], Yelena Adelfinskaya[1], Jeff Gilbert[1], Yasuto Yokoi[2], Yukihiro Fukamachi[2] and David A. Peake[3] , [1]Dow AgroSciences LLC, USA; [2]Mitsui

Knowledge Industry CO, LTD, Japan, [3]Thermo Fisher Scientific, USA

Plant lipids are a diverse and complex family of molecules typically characterized by their solubility in organic solvents. In this presentation, we describe the efficient extraction of such molecules and their separation by High Performance Liquid Chromatography (HPLC) using a C30 phase manufactured by Thermo Fisher Scientific (Acclaim™). The detection of the separated lipids was supported by a high-resolution mass spectrometer (Thermo Scientific Q Exactive Plus). The identification was based on high resolution accurate mass (HRAM) MS and corresponding MS/MS spectra to further characterize the acyl chains of the lipids. The recorded product ion accurate masses were compared to a database of known lipid ions and their predicted fragment ions to identify the corresponding lipid species. Data analysis was performed using Thermo Scientific LipidSearch™ software which allowed the batch processing of HRAM LC-MS and MS/MS data for identification and relative quantification. The results were normalized using class specific internal standards. Arabidopsis leaf lipids were extracted and analyzed via the HRAM LC-MS workflow. The efficiency of extraction and overall variability (biological and analytical) were calculated for all 225 lipids detected consistently. Chromatographically challenging acidic lipids such as phosphatidic (PAs) and lyso-phosphatidic acids (LPAs) were derivatized to allow more reproducible detection and quantification. Several molecules typical of plant lipids were added to the library (methylated PA, phytosteryl-glycosides and -acylglycosides). The glycerophospholipid content of sunflower seed oil extracted with different methods was assessed directly in the total oil. Finally, a collection of vegetable oils was analyzed to compare their TAG profiles.

Improvement of Current Official Methods for the Analysis of Non-hydrogenated High Value Oils
Pierluigi Delmonte*, Andrea Milani, and Shivani Bhangley, *US Food and Drug Administration, USA*

Recent changes in the regulatory status of partially hydrogenated oils and the desire for more nutritious foods have recently reshaped the composition of vegetable oils on sale in the US market toward a higher content of mono-unsaturated and poly-unsaturated fatty acids (MUFA, PUFA). Methods previously optimized for the quantification of trans fatty acids (TFA) in partially hydrogenated vegetable oils need to be revised and optimized for the measurement of TFA derived from the geometric isomerization of PUFAs during the refining process. This presentation will focus on the determination of the most suitable gas chromatographic conditions for the analysis of non-hydrogenated high value vegetable oils, by comparing separations provided by different stationary phases and elution temperature profiles. Elution patterns and the identity of unknown fatty acids contained in specific oils were studied by two dimensional gas chromatography, two dimensional gas chromatography with online reduction, high resolution mass spectrometry, and high performance liquid chromatography combined with gas chromatography.

Rapid and Sensitive Detection of Free Fatty Acids in Edible Oils Based on Simple Chemical Derivatization Coupled with Shotgun-electrospray Ionization-tandem Mass Spectrometry
Ming Liu[1], Fang Wei*[2], Xin Llv[1], Xu-yan Dong[1], and Hong Chen[1], *[1]Chinese Academy of Agricultural Sciences, China; [2]Oil Crops Research Institute, CAAS, China*

This study proposes a strategy based on simple chemical derivatization coupled with neutral loss scan-electrospray ionization-tandem quadrupole mass spectrometry (NLS-ESI-MS/MS) for rapid and sensitive detection of FFAs in edible oils without

complicated sample purification and enrichment was developed. A derivative reagent (N, N-diethyl-1, 2-ethanediamine, DEEA) was employed to selectively label carboxyl groups of FFAs to form an amino compoundwith a tertiary amino group, which enabled to analyze the DEEA derived FFAs under the positive ion mode of MS. The DEEA derivative products could lose characteristic neutral loss fragment of 73 Da in collision-induced dissociation (CID), which enabled us to discriminate and analyze the DEEA derived FFAs with neutral loss scan mode (NLS 73Da). After derivatization, the detection sensitivities of FFAs increased by 2200-6670 folds compared with underived FFAs detected with MS in negative ion mode with the limit of detection and quantitation were 0.1-0.5 nmol/L and 0.3-2.0 nmol/L, respectively. The established method was applied to determine dynamic FFA formation in seven types of edible oils subjected to a microwave heating treatment test. By using this method, oil samples can be simply diluted by n-hexane, derivated with DEEA, and then directly infused and analyzed by NLS-ESI-MS/MS without complicated sample purification treatments. The results showed that the abundance of oleic acid (C18:1) and linoleic acid (C18:2) in all investigated oil samples except linseed oil (LS) and sesame oil (SO) had significantly changes during microwave heating treatment; and lineoleic acid (C18:3) dramatically increased in all the C18:3 containing oil samples except corn oil.

Determination of Peroxide Values of Edible and Marine Oils by Nuclear Magnetic Resonance Spectroscopy Elina Zailer*, Bernd W.K Diehl, and Sascha Wiedemann, *Spectral Service AG, Germany*

Oil oxidation is an undesirable complex series of chemical reactions degrading the quality of the oil product. Primary oxidation processes mainly form hydroperoxides which are measured as the peroxide value (PV) by official titration methods or alternative techniques like the near-infrared spectroscopy. Being an indicator of the initial stages of oxidation, PV is one of the most frequently determined quality parameter during production, storage, and marketing. Very fresh oils show just a small PV leading to a demand of a very sensitive method for quantifying peroxides at low concentration levels. A novel and rapid method for measuring peroxides in edible and marine oils by using the NMR spectroscopy was developed. The method was based on the reaction of triphenylphosphine (TPP) with peroxides producing triphenylphosphine oxid (TPPO). The recalculation of the ratio of non-reacted TPP and TPPO provides a simple means for determining PV in several oil based matrices. The NMR method was not affected by the oil type and can be successfully applied to a variety of edible and marine oils. The proposed method is accurate, simple, and could be used as an alternative to the conventional method for both qualitative and quantitative analyses of PV in edible and marine oils.

Determination of Peroxide Values of Krill Oil and Lecithins by Nuclear Magnetic Resonance Spectroscopy Elina Zailer*, Bernd W.K Diehl, and Sascha Wiedemann, *Spectral Service AG, Germany*

Oil oxidation is an undesirable complex series of chemical reactions degrading the quality of the oil product. Primary oxidation processes mainly form hydroperoxides which are measured as the peroxide value (PV) by official titration methods or alternative techniques like the near-infrared spectroscopy. Being an indicator of the initial stages of oxidation, PV is one of the most frequently determined quality parameter during production, storage and marketing. Researches proved that krill oil has an impact on the oxidation stability leading to a slower peroxide formation process and very low PV. Such low PV demands a very sensitive method for quantifying peroxides at

low concentration levels. A novel and rapid method for measuring peroxides in krill oil and lecithins by using the NMR spectroscopy was developed. The method was based on the reaction of triphenylphosphine (TPP) with peroxides producing triphenylphosphine oxid (TPPO). The recalculation of the ratio of non-reacted TPP and TPPO provides a simple means for determining PV in several oil based matrices. The NMR method was not affected by the oil type and can be successfully applied to a variety of edible and marine oils. The proposed method is accurate and simple and could be used as an alternative to the conventional method for both qualitative and quantitative analyses of PV in krill oils and lecithins.

ANA 1.1 / LOQ 1b: Evaluation and Prediction of Oxidative Stability and Shelf-life

Chairs: Hong-Sik Hwang, USDA, ARS, NCAUR, USA; and Min Hu, DuPont Nutrition & Health, USA

Analyzing Multiple Lipid Oxidation Products—Required, or Not? Karen M. Schaich*, *Dept. of Food Science, Rutgers University, USA*

With increasing use of polyunsaturated lipids in foods, there is increasing challenge to prevent oxidation and loss of beneficial health effects as well as to maintain food quality. In addition, as BHA and BHT are replaced by natural antioxidants, resulting oxidation in foods must be tracked accurately to assess effectiveness of new formulations. Traditional approaches to analyzing lipid oxidation focus mostly on hydroperoxides and perhaps also volatile products such as hexanal. However, routine analyses of multiple lipid oxidation products from alternate competing pathways, generating epoxides, dimers, and hydroxylipids are almost never performed and detailed information about volatile products other than hexanal is seldom collected. These practices underestimate extent of lipid oxidation and misses important products, particularly epoxides and hydroxylipids, that have toxic potential and provide evidence for shifted mechanisms of lipid oxidation. This paper discusses these issues in the context of mechanism and antioxidant studies, with consideration of new assays for epoxides and hydroxylipids.

Explaining the Polar Paradox and Cut-off Effect for AO Distributions and Reactivity in Emulsions Laurence S. Romsted*[1], and Carlos Bravo Diaz[2], *[1]Rutgers University, USA; [2]University of Vigo, Spain*

The polar paradox and cut-off effect are two important general observations about antioxidant (AO) efficiencies and distributions in surfactant-based emulsions that have, until recently, evaded clear explanation. Both problems were solved using the chemical kinetic method. The results provides values for the distributions of AOs of different alkyl chain lengths between the aqueous and interfacial regions and the oil and interfacial regions of emulsions and also provide a method for comparing AO efficiencies in different emulsions. In addition, a maximum appears naturally in the distributions of AOs with increasing alkyl chain lengths that depends on AO hydrophobicity, matches AO efficiencies, and explains the cut-off effect. The chemical kinetic method is based on the use of a chemical probe and the results are interpreted using pseudophase kinetic models that were originally developed in homogeneous association colloids, but also work in kinetically stable emulsions for two reasons: (a) in kinetically stable emulsions, the totalities of emulsified surfactant, oil and aqueous regions act as separate reaction regions, i.e., the observed reaction rates depend on the total volume of each region and not on droplet size; and (b) reactant distributions are in dynamic equilibrium between the oil, interfacial and aqueous regions. The talk will include: (a) the logic and basic assumptions of the pseudophase kinetic model as applied to emulsions; (b) the important properties of the chemical probe, a hydrophobic arenediazonium ion; (c) a brief description of the dye derivatization method for monitoring reactions in emulsions; and (d) the explanation for the cut-off effect.

Oxidative Stability and Shelf-life of Bulk Oils and Fats Min Hu*, *DuPont Nutrition & Health, USA*

Bulk oils and fats can be used as ingredients to develop a variety of foods to increase nutritional

value, impact structures and enhance the flavor of the foods. The quality and oxidative stability of bulk oils and fats have big impacts on the oxidative stability and shelf-life of foods containing the bulk oils and fats. Examples of bulk oils and fats available in food industry are: vegetable oils such as soybean, canola, corn, sunflower, safflower coconut, palm and flaxseed oils, high oleic soybean, canola and sunflower oils, fish, algal and krill oils, and plant-based EPA and/or DHA oils; animal fats such as chicken and pork fats, and tallow. There are a number of methods that may be employed to assess the oxidative stability of bulk oils and fats. There are many antioxidants that may be selected to enhance oxidative stability of the bulk oils and fats. In the presentation, we will address fatty acid profiles of the bulk oils and fats, and will highlight how to select appropriate methods to evaluate the oxidative stability and shelf-life of bulk oils and fats, and to increase the oxidative stability of the bulk oils and fats.

Correlation of Sensory Evaluation with Chemical Assays in Oils/Fats and Oil/Fat-based Foods Robin Boyle, and Nora Yang*, *Kalsec, Inc., USA*

The most important method to assess the flavor quality of foods is sensory evaluation, which plays a key role to determine the shelf life of foods; however, sensory evaluation is subjective and can be very time and cost-consuming. Therefore, chemical analyses that generate more reliable and accurate information from the molecular level are attracting more and more interest in both the food industry and academia. The most crucial question is whether there is correlation between sensory evaluation and chemical assays. This talk will focus on examining whether the most commonly used chemical markers, such as free fatty acids, peroxides, anisidine values and volatiles as secondary oxidation compounds, can be utilized to predict the stability and quality of oils/fats and oil/fat-based foods. Both opportunities and challenges in this area will be discussed.

ANA 2a: Advances in the Sample Processing and Lipid Extraction Techniques

Chairs: Susan Seegers, Bunge Oils, USA; and Tiffanie West, USA

Sample Preparation Techniques for Oxidative Stability Measurements in Difficult Samples Scott S. Segro*, *Metrohm USA, USA*

Oxidative stability is an important parameter for estimating the rancidity and shelf life of oils. This can be accomplished using the Rancimat. Oil samples can be directly analyzed on the system following a simple procedure. Samples with more difficult matrices, such as cheeses, can also be evaluated using the Rancimat if a low temperature oil extraction is performed. In this talk, learn how the Rancimat works for oxidative stability, as well as the sample prep procedures needed for more difficult matrices, such as cheeses.

Factors Affecting the DNPH Reaction with Carbonyl Products of Lipid Oxidation Morgan Kandrac*, Chris Izzo, and Karen M. Schaich, *Rutgers University, Dept. of Food Science, USA*

There is great need to analyze soluble carbonyl products of lipid oxidation in oils and lipid extracts to track breakdown pathways and extent of degradation, and to provide a measure of secondary products. Soluble carbonyls traditionally have been quantitated by complexation with dinitrophenylhyrazine (DNPH) to form hydrazones. The reaction is difficult to follow in solution because the hydrazones absorb at wavelengths too near that of DNPH, but reversed-phase HPLC readily separates unreacted DNPH and individual hydrazones. Coupling HPLC with mass spectrometry detection adds identification of carbonyl and ketone components. Despite these advances, quantitation by DNPH remains problematic due to incomplete and inconsistent reaction. We have investigated effects of acid levels and type, carbonyl structure (chain length, unsaturation), and reaction time on formation and stability of aldehyde hydrazones. Some acid is required to form the DNPH carbocation for addition to aldehydes. However, if pH too low, DNPH is protonated to unreactive DNPH2, reducing carbonyl detection and necessitating use of huge excesses of DNPH for reaction. Some excess of DNPH is required to prevent aldol condensation of aldehydes, but more work is needed to identify minimum levels for complete reaction. Optimum pH appears to be about 3. Sulfuric acid (normally used) can be replaced with hydrochloric or formic acid for compatability with mass spec detection. Reaction rate slows with length of carbon chain and unsaturation due to inhibited formation of intermediate carbinolamines. One to four hours gives full reaction of all aldehydes, but also shifts syn/anti hydrazone equilibria, complicating quantitation.

Status and Recommendations on Analysis of Free and Ester-bound 2- & 3-MCPD- and Glycidol Jan Kuhlmann*, *SGS Germany GmbH, Germany*

From the perspective of food safety, fatty acid esters of glycidol as well as 2- and 3-Mono¬chloro-propane¬diol (MCPD) esters are known to be undesired process induced contaminants that occur in refined edible oils and fats. Free 2- & 3-MCPD might occur in other foods after heat treatment. 3-MCPD is classified as carcinogen whereas glycidol has shown genotoxic effects. Recent studies have raised strong indications that during digestion the free analytes are released in major parts out of the ester-bound form. In 2015 the EFSA Panel on Contaminants in the Food Chain (CONTAM) confirmed that the toxicity of 3-MCPD fatty acid esters should be considered equivalent (on a molar basis) to that of the parent compound. Since the occurrence of significant amounts of bound MCPD and glycidol in various edible oils was reported, several analytical methods have been published. But these methods mainly have not tested, if free MCPD being present in foods would have an impact on the results or not. Already in 2014 the European Commission released a recommendation to monitor the presence of free and bound MCPD/glycidol in edible oils & fats and

corresponding foods. In this regard there is an obvious need for a guideline which methods are available for different matrices. This presentation intends to focus on the availability of analytical methods and their applicability and comparability in regard to the determination of all moieties of 2- & 3-MCPD and glycidol in different types of edible oils & fats and in oil and fat containing foods.

ANA 2b: General Analytical

Chairs: Sanjeewa Karunathilaka, US Food and Drug Administration, USA; and Magdi Mossoba, US Food and Drug Administration, USA

Application of a Novel FT-NIR Spectroscopy and PLS Procedure to Predict the Authenticity of Extra Virgin Olive Oil Retail Products in the US Magdi Mossoba*[1], Hormoz Azizian[2], Ali Reza Fardin-Kia[1], Sanjeewa R. Karunathilaka[1], and John K.G Kramer[3], *[1]US Food and Drug Administration, USA; [2]NIR Technologies, Canada; [3]Guelph Food Research Center, Canada*

For the first time, a recently developed FT-NIR spectroscopy methodology in conjunction with partial least squares (PLS1) data analysis was applied to commercial products labeled extra virgin olive oil (EVOO) purchased in College Park, MD, US to rapidly predict, based on three criteria*, whether they are authentic, potentially mixed with refined olive oil (RO) or other vegetable oil(s), or of lower quality. Using pre-developed PLS1 calibration models, this methodology involved estimating the FT-NIR Index, predicting the concentration of five fatty acid (FA) markers according to published information and an AOCS Standard Procedure, and predicting the concentration of RO and the nature and concentration of other edible oils using gravimetrically prepared mixtures with EVOO. The following mixtures were evaluated: EVOO mixed with edible oils high in linoleic acid (OH-LA) or high in oleic acid (OH-OA), palm olein (PO), and RO. The FT-NIR Index provided an estimate of total volatiles characterized by an overtone band attributed to a carbonyl group absorbing near 5269 cm-1. A low index value negatively reflected on the purity and/or quality of EVOO. Of the 88 commercial products labeled EVOO that were analyzed, 33 (37.5%) satisfied the three published FT-NIR / PLS requirements identified for authentic EVOO products which included the purity test based on the limits established for OH-LA, OH-OA, PO, and/or RO contents. The remaining 55 samples (62.5%) did not meet one or more of the criteria established for authentic EVOO. *Lipids. 50:705-718 (2015); Lipids. DOI 10.1007/s11745-016-4195-0 (2016).

Fusty/Musty Off-flavor in Native Cold-pressed Rapeseed Oil: Sensomics, Principal Component Analysis, Development of a Quick Method for Quality Control Katrin Matheis and Michael Granvogl*, *Chair for Food Chemistry, Technical University of Munich, Germany*

The sensomics approach was used to clarify the formation of the fusty/musty off-flavor of native cold-pressed rapeseed oil using a “positive control” (PC) showing the desired sensory attributes and an oil eliciting a fusty/musty off-flavor (OF). Key odorants were quantitated via stable isotope dilution analysis using (headspace) GC-MS or GC/GC-MS. Main differences between both oils were obtained for compounds caused by microbial influence resulting from inappropriate storage conditions (moisture and/or temperature). As a consequence, a clear increase of 16 odorants in OF was observed. These compounds were analyzed in a further 7 native cold-pressed rapeseed oils, eliciting the same sensory defect (OF1-7), and in 5 oils showing the desired attributes (PC1-5) to verify the obtained data. Compared to PC, clearly higher concentrations were determined for 8 compounds in all OF oils, mostly pronounced for 2- and 3-methylbutanoic acid and 4-methylphenol representing aroma-active marker compounds for the fusty/musty off-flavor. For all oils, the concentrations of the 16 compounds were statistically evaluated using principal component analysis (PCA) showing a clear separation of both oil groups. Finally, a quick method (headspace GC-FID) was successfully established enabling a quality control of the seeds to prevent the production of oil eliciting the off-flavor.

Rapid Identification and Quantification of Nutraceutical Oils by Near-infrared Spectroscopy Kyle Hollister*, *Metrohm USA, USA*

The nutraceutical industry has experienced strong growth in the last few years, and recent news reports have highlighted challenges in delivering consistent products that meet advertised claims. As the industry grows, congressional, FDA, and FTC scrutiny intensifies, which is leading to a need for more stringent manufacturing and testing requirements. Near-infrared (NIR) spectroscopy provides fast and accurate information that can improve the production process, ensure product quality, and minimize costs associated with energy and chemicals. The use of NIR for reagent-free quantification of Vitamin E (5-75%) in nutraceutical oils, and for identification of five chemically similar fish oil blends, is demonstrated here. Chemometric development of prediction models and details of their performance are reported.

GC-VUV for the Analysis of Fatty Acid Methyl Esters. Inês C. Santos[1], Hui Fan[1], Jonathan Smuts[2], and Kevin A. Schug[1], [1]Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, TX, USA; [2] VUV Analytics, Inc., Austin, TX, USA

Vacuum ultraviolet (VUV) detector for gas chromatography (GC) has been developed to overcome some of the drawbacks of other GC detectors such as distinguishing isomers. Absorption spectra (115 to 240 nm) are acquired continuously, up to rates of 100 Hz. Due to the simple additivity of overlapping absorption spectra, the detector also allows for the deconvolution of co-eluting compounds. In this work, the GC-VUV was applied for the analysis of fatty acid methyl esters (FAMEs) in food samples such as vegetable oils, fish oil and butter.

Rapid Prediction of Low Levels of *trans* Fat in Edible Oils/Fats and Fast Foods by ATR-FTIR and PLSR Sanjeewa R. Karunathilaka*, Cynthia Srigley, Samantha Farris, and Magdi Mossoba, *US Food and Drug Administration, USA*

The United States Food and Drug Administration (FDA) ruled that partially hydrogenated oils (PHO), the major dietary source of industrially-produced trans fat (TF), were no longer “generally recognized as safe (GRAS)” for any use in human food. However, small amounts (<3.6% of total fat or <0.5 g per serving) of certain TF isomers may still be found in refined, bleached, and deodorized edible fats and oils due to the cis-to-trans isomerization of unsaturated fatty acids during high temperature processing . The objective of the present study was to develop a rapid screening tool, using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, for the quantitative prediction of low concentrations of TF for regulatory purposes to verify label declarations and in quality control monitoring during food processing. Calibration models were developed using a broad-based partial least squares regression (PLSR) approach by combining samples of neat edible oil and fats and fatty acid methyl esters (FAME) derived from fast food lipid extracts, which together permitted the analysis of a wide range of sample matrices and total TF concentrations. Predicted concentrations of TF showed good accuracies relative to the gas chromatography primary reference data with low standard error of prediction (SEP) values. Calibration models were also successfully transferred from a benchtop FTIR spectrometer to a portable FTIR device for the prediction of TF concentrations as low as 0.4% (weight/weight). This simple, fast, and nondestructive quantitative method may be used for the rapid screening of total TF at concentrations as low as 0.1% in edible oils and fats and 0.4% in processed foods.

ANA 3: Marine and Krill Oil—Analytical Advances

Chairs: Cynthia Srigley, US Food and Drug Administration, USA; and Erik Fuglseth, Orivo AS, Norway

Matrix Extension Validation of AOCS Official Method Ce 2c-11 for Foods and Dietary Supplements Containing Added Marine Oil
Cynthia Srigley*[1], Shaun P. Kotoski[2], and Ziyi Li[1], *[1]US Food and Drug Administration, USA; [2]University of Maryland, USA*

Marine oils are currently added to foods and dietary supplements to increase their contents of long chain omega-3 polyunsaturated fatty acids (PUFA), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have been shown to offer numerous potential health benefits. These products vary in sample matrix and formulation, using fish, cod liver, and algal oils to provide long chain omega-3 PUFA. Our objective was to conduct a matrix extension validation of Official Method Ce 2c-11 of the American Oil Chemists' Society (AOCS) for the determination of fatty acids in foods and dietary supplements containing added marine oil, especially those formulated with microencapsulated marine oil. Accuracy was tested in a series of spike-recovery experiments in which microencapsulated marine oil concentrate was added to five blank matrices (beef baby food, chocolate biscuit, gummy supplement, juice, and water) over the EPA and DHA spike ranges of 2.4–88 mg/g. Precision was verified by replicate analysis of 24 commercially-available products which contained EPA and DHA over the concentration range of 0.12–23 mg/g. Linearity and robustness were evaluated by analysis of a prenatal protein formula over the sample test portion range of 0.05–2 g. The limits of detection (0.01 mg/g) and quantification (0.04 mg/g) for DHA were determined by serial dilution of a powdered beverage product.

AOCS Official Method Ce 2c-11 showed acceptable accuracy (91–103% recoveries), precision (≤14.6% RSDr), and linearity ($r \geq 0.9988$), indicating that this method is suitable for the analysis of foods and dietary supplements containing added marine oil.

A Novel Tool for the Determination of Authenticity of Marine Ingredients
Erik Fuglseth*, *Orivo AS, Norway*

Adulteration in the marine ingredients industry has for a long time been a known challenge. In order to maximise profit, dishonest players in the value chain can dilute meal and oil based on high-quality sources with those of lower quality without declaring it. Until now, due to the lack of sufficiently accurate analysis tools, such product adulteration has been hard to reveal. Previous studies have shown that nuclear magnetic resonance (NMR) spectroscopy of the fat in marine species gives different patterns related to the distinct species and the geographic origin. By analysing a large number of marine oil samples by NMR spectroscopy, and combining the resulting data with multivariate data analysis techniques, it has been shown that it is possible to distinguish between several commercially relevant oil sources. The newly developed analysis method can for instance distinguish between sardine oil from Morocco and anchovy oil from Peru, and cod liver oil based on pure arctic cod (Gadus morhua) from Norway and oil from the same species from Iceland. Recently, the statistical algorithms have been implemented in a customised software, thereby automating the classification process and minimising the risk of human mistakes. The system is now being used on a commercial basis by several companies in the marine ingredients industry.

Vibrational Spectroscopy and PLS Procedures for the Rapid Prediction of Omega-3 Polyunsaturated Fatty Acid Concentrations in Marine Oil Dietary Supplements Sanjeewa R. Karunathilaka*, Jin Kyu Chung, Cynthia Srigley, and Magdi Mossoba, *US Food and Drug Administration, USA*

Long chain omega-3 (n-3) polyunsaturated fatty acids (PUFA) are predominantly found in marine-derived foods and dietary supplements, including fish oil (FO) supplements. FO supplements are among the most frequently consumed non-mineral, non-vitamin dietary supplements for health reasons in the United States (US) and other countries. The rapidly increasing demand for these FO dietary supplements warrants strict process control and quality assurance measures, which in turn require simple, fast, and accurate analytical techniques for monitoring FA concentrations. Infrared spectroscopy (IR) is one of the promising analytical techniques in research applications, including process control and monitoring, due to demonstrated advantages of simplicity, limited sample pre-treatment, and rapid measurement time. Our objective has been to rapidly predict the FA composition of FO supplements purchased from different US-based online retailers using mid-infrared spectroscopy (MIR), Near Infrared spectroscopy (NIR), and Chemometrics. A broad-based single calibration set consisting of all available representative samples of FOs was used for quantification using partial least squares regression (PLSR). Calibration models provided good reliable predictions for both major and minor FA components, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); the sums of saturated, branched chain, and monounsaturated FA; and n-6, n-4, n-3, n-1, and trans polyunsaturated FA. 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.

Physical and Oxidation Stability of Self-emulsifying Krill Oil-in-Water Emulsions Qian Wu*[1], Eric A. Decker[2], Sibel Uluata[3], Leqi Cui[4], Chao Wang[1], and Dongsheng Li[1], *[1]Hubei University of Technology, China; [2]University of Massachusetts Amherst, USA; [3]Dept. of Food Technology, Inonu University, Turkey; [4]Fuli School of Food Equipment Engineering and Science, Xi'an Jiaotong University, China*

Krill oil is a unique source of omega-3 fatty acids since it is a mixture of phospholipids and triacylglycerols. Due to the presence of phospholipids, it can form oil-in-water emulsions without additional food additives. In this work, the physical stability of krill oil-in-water emulsions was determined at various pH values (3–7) and NaCl concentrations (50–1000 mM). The initial particle size ranged from 150 to 165 nm. The emulsions were the most stable at pH ≥ 5.0 and salt concentrations below 100 mM. Lipid oxidation was accelerated by iron and inhibited by Trolox and α-tocopherol. Moreover, α-dicarbonyl compounds and advanced glycation end products were investigated using HPLC-MS2 during storage. It showed that Trolox was a more effective antioxidant than α-tocopherol. α-Tocopherol had a better inhibitory effect when it was added after homogenization than when added to the lipid prior to homogenization. These results indicate that krill oil emulsions could represent a self-emulsifying, oxidatively stable source of omega-3 fatty acids that may be used in functional foods.

Performance Assessment in Quantitative NMR Analyses of Krill Oils Elina Zailer and Bernd W.K Diehl*, *Spectral Service AG, Germany*

An interlaboratory comparison was organized with the aim to investigate the evaluation of krill oils analysis by nuclear magnetic resonance (NMR) spectroscopy. 14 international laboratories demonstrated their individual performance by presenting in total 17 analysis results (different

NMR spectrometers, different evaluators). Five krill samples were prepared by the organizer and analyzed by 31P NMR spectroscopy by the participant. Phosphatidylcholine (PC) and other phospholipids (PL) were quantified by adding Triphenylphosphate (TPP) as internal standard. Outliers were identified by the Grubb's test. The performance of each laboratory was assessed by a z-score which is related to the mean value and the standard deviation. Laboratories endowed with a |z|-score of < 3.0 were considered to be acceptable, z-values of |z| ≥ 3.0 unacceptable. The participation in an interlaboratory comparison is successful if 80% of all required results exhibit a z-score of |z| < 3.0. The interlaboratory comparison was successfully completed by all participants. The NMR results showed that NMR spectroscopy is a robust quantification tool for phospholipids in krill oil.

Qualitative Detection of Krill Oil Adulteration with Fish Species and Synthetic Oil by ^{13}C NMR Spectroscopy Yulia B. Monakhova*, Bernd W.K Diehl, and Elina Zailer, *Spectral Service AG, Germany*

Krill oil is currently among the most highly promoted products on the dietary supplements market, which, due to its high price, can be potentially adulterated by fish oil from different species. ^{13}C NMR spectroscopy was used to distinguish between krill and fish oil – based dietary supplements. The analysis included only dilution of a sample in CDCl3 and 20 minutes for measurement and evaluation. The adulteration can be detected by fatty acid distribution in the sn-2 TAG position. The sensitivity of the method is about 10 w/w% of fish content in blends. The same methodology can be used to recognize synthetically modified krill oil. The method was successfully applied to 30 commercially available krill and fish oil supplements.

Qualitative Detection of Fish Oil Adulteration by Nuclear Magnetic Resonance Spectroscopy Pattern Recognition Yulia B. Monakhova, Bernd W.K. Diehl*, and Elina Zailer, *Spectral Service AG, Germany*

Fish oil is a popular nutritional product consumed all over the world. Demand for product specification varies significantly, depending on the use of the product: health food, food ingredient, supplement product. Due to the high prices of high quality fish oil, producers, distributors and customers demand for a control of potential adulteration by low quality fish oil from other species. NMR spectroscopy in conjunction with multivariate analysis of fish oils have been used to provide discrimination concerning the nature, composition, and/or adulteration or authentication of the products. The analysis included only a dilution of a 200 mg sample in CDCl3. The adulteration could be detected by the fatty acid profile and the distribution in the fatty acids in TAG sn-position. The sensitivity of the method depended on the fish species in blends.

ANA 4: Minor Components in Specialty Oils—Analytical and Application Aspects

Chairs: Rakesh Kapoor, Bioriginal Food & Science Corporation, Canada; Sara Shinn, California State University, Fresno, USA; and Fereidoon Shahidi, Memorial University of Newfoundland, Canada

Minor Components of Edible Oils Affect Their Stability Characteristics Fereidoon Shahidi*, *Memorial University of Newfoundland, Canada*

Edible oils are mainly composed of triacylglycerols and some also contain mono- and diacylglycerols of up to 10% or so. Their minor components include tocols and chlorophylls, carotenoinds, sterols and oxidation products. The stability of the oils is dictated by the storage conditions and is affected by the presence or absence of light. While oils are generally stable under autoxidative conditions as such, when striped of their minor components are generally less stable as such but show a reverse trend when kept under photooxidative conditions generally experienced in the supermarkets under display light. Examples will be provided to demonstrate that in addition to the degree of unsaturation and positional distribution of fatty acids, minor components and storage conditions are important factors that play a major role in their stability characteristics.

Regression Analysis to Predict Impact of Confectioner's Sugar and Processing Conditions on Palm Oil Behavior Ryan West*, and Dérick Rousseau, *Ryerson University, Canada*

Fat-based confections are typically composed of a continuous fat phase and non-fat particulates such as confectioner's sugar. Quite often, research surrounding confections extrapolate from bulk fats and oils while overlooking the role of these particulates or any processing interactions. This is further complicated in systems containing palm oil (PO), which is exceptionally sensitive to compositional and processing conditions and is industrially characterized through empiricism. The effects of these conditions on the rheological and textural properties of a commercial PO and its mid-fraction (PMF), the latter of which is reduced in saturated and partial glyceride content, over four weeks of storage were explored by analysis of variance. At a low cooling rate (i.e., $1°C \cdot min^{-1}$), the solid fat contents (SFCs) of PO- and PMF-sugar blends were respectively 22.0±1.3 and 14.2±2.1%, a difference attributed by the inherent glycerides. As this rate was increased (i.e., $5°C \cdot min^{-1}$), the respective SFCs were 27.3±2.3 and 26.7±1.2%. This is particularly important considering SFCs of bulk oil counterparts showed no significant change with effect of cooling rate. As a result, oil-specific polynomial regressions were calculated for the various physicochemical properties of these systems as responses to the different treatment effects. These regressions should overcome the difficulties that palm-based confections impose when significant composition-processing interactions exist. Ultimately, it is our goal that this research establishes the ability to predict confectionery behavior while optimizing production and formulation in an industrial setting.

AOCS Method Ce 12-16 for the Determination of Plant Sterols/Stanols in Foods and Dietary Supplements Containing Added Phytosterols: Collaborative Study Results Cynthia Srigley*[1], Steven L. Hansen[2], Sean A. Smith[2], and Richard Cantrill[3], *[1]US Food and Drug Administration, USA; [2]Cargill Minneapolis R&D Center, USA; [3]AOCS, USA*

A multi-laboratory collaborative study was conducted to evaluate the performance of Method Ce 12-16 of the American Oil Chemists' Society

(AOCS) for the determination of plant sterols and plant stanols, collectively referred to as phytosterols, in foods and dietary supplements containing added phytosterols and the phytosterol food additive concentrates used to prepare those products. AOCS Method Ce 12-16 involves the extraction of free sterols/stanols and saponified steryl/stanol esters followed by the gas chromatographic separation and flame ionization detection of phytosterol trimethylsilyl ether derivatives. A total of 14 laboratories from six countries successfully completed the analysis of collaborative samples of foods (e.g., baked goods, beverages, margarine; n=11), dietary supplements (n=3), and phytosterol concentrates (n=4). Study results for the contents of total phytosterols (weight/weight) were 0.19–8.4% for foods, 8.7–49% for dietary supplements, and 57–97% for phytosterol concentrates. AOCS Method Ce 12-16 showed acceptable performance for major phytosterols (>1.3% weight/weight) with reproducibility relative standard deviation (RSDR) values of ≤12% for all but one test sample, indicating that this method was suitable for the determination of added phytosterols in the wide variety of products and concentrates that were studied from the United States (US) market. AOCS Method Ce 12-16 is also appropriate for the determination of the five major phytosterols (campesterol, stigmasterol, beta-sitosterol, campestanol, and sitostanol) that are the subject of the US Food and Drug Administration's health claim for phytosterols and the reduced risk of coronary heart disease.

GC-MS Analysis of the Volatile Constituents of Ocimum Tenuiflorum Noelle J. Fuller*, Ronald B. Pegg, and David Berle, *University of Georgia, USA*

With rising healthcare costs, there is an increased focus on the role of medicinal plants in health and wellness. Holy basil (Ocimum tenuiflorum) is an important medicinal used to reduce stress, regulate metabolism, and reduce inflammation. This study evaluated the volatile oil composition of O. tenuiflorum varieties to determine the best for commercial production. Plants from 14 holy basil varieties were selected from commercial catalogs and the USDA Germplasm systems. Plants were grown in the field over two growing seasons, harvested twice each growing season, and dried. Volatile oils were extracted by hydrodistillation. The volatile oils were analyzed qualitatively by GC-MS and quantitatively by GC-FID with external standards selected based on the relative abundance of the compound in the sample as well as its biological relevance, price and availability. GC-MS analysis of the volatile oils revealed 4 distinct chemical profiles: eugenol; eugenol, caryophyllene and β-elemene; methyl eugenol; and a profile with relatively equal quantities of eugenol, estragole, eucalyptol, and β-bisabolene. Also, quantification with GC-FID indicated that the amount of each compound in the volatile oil changes from year to year, and with successive harvests. The findings suggest volatile oil composition is highly variable over successive growing seasons and certain varieties of O. tenuiflorum differ greatly in their volatile oil composition. These findings should be considered when choosing a variety for commercial production.

A Streamlined Method for Quick Determination of Total and Individual Glucosinolates in Rapeseed Chuan Zhou*[1], Hai Ming Shi[2], Dian Ping Ma[2], Wen Ming Cao[2], and Yuan Rong Jiang[2], *[1]Wilmar, China; [2]Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China*

Glucosinolates are an important group of secondary metabolite in rapeseeds. Glucosinolates and their enzymatic degradation products such as isothiocyanates majorly contribute to the

characteristic flavor of rapeseeds. Previous analytical methods of glucosinolates are time-consuming and tedious. We here report on the optimization and validation of a streamlined method for quick determination of total and individual glucosinolates using spectrophotometry and high performance liquid chromatography, respectively. The rapeseeds were extracted using 70% methanol solution and purified by a strong anion exchange column. The total glucosinolates were hydrolyzed under alkaline condition, and the hydrolysate, 1-thioglucose, was oxidized by ferricyanide. The total glucosinolates content was determined by evaluating the loss of chromogenic ferricyanide in 96-well microtiter plates at 420 nm. Then the total glucosinolates were mixed with sulfatase solution for desulfuration within 1 h. The individual desulfoglucosinolate was analyzed by high performance liquid chromatography after a fast cleaning step on a homemade ion-exchange resins. The quantitative analytical method of total glucosinolates was fully validated with respect to linearity (r^2>0.995), sensitivity, precision, accuracy (the recovery was between 91.7% and 108%), and robustness (the RSD was less than 5.23%). The developed method was successfully applied to determine the individual glucosinolates without an internal standard. The newly established method is simple, fast and reliable on the quantitation of glucosinolates in rapeseeds.

Detecting Adulteration of Goat Cheeses with Cow Milk by Analysing the Triglyceride Profile Ignacio Vieitez*, Bruno Irigaray, Nicolas Callejas, Verónica González, Sofia Jimenéz, Añez Arechavaleta, Maria Grompone, and Adriana Gámbaro, *UdelaR, Uruguay*

Composition of fatty acids and triglycerides of fats from different commercial samples of goat cheeses currently marketed in Uruguay were analysed. Moreover, since goat milk is more expensive and less abundant than cow milk in Uruguay, the ability to detect adulteration of goat cheese with cow milk has interest. This is particularly important due to repercussions on the nutritional value. Consequently, also were determined the TAG composition in terms of the Partition Number (PN) in fats coming from pure goat milk, pure cow milk and blends thereof (90:10, 80:20, and 50:50, goat milk:cow milk). All Uruguayan goat cheeses showed high levels of conjugated linoleic acid and trans-Vaccenic acid, well above those reported in cheeses from other countries. This is advantage in terms of nutritional quality. In fats from goat milk in Uruguayan cheeses, the highest average percentages of triglycerides correspond to PN = 36 (13.2%) and PN = 42 (13.6%). The results demonstrate that the triglyceride profile changes when cow milk is added to goat milk. When the amount of cow milk added to goat milk increases, triglycerides with PN values of 38, 40, and 42 tend to decrease, while triglycerides with PN values of 46, 48, and 50 tend to increase. These results suggest that the triglyceride profile could be used to determine whether a given goat cheese was adulterated with cow milk.

Partially Hyrdrogenated Oils (PHO) are No Longer GRAS—A Method of Their Detection Sneh Bhandari*[1], and Ming Gao[2],[1]*Merieux Nutrisciences, USA;* [2]*Merieux NutriSciences, USA*

Last year FDA revoked the GRAS (Generally Recognized as Safe) status of partially hydrogenated oils (PHO) for any use in human food without its prior approval in an attempt to help in preventing cardiovascular diseases and resulting deaths every year. Foods containing PHOs are being reformulated in a variety of ways to eliminate PHO derived trans fat from food. Trans fatty acids (TFA) occurs naturally in meat and dairy products from ruminant animals. Non-

hydrogenated refined oils may also contain TFA at low levels due to heat processing. Differentiating TFA derived from PHO in food matrices containing trans fat also from meat and milk of ruminants and processed vegetable oils is difficult but is required to establish that the product is PHO free. We have developed a method to detect PHO in food matrices based on their pattern of TFA distribution. The method has been verified in a large number of food matrices. The accuracy of the method established by comparison of the results with the label information. The method could accurately detect trans fat from PHO at 0.5% - 1.0% level of total fatty acids in food matrices of varying fat levels.

Performance Assessment in Quantitative NMR Analyses of Edible Oils Elina Zailer*, Bernd W.K. Diehl, and Yulia B. Monakhova, *Spectral Service AG, Germany*

An interlaboratory comparison was organized with the aim to evaluate the applicability of the alternative oil analysis and to investigate the evaluation of edible oils analysis by nuclear magnetic resonance (NMR) spectroscopy. 17 international laboratories demonstrated their individual performance by presenting in total 21 analysis results (different NMR spectrometers, different evaluators). Five oil samples were analyzed by 1H NMR spectroscopy. 15 signals were integrated. Outliers were identified by the Grubb's test. The performance of each laboratory was assessed by a z-score which is related to the mean value and the standard deviation. Laboratories endowed with a $|z|$-score of < 3.0 were considered to be acceptable, z-values of $|z| \geq 3.0$ unacceptable. The participation in an interlaboratory comparison is successful if 80% of all required results exhibit a z-score of $|z| < 3.0$. The interlaboratory comparison was successfully completed by 15 participants, including 19 analysis results. The NMR results showed that NMR spectroscopy is a robust quantification tool for edible oil analysis.

Asarinin as a Specific Marker for Differentiating Pressed Sesame Oil from Refined Sesame Oil Wen Ming Cao*[1], Bin Xue[1], Chuan Zhou[2], and Yuan–Rong Jiang[1], *[1]Wilmar Biotechnology R&D Center (Shanghai) Co., Ltd., China; [2]Wilmar, China*

Sesame oil is a popular flavored vegetable oil in Asia, usually including pressed sesame oil (PSO) and refined sesame oil (RSO). Some illegal merchants adulterating RSO into PSO to get high profits. It's an extremely serious problem in the edible oil industry. In the past decades, many attempts have been exerted to detect the PSO adulteration. However, up to now, there is no reliable analytical method. This is partly because the fatty acid composition (FAC) of PSO and RSO is similar. The objective of this study was to develop a highly effective analytical method for the differentiation of PSO and RSO. The chemical profiling of sesame lignans for PSO and RSO was established by Reverse Phase High Performance Liquid Chromatography (RP-HPLC). Our study showed that PSO had high amounts of sesamin and sesamolin, while RSO contained an unknown substance besides sesamin and sesamolin. The unknown substance was extracted from RSO by Preparative Flash Chromatography, and further purified by Preparative HPLC. The unknown substance was identified as asarinin, an isomer of sesamin, by the means of NMR and LC-MS/MS. This study also indicated that the epimerization of sesamin mainly occurred during the acid-clay bleaching, resulting in a mixture containing an equal amount of sesamin and asarinin. Analyzing of 50 sesame oil samples from different origin indicated that the content of asarinin ranged from 57 to 128 mg/100g in RSO, while asarinin was not detected in PSO. Our study showed that asarinin

can be a specific marker for differentiating PSO from RSO.

Introducing the Vapor Pro® XL—A Chemical Free Karl Fischer Alternative Quincy Biamonte and Garrett Rowe*, *Arizona Instrument LLC, USA*

Moisture in frying oils causes more than just popping - it can also affect the frying process itself well as the consistency and flavor of the final product. Monitoring the moisture content of new and used frying oils can help you maintain quality and consistency of both your frying oils and your final products. So, whether you use peanut, olive, corn, mustard or an oil that is altogether unique, moisture analysis is essential to your production process. Moisture analysis of frying oils has traditionally been performed using Karl Fischer (KF) titration. Although the results are both accurate and precise, maintenance is both costly and labor intensive. The testing process itself is also complicated and requires special training to operate with the consistency needed to obtain accurate and repeatable results. An alternative is the Vapor Pro® XL by Computrac®. The Vapor Pro® XL (VPXL) is a chemical free alternative to Karl Fischer titration that is able to give moisture specific results without the use of hazardous chemical reagents or complicated test procedures. It requires little maintenance or training and allows users to test a variety of samples with the touch of a button. The Vapor Pro® XL is equipped with a touchscreen and redesigned navigational menus, making it the most user-friendly Computrac® to date. It also features an upgraded heater, is compatible with multiple sizes of sample bottles, and is equipped with stepped temperature testing capabilities for enhanced method development.

ANA 5 / H&N 5: Impact of Oil Processing on Health Outcomes

Chairs: J. Thomas Brenna, Cornell University, USA; and Sean Liu, USDA, ARS, USA

Introduction: Oil Processing or Fatty Acid Composition, What's More Important? J. Thomas Brenna*, *Cornell University, USA*

The controversy over health effects of saturated fat and health has raged since at least the 1950s with no signs of resolution. Early data pointed to cholesterol raising properties of saturated fats, initially understood as animal fats (butter, tallow, lard) and later as tropical oils, implying harm to heart health. Unequivocal evidence in experimental animals developed in the 1970s shows that highly refined coconut oil dramatically raises serum cholesterol. The epidemiology of saturated vegetable fats supports low, not high, levels of heart disease in native populations consuming, for instance, coconut as the predominate source of dietary fat. The recent widespread availability of virgin coconut oil prompted head to head studies showing virgin coconut oil does not raise serum cholesterol compared to refined coconut oil. Moreover, studies of the most prominent trans fatty acid in partially hydrogenated vegetable oil, elaidic acid, support lower not high serum cholesterol. Advances in chemical analysis enable sensitive measures of compounds that are created during processing or enter the oils during processing or storage. This symposium will introduce issues about how fats and oils processing may influence the healthfulness of fats and oils independent of fatty acid composition.

Impact of Industrial Processing and Mitigation on MCPD/Glycidyl Ester Concentrations in Oils and Foods Jessica K. Leigh*, and Shaun MacMahon, *US Food and Drug Administration, USA*

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are process-induced chemical contaminants found in refined edible vegetable oils. Formed during the deodorization step of the refining process, these compounds are considered potentially carcinogenic and/or genotoxic, making their presence in edible oils and processed foods containing these oils 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 determine levels of exposure. Validated methodology for the quantitative analysis of 3-MPCD and glycidyl esters in oils and various food products will be briefly described in this presentation, followed by a detailed look at the occurrence of these contaminants in a wide array of oils and infant formulas from the United States, Canada, and Europe. In addition, preliminary occurrence data for 3-MCPD and glycidyl esters in other complex food matrices, including chips, cookies, baked goods, and other food items containing refined oils, will be presented. Results from the occurrence studies show a wide range of 3-MPCD and glycidyl ester concentrations across various types of refined oils, as well as varying concentrations among similar infant formula varieties produced by different manufacturers. Finally, an evaluation of the potential impact of processing and mitigation on the concentrations of these contaminants in food products will be

discussed.

A Novel Method to Assess Health Effects of Oils: Virgin and Refined Coconut Oil Ruijie Liu*[1], Can Shi[2], Elizabeth Mendralla[3], Kumar S.D. Kothapalli[3], Xingguo Wang[2], and J. Thomas Brenna[3], *[1]Jiangnan University/Cornell University, China; [2]Jiangnan University, China; [3]Cornell University, USA*

Introduction. Head-to-head comparisons of virgin coconut oil and harshly processed copra oil of identical fatty acid profiles show liver cholesterol and triglyceride levels to be dramatically lower in the VCO group, and more similar to PUFA. We hypothesize that the cholesterol raising effect of tropical oils may be due chemical alteration during processing rather than their saturated fats. Our objective was to develop a rapid system to evaluate prompt effects on cholesterol metabolism that recapitulate these effects in vitro. Methods. Coconut oils of various degrees of processing but identical fatty acid profile were used as a test case. Oils were made into a test emulsion and cells were treated with 500 µM for 24 h. Gene expression measured by RT-PCR. Results. Cells took up the oils as indicated by changes in their fatty acid profiles. Two genes were responsive to degree of processing: HMGCR, the rate-limiting step for endogenous cholesterol synthesis, and CYP71A that catalyzes the initial step of cholesterol catabolism. HMGCR expression increased from 0.97 to 1.78 fold in a stepwise manner over four processing steps, normalized to untreated cells. At the same time, CYP7A1 expression decreased. The net effect suggest increasing cholesterol levels due to more processed oils. Conclusion. These data are consistent with an increase in cholesterol synthesis and a decrease in cholesterol degradation due to processing, probably attributable to generation of chemical factors specifically influencing cholesterol synthesis. This approach holds promise for rapidly assessing metabolic effects on humans.

Plasticiser Residues in Edible Oils and Fats–Relevance and Analysis Jan Kuhlmann*, *SGS Germany GmbH, Germany*

Plasticisers represent a complex group of world-wide and in large scale applied chemicals. They ensure important properties to plastic materials but they are also used as auxiliaries in medical and personal care products and in various other household items. Trace contamination of edible oils and fats might occur during harvesting, processing, bottling and storage. Specific plasticisers are suspected to have adverse health effects. In this regard some authorities have started to take action in terms of announcing recommendations or defining TDIs. Also NGOs have picked up the absence of certain plasticisers as quality criteria for foods such as edible oils and fats. Very likely these compounds will raise increasing intention of authorities and consumers in the future which might result in setting MRLs. In order to determine trace amounts of plasticisers for monitoring purpose or to control internal or official limits there is an obvious need for reliable and validated analytical methods. However, analysis of plasticiser residues in foods seems to be challenging as the number and diversity of compounds is increasing while at the same time the ubiquity of plasticiser containing utilities as source of background levels in laboratories raises the issue of cross-contamination. This presentation highlights the relevance of the issue in regard to product quality and market demands. A new analytical approach for the parallel determination of 24 plasticisers in oils and fats by on line coupled LC-MS² technique is introduced. Occurrence data for refined and non-refined edible oils and fats will be presented.

Analysis of Heavy Metals in Rice Bran Oil by Inductively Coupled Plasma (ICP) Spectrometry Robert O. Dunn*[1], Erica L. Bakota[2], and Sean Liu[3], *[1]USDA, ARS, NCAUR, USA; [2]Harris County Institute of Forensic Sciences, USA; [3]USDA, ARS, USA*

Rice is one of the most important staple crops in the world. Nevertheless, health-conscious consumers have expressed concern regarding the presence of heavy metals, specifically arsenic, in rice. The United Nations Food and Agriculture Organization (UNFAO) limits the arsenic concentration at 0.2 mg/kg in rice, but has no set limit in rice bran oil. Rice bran oil is known to have good antioxidant activity in foods. The study evaluates the use of inductively coupled plasma (ICP) spectrometry in determining the concentration of arsenic, cadmium, lead, mercury and zinc metals concentrations in crude and refined rice bran oils. Most analytical laboratories digest oil samples into an aqueous matrix before running the analysis for heavy metals. However, digestion or organic samples may increase the experimental error in the analysis. In the present work, the digestion step was omitted and the oil samples mixed with kerosene before ICP analysis. Comparison of the results with data from two independent laboratories indicated large deviations for the arsenic and mercury concentrations.

Quantifying Trans Fat in Foods: How Low Can We Really Go? Cynthia Srigley*, Sanjeewa R. Karunathilaka, and Magdi Mossoba, *US Food and Drug Administration, USA*

The intake of trans fatty acids (TFA) has been associated with numerous potential health risks, leading regulatory authorities, such as the United States Food and Drug Administration (FDA), to issue mandatory labeling regulations for the contents of total trans fat in foods. In June 2015, FDA issued its final determination that partially hydrogenated oils (PHO), the major dietary source of industrial-produced TFA, are no longer generally regarded as safe (GRAS) for any use in human food. However, low concentrations of trans fat (e.g.,

2016 Monitoring of MCPD Derivatives and Glycidyl Esters in German Foods—Outcome and Applied Methods Jan Kuhlmann*, *SGS Germany GmbH, Germany*

Monochloropropanediol (2- & 3-MCPD) and glycidyl esters have raised tremendous attention in the past years as they are world-wide occurring process-induced contaminants which might have adverse effects on health of consumers. Free MCPD can be generated when complex composed foods are heated. By contrast the more complex groups of fatty acid esters of MCPD and glycidol mostly are formed during deodorisation of edible oils and fats. This lead to an EU recommendation to monitor free and ester-bound 2- & 3-MCPD and ester bound glycidol in oils and fats but also in a broad variety of oil and fat containing foods. Limits for the Tolerable Daily Intake of free and bound 3-MCPD are set between 0.8 and 4 µg/kg bw d in the EU. Glycidol shows genotoxic properties so that consumers uptake should be As Low As Reasonably Achievable. Anyway, many retailers have set self-defined maximum levels as the issue of MCPD- and glycidol contamination of foods is in public´s perception. The release of MRLs recently is discussed by the EU commission. This presentation gives an overview on the occurrence of free 2- and 3-MCPD as well as ester-bound 2- & 3-MCPD and glycidol in different foods. The applied analytical method will be presented as it has been developed newly in order to have a method available that is on the one hand based on the validated approaches for oils and fats but also should be more sensitive and applicable to all different kinds of complex composed foods.

ANA 5.1 / PRO 5.1: Process Control Utilizing NIR and Similar Online Analytical Tools

Chairs: Chris Dayton, Bunge Limited, USA; and John Glenski, Automation Plus, USA

Process Optimization in the Edible Oil Industry with NIR-Online Measurements Dominik Margraf*, Yosra Allouche, and Michael Eckert, *BUCHI NIR-Online GmbH, Germany*

In general analytical equipment to be utilized directly in a process stream needs to be capable of coping with harsh industrial environments such as vibrations or extreme climate conditions in case of outdoor installations. To circumvent these challenges near infrared (NIR) spectrometers based on robust diode array technology without any moving parts have been developed. Light emitted from a halogen source is transflected from the sample, collected and diffracted on a stationary grating. Spatially separated light is then detected by means of a diode array followed by automated chemometric analysis. Thus, multiple key parameters may be measured non-invasive, simultaneously and continuously within milliseconds. Operators may therefore correct process deviations in real-time greatly reducing safety margins or out-of-specification batches leading to an increased overall production capacity. Here, we will present NIR-Online measurements at multiple installation points covering the entire value chain in the soy and rape seed industry: incoming raw materials, conditioning, flaking, extraction, degumming, soapstock and refined deodorized oil. Measured parameters include e.g. protein, moisture, oil content, residual oil, free fatty acids and phosphorous. Moreover, several payback calculations demonstrating the fast amortization of BUCHI NIR-Online® sensors will be shown.

Implementing Alarm Management (ISA 18.2)—Improving Efficiency and Limiting Risk Monte Vander Velde*, *Interstates, USA*

Alarms are a critical part of oil seed processing. Alarms come in various fashions. Some are critical while others can wait. One thing is certain, when alarms are sounding all the time from all directions, it is easy for even the most experienced operator to become overwhelmed and fail to perform the appropriate corrective action. The alarm management standard outlined in ISA 18.2 provides tools and resources to get a grip on your facilities alarms. Though the implementation of a proven alarm management process facilities have improved efficiency with reduced downtime. Alarm management done right is proven to reduce risk; both safety and compliance risk. This presentation will review alarm management best practices and the implementation of ISA 18.2.

FT-IR Analysis for Process Control Chris Dayton*, *Bunge Limited, USA*

Analytical measurements of lipids utilizing physical or instrumental techniques did not become truly viable until the advent of the personal computer (PC). The PC has allowed the use of strong mathematical tools such as Fourier Transform (FT) and Partial Least Squares (PLS) to be coupled with traditional analytical instrumentation tools. The utilization of these tools has given rise to a new field of analytical chemistry, chemometrics. Utilizing chemometrics allows analysis of samples with complex matrices and potential interferences without the need of "purifying" or concentrating the unknown analyte(s). The amount of useful information as well as the speed of the instrumental analysis is much faster than the

traditional wet methods. My presentation will cover the utilization of FT-IR for measuring Iodine Value (IV), Trans, Saponification Value, Solid Fat Content (SFC), and Fatty Acid Composition (FAC) of vegetable fats and oils.

Level Measurement of Industrial Oils. Philip H. McCain[1], Brent Frizzel[2], and Tim Thomas[2], [1]Automation Plus, USA; [2]Endress+Hauser, USA

The benefits and reasons for level measurement will be discussed. A summary of various level measurement technologies, including continuous and point level will be presented. The enhanced reliability of using two measurement devices will be discussed along with fail safe wiring design. Specific technologies for measuring oil products will be shown with emphasis on radar instrumentation.

At-line Near-Infrared Spectroscopy Monitoring Algal Fermentation Process Yao Lu*, *DSM Nutritional Products, USA*

Quick measurement of major components during fermentation is crucial to understand the process and make immediate operation adjustment. Algae is a sustainable source of omega-3 oil, like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). During traditional agal fermentation process, the fatty acid analysis is conducted after the whole process is finished, and the analysis takes about three days from drying the biomass to achieving the final fatty acid titers. NIR has been widely used in oil quality control, but analyzing fatty acid profile of fermentation broth could be challenging because of water content in the broth and complicated media. In this work, NIR with a transflectance unit was applied to collect spectra from broth samples at various time points during fermentation process, and partial least square (PLS) models were built to monitor fatty acid titers and other key parameters during fermentation process, like dry cell weight and glucose level. Furthermore, the at-line NIR models were transferred to fit the spectra collected with a NIR optic fiber probe, which shows the potential application of NIR probes for in-line monitoring algal fermentation process.

ANA-P: Analytical Poster Session

Chairs:

1. **Authentication of Andean Flours Using a Portable Mid-infrared Fourier Transform Infrared System Spectrometer** Mei-Ling Shotts*, and Luis E. Rodriguez-Saona, *The Ohio State University, USA*

Quinoa, kañiwa, amaranth, and kiwicha are high nutritional value grains grown in the Andean region. Their nutritional importance is based on their high protein content, essential amino acids, high fiber, bioactive compounds, minerals and gluten free. Due to their nutritional and economic importance, there is a risk for adulteration of these grains. Our objective was to develop a rapid untargeted approach to authenticate high-value Andean grains by infrared spectroscopy. Samples (n=106) were collected from Peruvian (n=89) and US (n=17) markets that included Andean flours and other common flours such as maize, soybean, and wheat. Samples were characterized by reference methods to identify potential adulteration by fat analysis by soxhlet (AOAC #960.39), protein analysis by Dumas (ICC Standard No. 167), and fatty acid profile by Gas Chromatography (AOAC Method 996.0). Spectra was collected by using a portable infrared system that was analyzed by Soft Independent Model of Class Analogies (SIMCA) and Partial Least Square Regression (PLSR) to develop classification and quantitative algorithms. Authentic flours formed distinct clusters allowing the evaluation of commercial samples from local markets in Peru showing some prevalence of adulteration. Spectral differences responsible for the separation of classes were associated with the fingerprint region (1100 – 900 cm-1). In addition, regression models were generated for non-destructive and rapid determination of fat (R=0.95 and SECV=1.92) and protein (R=0.96 and SECV=1.15) levels. New generation portable infrared devices provided a viable tool for chemical profiling allowing for the rapid, reliable, and "in-field" authentication, making it an alternative to time-consuming traditional testing methods.

2. **Evaluation of Color Adulteration of Green Table Olives with Copper Salts** Pierluigi Delmonte*, Bhakti Petigara Harp, Patrick Gray, Peter Scholl, and Todor Todorov, *US Food and Drug Administration, USA*

Green table olives, a delicacy characterized by a unique green coloration, must be processed with alkali to hydrolyze oleopein and remove the bitter taste prior to human consumption. Chlorophylls, the pigments that provide the color of fresh olives, are degraded to pheophytin a and other magnesium-free chlorins during processing and subsequent storage. This chemical transformation causes the color of green table olives to fade and change from green to brown or yellow. The addition of copper salts during the processing of green table olives results in the formation of stable copper complexes with degraded chlorophylls, such as pheophytin a, thereby imparting a bright green color to the olives and causing the olives to be adulterated according to U.S. authorities. In this study, the amount of copper chlorophylls in bright green table olives was measured by UHPLC combined with ICP-MS. The use of ICP-MS as the detector for the measurement of the elemental copper contained in copper chlorophylls, combined with post column online isotope dilution, allowed the quantification of these compounds in the absence of pure calibration standards. Identification of copper chlorophylls was achieved by UHPLC combined with an Orbitrap-MS.

3. **Gas Chromatographic Separation of Common Vegetable Oils with Highly Polar Capillary Columns** Andrea Milani*, Shivani Bhangley, and Pierluigi Delmonte,*US Food and Drug Administration, USA*
The composition of edible oils and fats on sale in the global market was recently modified to reflect the proposal or the introduction of new regulations. Also, consumers are progressively more aware of the health benefits provided by consuming oils rich in polyunsaturated fatty acids (PUFA) and newer technologies are applied to the production of food oils. In this study, the elution profiles of food oils and high value oils on sale in the U.S. market were acquired by applying experimental conditions optimized for the quantification of the trans fatty acids (TFAs) occurring in vegetable oils rich in PUFAs. Fatty acids (FA), as methyl esters (FAME), are separated with a 100 m x0.25 mm I.D. capillary column coated with 100% poly(biscyanopropyl siloxane) using a temperature gradient optimized for the separation of FAs with two or more double bonds. The proposed chromatographic conditions are also compatible with the measurement of the trans-monounsaturated FAs occurring in partially hydrogenated vegetable oils (PHVOs). This study allows the direct comparison between the FA compositions of most edible oils on sale on the U.S. market, and also serves as template for the analysis or identification of unknown oils.

4. **Glycidyl Esters, 2-monochloropropandiol and 3-monochlorpropandiol Content in Refined Olive Oil: Preliminary Results** Angelo Cichelli*[1], Lorenzo Cerretani[2], and Nicola d'Alessandro[3], *[1]University G.D'Annunzio, Chieti-Pescara, Italy; [2]Pizzoli SpA, Italy; [3]Dept. of Engineering and Geology, University G.D'Annunzio, Chieti-Pescara, Italy*

In 2016 EFSA issued an opinion regarding the risks concerning to public health caused by glycidol, 2-monochloropropane (2-MCPD), 3-monochloropropandiol (3-MCPD) and all the related esters of fatty acids present in fatty derivatices.1 Even if these contaminants have been discovered several decades ago, only after the recent EFSA report, the scientific community approved the idea to fix the safer threshold limits in related foods. For fatty foods, they have been proposed with limit threshold of 1.0 mg/kg for GE, 2.0 mg/kg for 3-MCPD while 2-MCPD has not been considered. Regarding the generation of such compounds, it was noted that they are formed during the processing phases where the temperatures are particularly high.2 In the case of vegetable oils, it can be attributable to the step of the refining where often the temperature exceeds 200 °C. A comparison of the above data with the GE contents observed in the typical fat used in the Mediterranean diet (olive oil), clearly demonstrate that the olive oil is safer.3 However, we retain that a careful investigation involving the typology of olive oils that have undergone any heat treatment must be done. Therefore, in the present study we will show the GE content of several olive oil samples taken from Italian companies operating in the field of oil refining ("lampante" and "pomace"). 1) EFSA Journal 2016, John Wiley and Sons Ltd, doi:10.2903/j.efsa.2016.4426 2) Pudel and al., Eur. J. Lipid Technol. 2011,113, 368-373 3) Ozdikicierler and al, Eur. Food Res. Technol. 2016, 242:805-813

5. **Immobilization of *Candida rugose* Lipase on Celite-545, Sephadex G-25, and Chitosan Using Physical Adsorption** Samuel A. Besong[1], Stephen E. Lumor[1], and Bhagya Sri Kaja*[2],*[1]Dept. of Human Ecology, College of Agricultural Sciences, Delaware State University, USA; [2]Delaware State University, USA*

The hydrolytic and esterification activities of *Candida rugose* lipase in its free and immobilized forms were investigated at different pH and temperature settings. The main objective of this

study was to understand how different support materials (Celite-545, Sephadex G-25 and chitosan) and immobilization techniques (acetone and alcohol immobilization) affect lipase activity and stability. Results indicated that hydrolytic activity was significantly enhanced with immobilization on Celite-545 and Sephadex G-25. In general, immobilization resulted in considerable improvements in the stability of the enzyme with variations in pH and temperature. Immobilization on Celite-545 resulted in the highest catalytic efficiency. *Candida rugose* lipase had intermediate esterification activity, which was not significantly improved upon immobilization. Remarkable improvements in the recovery and reusability of the immobilized lipases were noted. Comparatively, the acetone immobilization procedure resulted in higher activities compared to alcohol immobilization. In conclusion, the activity of *Candida rugose* lipase was enhanced the most when immobilized on Celite-545 using acetone as an adsorption solvent.

6. **The Effects of Emulsifiers on the Crystallization of Palm Oil and Palm Oil-sugar Blends** Ayse Ece Turan*, and Dérick Rousseau, *Ryerson University, Canada*

Product composition, processing, and storage conditions can significantly impact the crystallization, and ultimately perception, of fat-based confectionery products. For example, in our previous findings the presence of sugar reduced solid fat content of palm oil and increased viscosity. Emulsifiers are functional additives regularly used in colloidal fat-based products to promote texture and stability by way of altering the extent of crystallization, viscoelasticity, and reducing interfacial tension. Here, we investigate the effect of palm oil type, addition of sugar (50 wt.%), and soya lecithin (0.15 wt.%) on the physical properties of palm oil suited for confectionery applications. Specifically, solid fat content, polymorphism, microstructure, and rheological properties are characterized over a period of four weeks. During four weeks, the presence of emulsifiers that is dispersed within fat-sugar mixture will change the crystallization and rheological behavior of the composite. These results are expected to enhance our understanding of palm oil as it is becoming increasingly popular in usage of confectionery products.

7. **Chemical Components of Sumac (Rhus typhina L.) Seed Oil from Different Cultivars of China** Tao Zhang*[1], Ruijie Liu[2], Ming Chang[1], Qingzhe Jin[1], and Xingguo Wang[1],[1]*Jiangnan University, China;* [2]*Jiangnan University/Cornell University, China*

Sumac (Rhus typhina L.) was typically grown in non-agriculturally viable regions in North America, suggesting its commercial potential without competing for food production land uses. In 1959, Sumac was introduced to China and identified as main forestation species. Then, it was successfully used for the rehabilitation of degraded lands in many areas. In 2013, sumac seed oil (SSO) was declared as a new resource food by Chinese government. Nowadays, millions of tons of sumac fruit are produced annually. In this study, the components (fatty acid, triacylglycerol and tocopherol) of SSO from five provinces (Anhui, Henan, Qinghai, Guangdong and Jiangxi) were analyzed and compared. Gas chromatography result indicated that the SSO with a content of 9.02–10.26% in sumac seed was predominately composed by palmitic acid (8.39–12.73%), oleic acid (1.89–10.99%) and linoleic acid (31.21–72.90%). The tocopherol content of α-, β-, γ-, and δ- isomer was varied from 139.46–192.03 μg/g, 156.11–573.70 μg/g, 237.04–624.69 μg/g, 376.72–428.67 μg/g, respectively. Furthermore, a total of 22 species of TAG in SSO were identified by ultra performance liquid chromatography-mass

spectrometry for the first time. Major triacylglycerols in SSO were C18:2-C18:2-C18:2 (5.83–26.68%), C18:1-C18:2-C16:1 (13.15–16.47%) and C18:2-C18:2-C18:1 (9.58–18.82%). The SSO from Qinghai province showed a unique composition, which contained eicosaenoic acid (13.53%) and eicosadienoic acid (C20:2, 17.15%). And the C20:2-C20:2-C20:2 (4.89%) made it obviously distinguished. The results revealed that the SSO could be served as a reliable resource of edible oil or functional ingredient in the food industry.

8. **Quantitation of Trans-fatty Acids in Human Plasma from the National Health and Nutrition Examination Survey** Dickson M. Wambua*[1], Heather C. Kuiper[2], Na Wei[2], Samantha L. McGunigale[2], Samuel P. Caudill[2], and Hubert W. Vesper[2],[1]*Centers for Disease Control and Prevention, USA;* [2]*CDC, USA*

Trans-fatty acids (TFA) are geometric isomers of naturally occurring cis-fatty acids, formed industrially via partial hydrogenation of vegetable oils or naturally in ruminant animals. High TFA intake has been associated with increased risk of cardiovascular disease. However, little is known about the TFA levels in humans. Many fatty acid (FA) isomers have been reported in humans, but some are not fully resolved by current methods. This can lead to inaccurate results for certain FA and incorrect interpretation of human exposure to TFA, creating the need for a new approach to assess TFA concentrations in humans. We developed and validated a new isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry method for the quantitation of 27 FA including four major TFA in 100 µl of human plasma. FA were derivatized with pentafluorobenzyl-bromide, resolved on a 200 m Select-FAME column with hydrogen as the carrier gas, and analyzed in selected ion monitoring mode using negative chemical ionization. The limits of detection were 0.07 µM for palmitelaidic acid, 0.28 µM for elaidic acid, 0.43 µM for trans-vaccenic acid, and 0.02 µM for linoelaidic acid. The intraday and inter-day percent coefficients of variation ranged from 1-11%CV and 6-15%CV respectively. The mean accuracy for all four TFA was 102% (95%CI: 98%-107%). We used this method to quantitate TFA in fasted adults in the U.S. population from the National Health and Nutrition Examination Surveys of 1999-2000 and 2009-2010, finding an overall reduction of TFA levels.

9. **Similarity Evaluation of sn-2 Fatty Acid Composition Between Commercial Infant Formulas and Human Milk** Cong Sun*[1], Xiaoqiang Zou[2], Qingzhe Jin[1], and Xingguo Wang[1], [1]*Jiangnan University, China;* [2]*School of Food Science and Technology, Jiangnan University, China*

In human milk fat (HMF), approximately 70% of saturated fatty acids (SFA) are located at the sn-2 position, especially palmitic acid (PA) which accounts for more than 50%. This unique fatty acid distribution of HMF has a large effect on their digestion and intestinal absorption in infants. At present, vegetable oils and/or milk fat are added to commercial infant formulas, in which PA are predominantly esterified at the sn-1,3 positions. In this study, sn-2 fatty acid composition of 180 commercial formulas with different fat sources (plant oil, cow milk and goat milk) and stages (infant, follow-on and growing-up) were analysed and compared with that of HMF, and their degrees of similarity were digitized by an established evaluation model. The resluts showed that sn-2 fatty acid composition was more susceptible to fat sources than stages. All formulas had significant lower levels of SFA and PA, and higher levels of oleic acid and linoleic acid at the sn-2 position than those of HMF. The PA contents at the sn-2 positions in the cow milk and goat milk formulas

were approximately twice times than those of plant-oil formulas. There was little chemical difference among formulas for the three stages except for the goat milk formulas. Based on the model, the similarity of the growing-up formulas with cow milk was 58.53, which was higher than the other formulas. These results suggest that more attention should be paid to stereospeicific structure of fat in infant formulas during production to better mimick human milk.

10. **Determination of Iodine Value (IV) in Fully Hydrogenated Oils** Shaun P. Kotoski[1], and Cynthia Srigley*[2], *[1]University of Maryland, USA; [2]US Food and Drug Administration, USA*

Fully hydrogenated oils (FHO) are produced by the hydrogenation of food grade oils to achieve an iodine value (IV) of no more than 4. Hydrogenated oils with IVs of greater than 4 are considered to be partially hydrogenated oils (PHO), which the United States Food and Drug Administration (FDA) has determined are no longer generally regarded as safe (GRAS) for any use in human food. The accurate determination of IV is important for discriminating if an edible oil has been fully or partially hydrogenated. This project examined the determination of IV in seven FHO (coconut; cottonseed, n=2; palm kernel; palm stearine; and soybean, n=2 oils) according to Official Method Cd 1d-92 of the American Oil Chemists' Society (AOCS; Titration Method) and AOCS Recommended Practice Cd 1c-85 (Calculated IV Method). The Calculated IV Method involved AOCS Official Methods Ce 2-66, for the preparation of fatty acid methyl esters, and Ce 1j-07 for gas chromatographic separation. Three of the samples (cottonseed, coconut, and palm stearine) showed comparable determinations of IV in the range of 0.3–1.4. For the remaining four samples, IVs were significantly higher when analyzed according to the Titration Method than the Calculated IV Method. The Titration Method proved to be cumbersome, due to the large volumes of reagents used, tendency for oils to curdle, and requirement for extensive shaking of samples. Such challenges may lead to an overestimation of IV in FHO for laboratories with little experience in running the Titration Method.

11. **Rosin Crystallinity Measured by Modulated DSC** Kun Cheng*, Lien Phun, Ellen Nagy, and Phillip W. Hurd, *Georgia-Pacific Chemicals, USA*

The term rosin refers to as a blend of eight closely-related tricyclic diterpene acids ($C_{20}H_{30}O_2$). One major type of rosin produced commercially is tall oil rosin (TOR), obtained from the distillation of crude tall oil. TOR tends to crystallize upon cooling, making it more difficult to handle. Highly crystalline TOR requires additional energy to remelt for further processing, resulting in undesired effects, especially darker colors, in the downstream applications of this important natural product. X-ray diffraction was used to study the property of rosin crystals by Chinese and Russian scientists a few decades ago. However, DSC (differential scanning calorimetry) is an easy to use technique which provides a quantitative analysis of thermal transitions such as glass transition, melting and crystallization, as a function of temperature and time. In the present study, a modulated differential scanning calorimetry (MDSC) method has been developed to measure the crystallinity of rosin acids in TOR and found to be highly reliable and much improved over the limitations of standard DSC. MDSC revealed that TOR generally has three thermal transition regions: a) 50-80°C (softening or glass transition); b) 80-120°C (crystallization), c) 140-160°C (melting). This observation from the MDSC study agrees with literature. The energy absorbed in the melting of TOR and released in its crystallization becomes measurable only in MDSC due to the high resolution and sensitivity of this

analytical method. The initial crystallinity is quantified with energies associated with melting and crystallization.

12. **Simple, Successful High-temperature Analysis of Triglycerides by GC** Kristen Parnell, Timothy Anderson, and Ramkumar Dhandapani*, *Phenomenex, USA*

Triglycerides are esters of glycerol with three fatty acids, and are naturally occurring in food. These compounds have relatively high molecular weights that increase with the degree of unsaturation, and are typically analyzed using low polarity GC stationary phases. Though conventional methods require high oven temperatures for analyte elution, the temperature limitations of many common GC columns result in compound carryover, excess column degradation, and increased bleed, which leads to both reduced sensitivity and increased cost due to consumable replacement. This work presents successful high temperature analysis of triglycerides in butter, olive oil, peanut oil, and canola oil using GC oven temperatures as high as 400 °C. Methods for high boiling compounds utilized optimized ramp procedures and a thin film, low-polarity Zebron™ ZB-5HT Inferno™ GC column, which provided stability to 430 °C for the high oven ramp programs used. The study revealed preferred materials and methods that avoid column degradation, prevent carryover, and improve sensitivity.

13. **FAMEs Analysis in Less Than 12 Minutes! Reducing Analysis Time Using The Magic of GC Column Parameters** Kristen Parnell, Matthew Trass, Timothy Anderson, and Ramkumar Dhandapani*, *Phenomenex, USA*

Increased resolution and efficiency are every analytical chemist's desire. Though a 60 minute analytical run has traditionally been common for analysis of fatty acid methyl esters (FAMEs) due to their unique structures, analysts in production environments often mention the need for faster run times to improve productivity. Using several key GC column parameters including selectivity and dimensions, run time for a typical 37 component FAMEs sample can be reduced to as short as 12 minutes. Using a high-cyano Zebron™ ZB-FAME GC column, demonstration of successful tactics for achieving short, successful FAMEs testing methods for real food samples is explored.

14. **Improved Methods for Fast and Efficient Separation of Simple and Complex FAMEs** Kristen Parnell, Timothy Anderson, and Ramkumar Dhandapani*, *Phenomenex, USA*

Analysis of fatty acid methyl esters (FAMEs) is critical to accurately characterizing, fingerprinting, and characterizing commonly adulterated food oils. In the present study, we discuss various FAMEs separation methodologies using gas chromatography. Improved resolution of FAMEs isomers is explored through optimization of the GC column stationary phase. A progression of simple to complex analyses is performed using commercially available Zebron™ GC columns typically used for FAMEs testing: ZB-FFAP, ZB-WAXplus™, and a novel chemistry, ZB-FAME. Evaluation of the various methods demonstrates that short run times, complete resolution of 37 complex FAMEs compounds, and efficient separation can be achieved using a short 30 meter column.

15. **Optimized Column Selectivity for Orthogonal Separation of Fatty Acid Methyl Esters Using GCxGC** Kristen Parnell[1], Timothy Anderson[1], Ramkumar Dhandapani*[1], Anumeha P. Muthal[2], and Nicholas Snow[2], *[1]Phenomenex, USA; [2]Seton Hall University, USA*

The use of multidimensional gas chromatography such as GCxGC is an advancement

that aids in maximizing peak capacity through the use of two orthogonal selectivities. In this study, we discuss GCxGC separation of fatty acid methyl esters (FAMEs) using two unique and highly efficient GC columns: Zebron™ ZB-FAME and Zebron ZB-5MSplus™. To optimize separation, exploration of polar/non-polar and non-polar/polar configurations are explored, in addition to adjustment of modulator parameters such as hot/cold jet pulse time. The results of the experiment demonstrate good peak shape and a successful GCxGC method for FAMEs separation.

16. **AOCS Method Ce 6-86 Antioxidants: Interfering Peak in the Analysis of TBHQ in Crude Canola / Rapeseed Oil** Mark W. Collison*, Michael R. Blumhorst, Travis A. Mahan, Kathryn M. Stanley, and Aaron P. Griffith,*Archer Daniels Midland Co., USA*

AOCS Official Method Ce 6-86 Antioxidants – Liquid Chromatographic Method was originally intended to confirm that the correct antioxidant was added at the specified concentration into refined oils. Today this method is increasingly utilized to assure that antioxidants are absent from oil products. False positive results can have a significant impact on the ability to sell product in specific markets and can impart additional business expenditures for conclusive secondary analyses. In the current work, we describe an interference in the determination of tert-butylhydroquinone (TBHQ) using AOCS Ce 6-86, where false positive results are observed in crude canola / rapeseed oil. Analysis by GC-MS and high-resolution LC-MS identified the interferent as 2,6-dimethoxy-4-vinylphenol (canolol), an endogenous compound present in crude canola / rapeseed oil. Resolution of canolol and TBHQ can be achieved via minor modification of the method conditions.

17. **Quantitative Determination of Polyphenol Content in Olive Oil by HPLC-MS** Dana E. Walkenhorst*, John Reuther, and Cheryl D. Stephenson,*Eurofins Central Analytical Laboratories, USA*

Renewed interest in the health benefits of polyphenols in olive oil and recent regulations regarding the right to claim these benefits have led to an increased need for identification and quantification of individual polyphenols. The aim of this study was to detect and quantify thirteen individual polyphenols that are most commonly found in olive oils by means of HPLC-MS. Reversed phase C18 column with 5 µm particles as stationary phase was employed and quantification was performed using available commercial standards.

18. **Identification of Sulfur Species in Lightweight Fractions of Biodiesel Distillate** Michael D. Hughes*, *Delaware State University, USA*

Environmental pollution has become one of the biggest issues facing this planet. Pollution causes several health related disorders and it is almost impossible to prevent exposure. Petroleum-based fuels, a major pollutant, not only releases harmful chemicals and greenhouse gases into the atmosphere and water supply, but also cause the depletion of a nonrenewable natural resource. Biodiesel has rapidly increased in usage over the past few years serving as a more environmentally friendly fuel source. However, its sulfur content poses significant health risk to humans. In order to properly utilize biodiesel and to continue to take steps towards the production of clean burning fuel, the study aims to identify the sulfur species in lightweight fractions of biodiesel for extraction. Solid phase extraction (SPE) will be used as a means of concentrating sulfur species present in biodiesel. Solvents ranging from least to most polar will effectively separate polar sulfur species from non-polar fatty acids and other constituents,

creating a concentrated sulfur sample. The samples will then be tested using TS 3000 Total Sulfur Analyzer connected to a TS-UV module to determine sulfur concentration in parts per million. Finally, specific sulfur molecules will be determined using mass spectrometry.

19. **Total Lipid Contents and Fatty Acid Composition of Some Marine and Freshwater Fish in Turkey** Ilkay Turhan Kara*[1], Ugurcan Bashan[1], Mehmet Bashan[2], Veysi Kizmaz[3], and Sevil Yucel[4],[1]*Istanbul Arel University, Turkey;* [2]*Dicle University, Turkey;* [3]*Artuklu University, Turkey;* [4]*Yildiz Technical University, Turkey*

In this study total lipid contents and fatty acid compositions of total lipids in the muscles of 3 freshwater fish species and 10 marine fish species were examined by gas chromatographic (GC) method. The total lipid contents in the muscles of marine fish species and freshwater fish species varied from 0.53 % to 4.88 % and from 1.86 % to 2.34 %, respectively. Nineteen different fatty acids were determined in all examined fish. Carbon number of fatty acids compositions were found between C:14 and C:22. The major fatty acids of total lipids of marine fish were palmitic acid (16:0) (% 19.30 - 27.89) in SFA, oleic acid (18:1) (% 14.87 - 31.28) in MUFA and docosahexanoic acid (22:6, DHA) (% 9.30 - 26.33) in ω3-PUFA. The proportion of eicosapentaenoic acid (EPA) of Red Mullet, Picarel and European Pilchard and the proportion of docosahexaenoic acid (DHA) of Horse Mackerel, Mackerel, Red Mullet and European Pollock was found very high rate. The ω3/ω6 ratio in total lipids of wild marine fish species was determined from 0.95 to 6.93. The dominate components in total lipits were found similar both marine and freshwater fish species. Nevertheless, DHA (22:6-ω3) percentage of wild marine fish species were found higher than the freshwater fish species and α-linolenic acid (18:3-ω3) and arachidonic acid (20:4-ω6) of wild marine fish species were found lower than the freshwater fish species. It was shown that total lipit contents and fatty acid compositions in the muscles of fish were significantly influenced by habitat.

20. **Quality Characteristics of Olive Oils Extracted from "Hurma" Olives** Dilek Kaçar[1], Esmaeil Ghanberi Shendi[2], Didar Ucuncuoglu[3], and Dilek Sivri Özay*[2],[1]*Çaycuma Vocational School, Bülent Ecevit University, Turkey;* [2]*Hacettepe University, Turkey;* [3]*Cankiri Karatekin University, Turkey*

Olives cannot be consumed after harvest because of the bitter taste. Several methods are used such as cracking, fermentation, curing with water, brine and lye in order to debittering. Olive variety, mostly "Erkence" grown in Karaburun peninsula of Izmir (Turkey) are debittered on the tree with a fungus (Phoma spp.) naturally. This olive is known as "hurma" olive and processed for olive oil production. The aim of this study was to investigate the quality of olive oil extracted from hurma olive. The olive oil samples were extracted by a laboratory scale extraction system (HAUS Centrifuge Technologies, Turkey). Free fatty acid, peroxide and UV absorbance values, moisture content and fatty acid profile were performed according to the methods of IOC. The total phenols were determined by the Folin&Ciocalteu reagent. Polar fractions of MVOO obtained by solid phase extraction (SPE). Phenolic and tocopherol profiles were obtained by ultra high performance liquid chromatography (UHPLC). Volatile compounds were detected by SPME-GC/MS. The amounts of α-tocopherol and β-tocopherol were 67 and 0.4 ppm, respectively. Hurma olive oils had high range of phenolic compounds and luteolin was the most abundant (35-111 ppm). Aldehydes (0,53 ppm), ketones (0,38 ppm), acids (0,05 ppm) were found surprisingly low. Some off-flavor compounds due to microbial activity and lipid autoxidation were also

detected. It can be concluded that MVOO extracted from hurma olives can be classified as "ordinary virgin olive oil" according to their free fatty acid, peroxide values and fatty acid composition according to the IOC trade standarts.

21. **Gas Chromatography of Non-conjugated Cis/Trans 18:2 Isomers Using 100 m Biscyanopropyl-polysiloxane and SLB-IL111 Columns** Payam Vahmani*[1], David C. Rolland[1], Katherine K.E Gzyl[2], and Michael E.R Dugan[1],[1]*Lacombe R&D Centre, Agriculture and Agri-Food Canada, Canada;* [2]*University of Lethbridge, Canada*

Several positional trans(t)-18:1 isomers (t4-t16-18:1) are formed during ruminal biohydrogenation of dietary unsaturated fatty acids and get incorporated into ruminant fats (e.g. beef and dairy). In the present study, liver cells were cultured with individual t-18:1 isomers and GC chromatograms of their triacylglycerol fatty acid methyl esters (FAME) were compared to beef fat chromatograms, and objectives included either confirming the presence of t-18:1 Δ-9 desaturase products in beef fat or tentatively identifying novel 18:2 isomers and their retention times. Individual t-18:1 isomers including t12-, t13-, t14-, t15- and t16-18:1 were isolated from beef fat using a combination of Ag+SPE and Ag+-HPLC, while t6-18:1 was sourced commercially. HepG2cells were cultured with individual t-18:1 isomers, and retention times of their Δ-9 desaturation products were determined using 100 m biscyanopropyl-polysiloxane and SLB-IL111 columns. Corresponding peaks were found in beef adipose tissues (i.e. subcutaneous fat and perirenal fat) known to have different delta-9 desaturase activities. Further lines of evidence indicating the presence of Δ-9 desaturation products of t-18:1 isomers in beef fat were developed by analysis of FAME fractionated using Ag+-TLC, and by GC/MS. Some of the Δ-9 desaturation products of t-18:1 have been previously identified in ruminant fat including cis(c)9,t12-18:2 and c9,t13-18:2. Some of the Δ-9 desaturation products of t-18:1 (c9,t14-18:2 and c9,t15-18:2) have been previously tentatively identified as different fatty acids, and for the first time we provide evidence of the presence of c9,t16-18:2, and where t6,c9-18:2 may elute during analysis of FAME from beef fat.

22. **Novel, Rapid FT-NIR, and PLS1 Proposed Procedure for Predicting Authenticity of Extra Virgin Olive Oils** Magdi Mossoba*[1], Hormoz Azizian[2], Ali Reza Fardin-Kia[1], Sanjeewa R. Karunathilaka[1], and John K.G. Kramer[3], [1]*US Food and Drug Administration, USA;* [2]*NIR Technologies, Canada;* [3]*Guelph Food Research Center, Canada*

A new, rapid Fourier-transform near-infrared (FT-NIR) spectroscopic procedure* is described to screen for the authenticity of extra virgin olive oils (EVOO) and, based on three criteria, to predict the nature and amount of refined olive oil (RO) or a potential adulterant in EVOO in minutes. Partial least squares (PLS1) calibration models were developed (1) to estimate a newly created FT-NIR index based mainly on the relative intensities of two unique NIR carbonyl overtone absorption bands attributed to volatile (5280 cm−1) and non-volatile (5180 cm−1) components; a low index value negatively reflected on the purity and/or quality of EVOO, (2) to predict the fatty acid (FA) composition, and (3) to predict the type and the quantity of refined olive oil (RO) or one of three potential adulterants (oils high in linoleic or oleic acid, or palm olein) each with a characteristic FA composition. The latter was achieved by preparing gravimetric mixtures of EVOO spiked with these edible oils. Plots of predicted vs. gravimetric concentrations for each of these oils mixed with EVOO yielded linear regression functions with four unique sets of slopes. Four corresponding slope

rules were defined and allowed the prediction of the type of oil potentially mixed with EVOO. The 66 multi-national, authentic EVOO varieties or blends analyzed were found to belong to four distinct "Groups," each with a characteristic linoleic acid concentration range, and required the development of additional sets of group-specific PLS1 calibration models. *Lipids. 50:705-718 (2015); Lipids. DOI 10.1007/s11745-016-4195-0 (2016).

23. **Rapid Screening of Extra Virgin Olive Oil Products for Authenticity Using Near-infrared Spectroscopy in Combination with Chemometrics** Sanjeewa R. Karunathilaka*, Ali Reza Fardin-Kia, Cynthia Srigley, Jin Kyu Chung, and Magdi Mossoba,*US Food and Drug Administration, USA*

A rapid tool for evaluating authenticity was developed and applied to the screening of extra virgin olive oil (EVOO) retail products by using Fourier-transform near infrared (FT-NIR) spectroscopy in combination with univariate and multivariate data analysis methods. Using disposable glass tubes, spectra for 62 reference EVOO, 10 edible oil adulterants, 20 blends consisting of EVOO spiked with adulterants, 88 retail EVOO products and other test samples were rapidly measured in the transmission mode without any sample preparation. The univariate conformity index (CI) and the multivariate supervised soft independent modeling of class analogy (SIMCA) classification tool were used to analyze the various olive oil products which were tested for authenticity against a library of reference EVOO. Better discrimination between the authentic EVOO and some commercial EVOO products was observed with SIMCA than with CI analysis. Approximately 61% of all EVOO commercial products were flagged by SIMCA analysis, suggesting that further analysis be performed to identify quality issues and/or potential adulterants. Due to its simplicity and speed, FT-NIR spectroscopy in combination with multivariate data analysis can be used as a complementary tool to conventional official methods of analysis to rapidly flag EVOO products that may not belong to the class of authentic EVOO.

24. **Fast GC Analysis of *trans* Fatty Acids in Food Products** Marc Gregerson*, and A. Carl Sanchez,*Phenomenex, USA*

Edible oils are common ingredients in food products and are often industrially processed to improve texture, shelf life and other functional properties. This processing typically increases the amount of trans unsaturated fatty acids. The amount of these industrially produced trans fatty acids in foods is under increasing regulatory scrutiny around the world due to their linkage to coronary heart disease. Recent legislation in the USA and EU limits the amount of industrially produced trans fatty acids allowed in foods and edible oils. Fatty acids are typically analyzed as the methyl ester derivatives, or FAMEs, using long (100m) GC columns coated with high polarity cyano-based stationary phases. One of the drawbacks of long GC columns is the accompanying long run time. In this study, we present a unique cyano-based stationary phase optimized to improve resolution and speed of analysis of complex FAME mixtures. A 30m column coated with this new stationary phase resolved all 37 components in a common 37-componenent FAMEs standard with resolution of ≥ 1.3 in 25 minutes. Furthermore, the fatty acid profile of several foods was determined using this new stationary phase as well as several conventional cyano-based GC columns. A 30m column resolved all the fatty acids of regulatory interest in the food extracts allowing a complete profile of the cis/trans fatty acids in each food item in almost half the time of

conventional cyano-based GC columns. Additionally, the improved cyano-based stationary phase has a higher maximum operating temperature than other commonly used cyano-based columns.

25. **Verification of Perilla Oil Authenticity Using Carbon Stable Isotope and Fatty Acid Analyses**

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The aim of this study was to verify the authenticity of perilla oils currently distributed in Korea using the analysis of carbon stable isotope ratio or fatty acid profile of the oils. No significant difference was found in the $\delta^{13}C$ value between the authentic (n = 27) and adulterated perilla oils (n = 10), whereas the content values of 11 different fatty acids in the adulterated perilla oils were significantly ($p < 0.05$) different from those in the authentic perilla oils. By using the orthogonal projection to latent structure discriminant analysis technique, the 18:2*n*-6 and 18:3*n*-3 content values were selected as variables that most effectively verify the perilla oil authenticity. A blind trial using 74 additional oil samples demonstrated that the perilla oils with foreign edible oils added at concentrations ≥3 v/v% could be distinguished by applying the range of the two variables observed in the authentic perilla oils.