ANA 1: Algal and Marine Oils Analysis

Chairs: K. Persons, Eurofins, USA; and T. Haines, Archer Daniels Midland Co., USA

Analysis of Hydrotreated Algae Oil Composition and Properties with the Advanced Distillation Curve Method. P. Hsieh*, J. Widegren, and T. Bruno, National Institute of Standards and Technology, Boulder, CO, USA.

Second-generation alternative fuels are synthesized thermochemically from non-food biomass feedstock, including microalgae and agricultural by-products. Hydrotreated alternative fuels are more chemically stable and less corrosive than fatty acid ester and alcohol fuels, being closer in composition to aliphatic Fischer-Tropsch synthetic fuels. We applied the Advanced Distillation Curve method to an algae oil sample that has been hydrotreated to yield a middle distillate fuel, and measured its boiling temperature as a function of distillate volume fraction. The bulk fuel and distillate compositions were characterized through nuclear magnetic resonance spectroscopy, gas chromatography and mass spectrometry. The results suggest that the empirical equation for calculated cetane index, based on petroleum distillate data, may need to be adjusted for second-generation alternative fuels such as hydrotreated algae oil. The quantitative data can be used to improve formulation of hydrotreated algae oil and other second-generation alternative fuel blends.

Novel Methoxylated Phospholipid Fatty Acids from the Caribbean Sponge *Asteropus niger*. N.M. Carballeira*, N. Montano, C. Adorno, and A. Rodríguez, Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico, USA 00931.

Sponges continue to be the source of some of the most unusual phospholipid fatty acids reported in recent years.[1] Over the years, our search for novel phospholipid fatty acids from marine sponges has unveiled a new class of a-methoxylated phospholipid fatty acids with a myriad of chain lengths, unsaturation and methyl branching patterns, and more importantly, enhanced biological activities (such as antiprotozoal and anticancer) as compared to the most traditional mammalian fatty acids.[2-3] In this work we present the complete phospholipid fatty acid composition of the rare Caribbean sponge *Asteropus niger* where a new family of a-methoxylated fatty acids have been just discovered. Some of these unusual fatty acids include a novel a-methoxylated octadecatrienoic fatty acid and several iso/anteiso a-methoxylated Δ5,9 fatty acids with chain lengths between 27 and 29 carbons. The characterization of the phospholipids (mainly PtdIns and PtdCho) that are responsible for these intriguing fatty acids, the complete characterization of the novel fatty acids by several analytical techniques such as gas chromatography-mass spectrometry, and the biological implications of these results will be presented.

A Method to Evaluate Lipid Oxidation in Encapsulated Systems. M.L. Herrera*[1] and R.J. Candal[2], [1]Instituto de Tecnologia de Polimeros y Nanotecnologia, UBA-CONICET, Buenos Aires, CABA, Argentina, [2]Instituto de Investigacion e Ingenieria Ambiental, San Martin, Buenos Aires, Argentina.

Encapsulation has been widely used to protect sensitive materials against oxidation and chemical reaction. However, when physical properties of encapsulating materials are evaluated, most authors use the extractable material for analysis. In other cases, encapsulated lipids suffer more damage for oxidation than the extractable fraction. Most likely oxidation occurred during the different steps (heating and agitation) involved in traditional methods. The aim of the present work was to develop a method to study oxidation of encapsulated lipid materials that not involves oxidative treatments. A concentrate of fish oil, coming mainly from salmon, enriched in DHA, EPA, and DPA was encapsulated in a sodium caseinate/trehalose matrix. The obtained powders were analyzed for physical chemical properties. Lipid material coming from washed powders dissolved in chloroform and not subjected to any other treatment, and fish oil extracted from powders with a traditional method were analyzed by MALDI-TOF. The extracted oil was oxidized as evidence from MALDI-TOF pattern and confirmed by peroxide value. On the contrary, encapsulated oil evaluated by direct dissolution of powder showed a MALDI-TOF pattern similar to the pattern of original oil. MALDI-TOF technique allowed to demonstrate something generally accepted but not well-proved: encapsulation protects lipids against oxidation.

Direct Methylation and Extraction for Fish Oils. C. Hyun*, M. Dan, and C. Choi, Amway, Buena Park, CA, USA.

With the growing awareness of health conditions such as obesity, diabetes and cardiovascular disease that may be caused by unhealthy diets, consumers are becoming more conscious of the importance of maintaining a good diet. Consequently, consumers are becoming more aware of the fat content in foods. However, there are differences between 'good fat' and 'bad fat' and determining the composition of fats in food for nutritional labeling purposes can be challenging. In this method, the fat composition of a variety of food products are determined by direct extraction methylation (DEM) of the sample and ultimate conversion of the fatty acids to their fatty acid methyl ester (FAME) forms which are simultaneously extracted in hexane. The FAME levels are quantitatively determined by capillary gas chromatography (GC) using FAME reference standards and C13 as an internal standard. Individual FFA concentrations or total FFA concentrations are then expressed as triglyceride equivalents. This method can be used in a wide variety of products for the identification and measurement of Linoleic Acid (LA), Gamma linoleic Acid (GLA), Alpha Linolenic Acid (LNA), EicosaPentaenoic Acid (EPA), and DocosaHexaenoic Acid (DHA), along with other fatty acid components present in the FAME reference standard.

The Composition of a New Algal Oil that Contains Eicosapentaenoic Acid (EPA, 20:5n-3) and Palmitoleic Acid (16:1n-7). A. Thompson*, M. Collins, J. Hippler, A. Ryan, and J. Astwood, Aurora Algae, Inc., Hayward, CA, USA.

Algae can be grown photoautotrophically using sunlight as energy and carbon dioxide (CO_2) as the building block for organic material. Alternatively, they can be grown heterotrophically using preformed organic substrates such as glucose for energy and the material from which to build organic material. Heterotrophically grown algae require cultivation of crops from which the organic substrate is derived. In contrast, photoautotrophic cultivation of algae have fewer environmental requirements than those needed for heterotrophic cultivation. Sunlight is freely available as the energy source as are waste streams of CO_2 to form the organic substrate. Arid land not cultivatable for agriculture can be used for ponds to cultivate the algae. Seawater can be used as the water source. Aurora Algae, Inc. has developed a unique composition of algal oil (Algal-EE) developed from photoautotrophic algae grown in outdoor open pond systems. This study describes the composition of the new algal oil ethyl esters (Algal-EE) derived from *Nannochloropsis sp.* Algal-EE contains the ethyl esters of EPA (25-30 wt%), palmitoleic acid (20-25 wt%), a small amount of linoleic and arachidonic acid (= 6.0 wt%) and no docosahexaenoic acid (DHA, = 0.1wt%). The purity standards conform to the GOED specifications for EPA and DHA.

Dynamic Lipid Profiling in Algae: Ultrahigh-resolution Mass Spectrometry Discovery of Novel Components of Advanced Biofuels Intermediates. L. Laurens*[1], E. Christensen[1], B. Black[1], A. Nag[1], and T. Schaub[2], [1]National Renewable Energy Laboratory, Golden, CO, USA, [2]New Mexico State University, Las Cruces, NM, USA.

Algal lipid yields are unmatched by any terrestrial feedstock, which makes their use ideal for renewable fuels and chemicals. Using algal lipids as fuel precursors has proven challenging due to the lack of complete characterization, there remain many lipid components unidentified or difficult to study. We present data on the utilization of ultra-high-resolution, fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) for the identification and quantification of novel components. Using a combination of Kendrick mass sorting and elemental characterization of molecular ions, series of compounds have been identified. Thousands of components can be identified from their exact mass, with validation of the lipid annotation using quantitative HPLC-MS. The superior mass accuracy and resolving power of the FT-ICR-MS has proven to be a necessary tool for creating a reference library of novel compounds for algae. We present data on the use of multivariate statistical analysis of high-resolution MS data to reduce the complexity of the data. Our results illustrate a dynamic lipid profile and class distribution as two algae are transitioning from early to late stage of cultivation.

Fungal Oil with EPA in *Mucor circinelloides* Cultivated on Thin Stillage from Corn-to-Ethanol Production. S. Ravi*[1], L. Yao[1], T. Wang[1], J. van Leeuwen[1], D. Mitra[2], and H. Duygu Özsoy[3], [1]Iowa State University, USA, [2]Intel Corporation, USA, [3]Mersin University, Turkey.

This study demonstrates the potential of the oleaginous fungus *Mucor circinelloides* for synthesizing oil rich in polyunsaturated fatty acids

when cultivated in thin stillage (TS) and centrifuged thin stillage (CTS) from corn-ethanol production. The fungal oil yield and fatty acid profile were studied by changing duration of cultivation and carbon to nitrogen (C: N) ratio of the growth media. Glycerol as a carbon source and urea, potassium nitrate and ammonium sulfate as nitrogen supplements were used to alter the C: N ratios. The fungal biomass yield (g/L) and its oil content (g/L) were found to be twice as high in TS as in CTS when harvested after two to six days. Glycerol supplementation of 20g/L led to an 18% increase and 63.6% decrease in fungal oil yield in TS and CTS respectively. Adding 5g/L of ammonium sulfate as a nitrogen source enhanced the fungal oil yield by 34% in TS and 26% in CTS. Similarly 0.5 g/L of urea addition increased the fungal oil yield by 12% and 13% in TS and CTS respectively. The fungi were found to be capable of synthesizing eicosapentaenoic acid in all conditions tested with longer retention times.

ANA 1.1/LOQ 1: Evaluation and Correlation of Sensory and Analytical Methods for Assessing Rancidity

Chairs: M. Collison, Archer Daniels Midland Co., USA; and C. Jacobsen, Technical University of Denmark, Denmark

Using Secondary Oxidation Products to Evaluate the Sensory Stability of Foods with Different Antioxidants. X. Yang*, P. Joseph, L. Burroughs, R. Boyle, J. McKeague, M. Wolf, and R. Nahas, Kalsec, Inc., Kalamazoo, MI, USA.

The most reliable method to evaluate food product quality is real life sensory evaluation, which determines whether a product still has acceptable sensory characteristics in a given time period. However, when there are multiple product formulations to be assessed, for example when different antioxidants need to be tested to improve the product shelf-life, sensory evaluation could be both time and cost-consuming. Therefore, faster analytical methods are favorable in this case. Here, the concentrations of secondary oxidation products measured by headspace SPME GC-MS were found to follow closely the development of oxidized flavors and aromas evaluated by sensory analysis in foods with different antioxidants. The correlation between sensory scores and secondary oxidation product levels revealed that the measurement of the appropriate secondary oxidation marker may serve as a simple, rapid and effective analytical tool to evaluate various antioxidants performance in different food applications.

Can Volatile Lipid Oxidation Products Be Used to Predict Sensory Qualities of Fish Oils? S.M. Budge* and J.C. Sullivan Ritter, Dalhousie University, Halifax, NS, Canada.

Polyunsaturated fatty acids (PUFA) found in fish oil are well known for both their health benefits and oxidative instability. This had led to the development of a number of simple chemical tests, such as peroxide and *p*-anisidine values, to evaluate the extent of lipid oxidation in fish oils. However, these tests rarely agree with the sensory perception of oxidation and a number of alternative approaches to track lipid oxidation have been proposed. The simplest methods measure individual compounds arising from lipid oxidation. More complex methods involve multivariate statistical techniques to allow correlation of concentrations of volatile lipid oxidation products with sensory evaluation. Here we describe a method that uses solid phase microextraction coupled with GCMS to acquire concentrations of lipid oxidation products; principal component analysis is then used to identify the volatiles that best correlate with the sensory perception of fish oil rancidity.

New Challenges for Evaluation of Oxidative Stability and Shelf Life of Oils/Fats and Oils/Fats Contained Products. M. Hu* and J. Erdmann, DuPont Nutrition & Health, Four New Century Packway, New Century, KS, USA.

In food and pet food industry, a business decision may be made based on oxidative stability data of products. Obviously, a number of analytical methods can be selected to evaluate oxidative stability and shelf life of oils/fats and oils/fats contained products. Also there are advantages and disadvantages for each analytical method. Thus, it is critical to select right analytical method for evaluating oxidative stability of a product and the efficacy of an antioxidant in a specific food system. In the oral presentation, we will use new data to show how to select right analytical method for a target food system and to use the data for a business

decision. For instance, we will evaluate oils based on OSI data in high temperature and headspace oxygen levels over ambient storage time, and assess solid samples based on Oxipres at 100°C and PV and hexanal level over ambient storage time. In addition, the following issues will be discussed: 1, how to evaluate heat sensitive antioxidants. 2. how to select accelerated and ambient storage testing. 3. how to compare oxidative stability of different foods with different matrixes, 4. how to avoid pitfall when comparing data from different methods or same method but different pretreatments and analytical procedures as well as different units used.

Thinking Beyond Traditional Theory in Lipid Oxidation: Alternate Pathways that Compete with Hydrogen Abstraction. K.M. Schaich*, B. Bogusz, and J. Xie, Rutgers University, New Brunswick, NJ, USA.

Current attempts to stabilize foods reformulated with polyunsaturated fatty acids as well as to elucidate the role of lipid oxidation in pathology challenge traditional understanding of lipid oxidation mechanisms. For more than 50 years, lipid oxidation has been explained simply as a free radical chain reaction composed of series of hydrogen abstractions, with radical recombinations afterwards leading somehow to products. However, observed kinetics and distributions of products do not always fit this mechanism. This paper presents evidence for multiple alternate pathways of peroxyl and alkoxyl radicals, including internal rearrangements, additions to double bonds, and scissions that compete with hydrogen abstraction in lipid oxidation and alter both observed kinetics and products. Questions are raised also about dominance of hydroperoxide positions and a vs b scissions of alkoxyl radicals as currently accepted. A concerted mechanism integrating the alternate pathways is proposed to stimulate new research in lipid oxidation mechanisms.

Proton NMR Can Be Used to Measure Epoxides Derived from Lipid Oxidation. W. Xia*, S.M Budge, and M. Lumsden, Dalhousie University, Halifax, NS, Canada.

Hydroperoxides and carbonyl compounds are typically viewed as the main products of lipid oxidation. Recently, epoxides have also been suggested as important intermediates but there is a lack of suitable methods for their determination in oxidized oils. Here we describe a method to quantify epoxide yield during lipid oxidation using ^{1}H NMR. To investigate the chemical shifts of mixed epoxides derived from polyunsaturated fatty acids, fresh fish oil was epoxidized using formic acid and hydrogen peroxide. The chemical shifts of mixed epoxides in epoxidized fish oil were found to be between 2.9-3.3 ppm. The peaks associated with glycerol remained constant during both oxidation and epoxidation of fish oil, allowing them to be used as an internal reference for the quantification of epoxides. Oil samples with different epoxide concentration were made and standardized using the AOCS method for oxirane oxygen. These were analyzed by ^{1}H NMR under standard conditions to generate a calibration curve. To demonstrate the utility of the method, commercial oils were then oxidized under a variety of conditions and epoxides were determined by ^{1}H NMR.

Development of Defective Aroma References for Sensory Evaluation of Virgin Olive Oil. H. Zhu*[1], S. Langstaff[2], C. Shoemaker[1], and S. Wang[2], [1]Department of Food Science and Technology, University of California Davis, Davis, CA, USA, [2]University of California Davis Olive Center, Davis, CA, USA.

Negative flavors and aroma attributes such as rancid, fusty, musty, muddy-sediment, winey-vinegary are sensory attributes of defective virgin olive oil recognized by the United States Department of Agriculture. Solid phase microextraction-gas chromatography/mass spectrometer (SPME-GC/MS) is employed to establish a flavor profile for each defective oils and the significant compounds in certain concentration are selected for each defective flavor to develop sensory reference standards. For example, E-decenal (120 mg/kg), E-2-octenal (35 mg/kg), E-2-heptenal (10mg/kg), nonanal (120 mg/kg), and E-2-nonenal (30 mg/kg), etc. are identified as significant compounds to reproduce the flavor and aroma of rancidity. The reference standard of each defective flavor can be reproduced with ease in the laboratory and used for sensory panel training. To the best of our knowledge, currently there are no reference samples available for olive oil though they exist for some other foods and beverages such as wine and beer.

Monitoring Lipid Oxidation of Canola Oil Using Surface Enhance Raman Spectroscopy (SERS). M. Driver* and L. He, University of Massachusetts, Amherst, MA, USA.

Lipid oxidation is a major challenge in the food industry's attempts to incorporate nutritionally

beneficial lipids in food products. However, conventional methods are time consuming and not as sensitive as the human nose in detecting oxidative rancidity. Surface-enhanced Raman spectroscopy (SERS) is combination of Raman spectroscopy and nanotechnology. Raman signals can be enhanced tremendously using gold nanoparticles, thus increasing sensitivity greatly. The objective of this study is to develop and apply a SERS based method for rapid detection of lipid oxidation. Gold nanoparticles fabricated in citrate buffer were firstly modified with alkanethiols to change the hydrophobicity, so that they can be dispersed well in hexane diluted canola oil. The oxidation of canola oil was monitored daily at 55° C for two weeks using conventional methods that measured conjugated dienes, hydroperoxides, and hexanal, conventional Raman spectroscopy and SERS. The significant increase of conjugated dienes, hydroperoxides, and hexanal were observed at day 10, 7, 14 respectively. The conventional Raman spectra of 100% oil didn't change significantly over time, while the SERS spectra of 3% oil changed significantly at day 2 analyzed by principal component analysis. This study demonstrated the potential of the SERS method for rapid and sensitive detection of lipid oxidation.

Improved Gas Chromatography-flame Ionisation Detector Analytical Method for the Analysis of Epoxy Fatty Acids. E. Mubiru*, K. Shrestha, A. Papastergiadis, and B. De Meulenaer, Ghent University, Ghent, Belgium.

In this study an improved method for analysis of epoxy fatty acids is reported. Data obtained from analysis of polar fatty acids has previously been presented, but due to the high number of compounds that co-elute in the polar fraction, the resultant chromatograms are complex which may lead to compromising the accuracy of the data. A three steps separation of fatty acid methyl esters (FAMEs) by solid-phase extraction (SPE) on a silica gel column to remove hydroxy fatty acid interferences was proposed. This approach is opposed to a two step separation procedure that has been often used to prevent analytical interferences caused by non-altered fatty acids. A gas chromatograph with a flame ionisation detector (GC-FID) equipped with a polar CP-Sil 88™ column was used. Quantification was based on the use of methyl nonadecanoate (C19:0), as an internal standard. Individual mono epoxy fatty acids were well separated without co-eluting compounds. The optimised method was finally applied to screen epoxy fatty acids in 37 fresh oil samples. Results obtained for the total epoxy fatty acids were in the range 0.03 – 2 mg g^{-1} of oil with repeatability coefficient of variation (CV) ranging from 2.8 to 9.9 % for duplicate analysis showing that the results obtained are repeatable.

Shelf Stability of Vegetable Oils. A. Syed and M. Evenson*, Dow AgroSciences, Indianapolis, IN, USA.

A 2-year long shelf stability study was conducted on common vegetable oils. Chemical and sensory analyses were conducted at intermittent time points. Resulting data suggest that each oil has a characteristic behavior of its own, and that using the one analytical criteria to grade them may not be exactly instructive.

ANA 2: Trace Contaminants

Chairs: S. MacMahon, US, FDA, USA; and J.D. Pinkston, Kellogg Company, USA

LC-MS/MS Detection of 2-MCPD Esters in Edible Oils. S. MacMahon* and T.H. Begley, U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA.

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD) and glycidol are potentially carcinogenic contaminants formed during the processing of commonly consumed edible oils. While method development and risk assessments have primarily focused on 3-MCPD and glycidyl esters, 2-MCPD esters have not been studied as extensively. Currently, the only validated methods for the detection of 2-MCPD esters in edible oils are so-called "indirect" methods. These methods require hydrolysis followed by chemical derivatization prior to analysis, resulting in the loss of any information about the structures of the intact esters. The development of a quantitative method which detects intact 2-MCPD is required.

The approach described herein involves rapid SPE cleanup of solid and liquid oils followed by LC-

MS/MS detection using electrospray ionization and quantitation of intact 2-MCPD in edible oils. The synthesis of 2-MCPD ester standards and the development of this method will be discussed. This rugged, sensitive and specific method allows for the direct determination of fatty acid esters of 2-MCPD and is suitable for regulatory analysis.

A Rapid Indirect Method for Simultaneous Determinations of 2-/3-MCPD Esters and Glycidyl Esters in Edible Oils. K. Koyama*, K. Miyazaki, S. Nakata, and M. Ebina, House Foods Group Inc., Japan.

Esters of monochloro-1,2-propanediol (MCPD) and glycidol that occur in refined edible oils are causing public health concerns. A number of indirect and direct methods for monitoring these esters have been proposed. Indirect methods, which determine total amount of 2-/3-MCPD and glycidol after hydrolysis of the esters, have an advantage over direct methods. The existing indirect methods, however, require long hours of hydrolysis. The aim of this study was to develop a more rapid indirect method through the use of a lipase in the hydrolysis step. Among the several lipases from different sources we tested, the lipase from *Candida rugosa* gave the best results and hydrolyzed MCPD-diesters, -monoesters and glycidyl esters satisfactorily within 30 min. By using *C. rugosa* lipase and sodium bromide for the hydrolysis / bromination step, 2-/3-MCPD esters and glycidyl esters were transformed, respectively, to 2-/3-MCPD and 3-bromo-1,2-propanediol without spontaneous interconversion. With a sample preparation time of about 5 hours for ten samples and a calibration curve, GC/MS analysis of 2-/3-MCPD and 3-MBPD derivatized with phenylboronic acid gave recovery rates and repeatability comparable to those of the existing methods for all the analytes spiked in edible oils.

Pitfalls in the Analysis of MCPD Esters and Glycidyl Esters in Foodstuffs. K. Hrncirik* and A. Ermacora, Unilever R&D, Vlaardingen, The Netherlands.

Reliable quantification of MCPD esters and glycidyl esters is of high importance as it enables mapping their occurrence, performing the exposure assessment, studying the mechanism of formation, developing mitigation technologies and establishing monitoring programs. A significant effort was spent in the development of analytical methodology for these process contaminants over the past years. As a result, several methods have been compared, validated and adopted as AOCS Official Methods. These methods, however, are suitable solely for the analysis of MCPD esters and glycidyl esters in oils and fats. Some attempts have already been made to apply these methods to the analysis of various foodstuffs. This is a risky endeavor though, since such applications bear considerable risks, and meticulous effort is required to avoid false results in food surveys and routine screening. In fact, the analysis of food products is complicated by several factors, namely the lack of a uniform technique for the extraction of MCPD esters and glycidyl esters from the food matrix, and the lack of a reference material suitable for method validation. This speech addresses the major challenges in the development of analytical methods suitable for various food products, and several options on how to deal with these challenges are discussed.

Analysis of Free and Bound MCPD and Glycidol in Edible Oils and Related Foods – Possibilities and Limitations. J. Kuhlmann*, SGS Germany GmbH, Hamburg, Germany.

3-Chloro-1,2-propandiol (3-MCPD) has been known for a long time as a food contaminant that might be generated during food processing. Hence acid hydrolysed vegetable protein and seasonings as well as roasted, toasted, grilled, barbecued and smoked foods were prone to contain free 3-MCPD. More recent findings have shown the formation of fatty acid ester bound 2- & 3-MCPD and 2,3-epoxy-1-propanol (glycidol) during the refining process of edible oils & fats. As MCPD and moreover glycidol are undesired components in regard to their potential impact on consumers health, the necessity for methods that allow reliable determination of these analytes is obvious. Major progress has been made in this field of trace analysis so that several different approaches for the determination of bound MCPD and glycidol mainly in oils and fats have been established in the last years. As European supervising authorities are currently discussing the aim of monitoring the contents of free and bound MCPD and glycidol not only in oils & fats but also in heterogeneous processed foods, the question arises, if the present analytical methods are applicable for this.

In regard to the determination of MCPD and glycidol this presentation focuses mainly on the comparability of the most commonly applied methods and their options and limitations.

Impact of the Oil Composition on the Formation of 2- and 3-MCPD Esters Under Conditions Mimicking Oil Deodorization. A. Ermacora* and K. Hrncirik, Unilever R&D Vlaardingen, Vlaardingen, The Netherlands.

2- and 3-monochloropropanediol (2-/3-MCPD) esters are food-borne contaminants formed during high temperature processing of fat-based matrices. Their occurrence in different types of food products is still poorly known, but significant levels of these contaminants were repeatedly reported in refined oils and fats. Since refined oils and fats were identified as the major contributors to the human exposure to 2- and 3-MCPD esters, substantial effort is currently made to mitigate the level of these contaminants during oil processing.
Fundamental pre-requisite for the development of effective mitigation strategies is the knowledge of the mechanisms of formation and degradation of these undesired compounds during processing. Although several studies addressed this subject, there are still substantial gaps concerning the identification of the precursors and the evaluation of their relative contribution to the final level of 2- and 3-MCPD esters in the samples.
This speech will focus on the evaluation of the effect of the oil composition (i.e. acylglycerols and chlorinated minor components) on the formation yield of 2- and 3-MCPD esters in model systems simulating the oil refining process. The level of precursors in the oils was modified by purification on silica gel and/or enzymatic modification, to realistically mimic the natural variations in oil composition.

Post Refining Formation and Decomposition of Fatty Acid Bound 2- & 3-MCPD and Glycidol in Edible Oils. J. Kuhlmann*, SGS Germany GmbH, Hamburg, Germany.

Since the generation of fatty acid esters of Chloropropandiol isomers (bound 2- & 3-MCPD) and of fatty acid esters of 2,3-epoxy-1-propanol (bound glycidol) during the refining process of edible oils and fats was reported, this topic has received much attention, as the core compounds, MCPD and glycidol, might have a negative influence on consumers health. Moreover the formation seems to occur principally but to a different degree in any kind of edible oil during high temperature treatment which obviously seems to be unavoidable in case of the deodorization step. Based on these findings a broad range of analytical, toxicological, formation and mitigations studies have been launched. Thereby major progress in knowledge has been achieved in all afore mentioned fields, but still open questions remain, as for example the formation and degradation seems to be dependent on several and in parts unknown factors. Various investigations have been performed in this field but not many trials have been made to clarify the formation and decomposition of bound MCPD and glycidol in already refined edible oils.
This presentation focuses on the determination of bound MCPD and glycidol in small-scale post refinement trials, for instance oil storage or food frying as well as laboratory chemical and heat treatment.

Hydrolysis of 2-MCPD Di-esters by Porcine Pancreas Lipase. N. Kaze[1], H. Sato[2], H. Yamamoto[1], and Y. Watanabe*[2], [1]Ueda Oils and Fats MFG Co., Kobe, Japan, [2]Osaka Municipal Technical Research Institute, Osaka, Japan.

In addition to 3-MCPDs, their isomer, 2-MCPDs are of the interest. It was previously presented that 2-MCPDs were indirectly quantifiable simultaneously to 3-MCPDs by DGF standard methods C-VI 18(10) with reference to 3-MCPD-d_5, and 2-MCPD-d_5 might not be required for ISD (JAOCS, 90, 1121-1130). It was also revealed that glycidol was converted into 2-MCPD in several percentages in addition to 3-MCPD at the extraction step under acidic conditions of the methods, and that the conversion should also be considered for the estimation of glycidol amount. The detected amount of 2-MCPDs were 1/10~1/4 of in crude palm oil by the methods, Assay A. The detected amounts of MCPDs, including glycidol derived ones, were nearly halved, when MAGs in the crude palm oil was removed by short-path distillation prior to the deodorization. The impact of MAG contamination on generations of MCPD/glycidyl esters at the deodorization step was larger than that of DAG. Meantime, 2-MCPD di-esters were newly synthesized and their degradability by pancreas lipase was investigated. The release of FAs from 2-MCPD di-esters went at the similar degree to that from 3-MCPD. Interestingly, 2-MCPD di-esters were converted to free form mainly, whereas 3-MCPD di-esters were converted to monoesters and free form.

Bioavailability of Esters of 3-MCPD: *in vitro* and *in vivo* Studies. F. Joffre*[1], S. Amara[2], F. Carriere[2], L. Couedeloc[1], C. Vaysse[1], and F. Lacoste[1], [1]ITERG, Pessac, France, [2]Laboratoire « Enzymologie Interfaciale et Physiologie de la Lipolyse», UMR 7282

CNRS-IMBL, Marseille, France.

In 2011, Barocelli et al. showed that free 3-MCPD presented a higher toxicity than diesters of 3-MCPD (MCPD-E) in rats. In a recent report dealing with human exposure to 3-MCPD, the European Food Safety Authority does the assumption of equivalent oral bioavailability between free and bound 3-MCPD. However, to date, no study on bioavailability of MCPD-E has been performed. Our work focused on the lipase activity (*in vitro* two-step digestion model) and absorption (*in vivo* lymphatic duct fistula model) of MCPD-E. A 4-weeks daily intake study of MCPD-E in rats gives information concerning theirs accumulations in organs.
Our results showed that MCPD-E are hydrolyzed by gastric and pancreatic lipases but the level of hydrolysis (close to 20%) is very low as compared to triglyceride (around 60-70% using the same two-step *in vitro* digestion model). This result was correlated however to the rate of absorption calculated in the lymphatic duct fistula model: only 25% of MCPD-E were absorbed after 24h. Finally feeding MCPD-E induce an accumulation of MCPD-E in gonads, kidney, liver and more surprisingly in heart.

In conclusion, it appears that MCPD-E were partially digested and absorbed and then accumulated in different target organs. Females had higher rates of accumulation that may explain the greater damage observed in toxicological studies.

Release of Toxicologically Relevant 2- and 3-MCPD as well as Glycidol from the Respective 2- and 3-MCPD as well as Glycidyl Fatty Acid Esters by Enzymatic or Thermal Degradation. M. Granvogl* and P. Schieberle, Technical University of Munich, Freising, Bavaria, Germany.

After the presence of 3-MCPD esters and glycidyl esters have been proven in food, especially in refined edible oils and fats, lots of efforts have been undertaken by industry, research institutes, and academia to mitigate their concentrations due to the fact that after consumption an ester cleavage to toxicologically relevant free 3-MCPD and glycidol is possible.

The lecture will give deeper insights into the stability of 2- and 3-MCPD esters (using 2-MCPD-1-monoester, 2-MCPD-1,3-diester, 3-MCPD-1-monoester, 3-MCPD-2-monoester, 3-MCPD-1,2-diester) as well as glycidyl esters. Therefore, first, a simple model was established using pancreas lipase. Next, a more complex model consisting of four artificial digestive juices (containing typical enzymes, organic compounds, and inorganic salts) to simulate digestion by saliva, gastric juice, duodenal juice, and bile was applied on the different esters. Finally, the influence of temperature on the cleavage of the esters was studied. Obtained data about free 2- and 3-MCPD and free glycidol as well as about the remaining esters will be discussed.

ANA 3: Analysis of Vegetable Oil Authentication and Adulteration

Chairs: P. Delmonte, US FDA, USA; and L. Reimann, Eurofins, USA

Detection of the Addition of Vegetable Oils to Olive Oils by Comparison of Theoretical and Experimental TAG. W. Moreda*, M.C. Pérez-Camino, and R. Gómez-Coca, Instituto de la Grasa (CSIC), Seville, Spain.

The fraudulent addition of vegetable oils to olive oil has been produced in the last years. The analytical methods and maximum limits included in the European Union Commission regulations (EEC/2568/91) referred to the olive oil allow the detection of some vegetable oils oil only in high proportions. The triacylglycerol (TAG) composition of seed oils shows differences respecting to the olive oil, in particular the increase of TAG containing linoleic acid and the decrease of those containing palmitic and linolenic acid.

The method compare the TAG composition determined by HPLC and that theoretically calculated from the fatty acid composition, assuming a 1,3-random 2-random distribution of fatty acids in the glycerol moiety, with a restriction for saturated fatty acids in the 2-position. In olive oils, differences between experimental and theoretical values were negligible, whereas in seed oils, significant differences were found in some TAG.
Taking in account the differences the following algorithms were calculated: K1, ΔR3, L3, R1exp, ΔR1, L4 and R2. An excel file was developed to perform all the calculations and comparisons, resulting in a genuine or non-genuine answer. The percentage of detection is reduced considerably to 2-12% depending of the percentage of linoleic acid of the olive oil.

Evaluation of the Authenticity of Olive Oil in the U.S. Market Through the Analysis of the Triglyceride Composition. P. Delmonte*, L. Vaclavik, A.R. Fardin Kia, M.M. Mossoba, and A. Krynitsky, U.S. Food and Drug Administration, College Park, MD, USA.

Olive oil, in its various grades, is an expensive commodity often economically adulterated by addition of less expensive oils. Among the various methodologies developed over the decades for the detection of extraneous oils in olive oil, the evaluation of the triglyceride profile has been the most promising approach. In this study olive oil samples were collected from the U.S. market and analyzed according to the International Olive Council "Global Method", and also the calculation of the equivalent carbon number 42 (ECN 42), and the determination of the fatty acid composition. The chromatographic conditions were then adjusted to improve the resolution of partially or fully co-eluting triglycerides. In addition to the chromatographic separation-based approach, a rapid fingerprinting of intact triglycerides was performed using the direct analysis in real time ambient ionization technique coupled to high resolution mass spectrometry (DART–HRMS). The simple dilution of olive oil samples with non-polar solvent was the only sample preparation needed prior to DART–HRMS analysis. The data obtained by the above methods were compared using multivariate statistical tools.

Rapid Analysis of Olive Oil Using FTIR and FTNIR. K. Kramer, N. Wang, and K. Ma*, Eurofins QTA Inc., Cincinnati, OH, USA.

The capability of FTIR and FTNIR for olive oil for chemistry analyses and authentication identification were investigated. Olive oils with various grade were received from various origins. These samples were scanned on FTNIR and FTIR instruments with optimized condition. Typical traits such as Total polyphenols, FFA, Pyropheophytins (PPP), Peroxide value, Tocopherols, and Diacylglycerides were evaluated. This report is the first part of the series study of rapid analysis of olive oil quality.

Using Stable Isotope Ratio Analysis of C, O, and H to Determine the Origin of Extra-Virgin Olive Oil. F. Camin*[1,2], [1]FEM-IASMA Research and Innovation Centre, San Michele all'Adige (TN), Italy, [2]ICQRF MIPAAF, Roma, Italy.

The isotopic composition of plant material is related to the climatic conditions and geographical characteristics of the area in which the plants are grown. The isotope ratio of C depends on botanical origin, availability of water, relative humidity and temperature; whereas the isotope ratios of H and O reflect the isotopic composition of groundwater, average precipitation and the extent of evapotranspiration.

In this talk, I will show how stable isotope ratio analysis can be applied to determine the origin of extra-virgin olive oil, discussing the advantages and drawbacks. I will present the analysis of bulk oil using Isotope Ratio Mass Spectrometry (IRMS) and of certain sub-components using GC-IRMS, discussing combination with other techniques (such as trace element and fatty acid analysis and ^{1}H-NMR profiling). The possibility of discriminating between PDO/PGI extra-virgin olive oils in Italy, between different countries of origin in Europe (Italy, France, Spain, Portugal, Greece, Cyprus) and between Italian and Tunisian olive oils will be investigated.

A Combined ^{1}H and ^{13}C NMR Method to Determine All Characteristics and Origin Tests of Edible Oils. B.W.K. Diehl*, Spectral Service AG, Cologne, Germany.

The combination of 1H and 13C NMR using high field cryo NMR devices enables the simultaneous determination of most quality parameters in the analysis of edible oils. The following items can be determined within a low cost 30 minutes analysis: Peroxide value (POV), Anisidinic value (AV), Iodine value (IV), Acid value (FFA), fatty acid composition, Sterols, Tocopheroles, mono- and diglycerides, wax, phospholipids, secondary compounds like sesamol, cinnamic acid, terpenes etc. The analysis is useful for the origin test of edible oils and for the routine quality control.

Characterization of Volatile Compounds of Virgin Olive Oil Produced in the United States. H. Zhu*[1], S. Tang[1], C. Shoemaker[1], and S. Wang[2], [1]Department of Food Science and Technology, University of California Davis, Davis, CA, USA, [2]University of California Davis Olive Center, Davis, CA, USA.

The unique and desirable flavors of virgin olive oil are mainly contributed by volatile compounds, which are highly affected by the olive variety, olive ripening stage and geographical origin. Several studies have been done to classify virgin olive oils according to their geographical origins by volatile profile, however, such information has yet to be collected for olive oils that are produced in the United States. In this study, major volatiles in single variety virgin olive oils from different producing

regions in the United States are characterized and quantified by solid phase microextraction-gas chromatography/mass spectrometer (SPME-GC/MS). The volatile compositions are compared with those from previous studies on the European olive oils. Statistical analyses are applied to determine the key volatile compounds that are able to discriminate the oils from varieties, ripeness, and cultivation regions.

Multiple Analytical Approaches for Discriminating the Geographic Origin of Asian Sesame Oils. B.H. Kim*, Chung-Ang University, Anseong, Gyeonggi-Do, Republic of Korea.

False indications of the geographic origin of sesame products became an issue of public concern in Korea. Chinese and Indian sesame seeds occupy ~81% of Korea's sesame imports during 2009-2010. They frequently appear in Korean local markets as domestically cultivated products. This has created the need for analytical methods to accurately distinguish Korean sesame oil from the oil obtained from imported sesame seeds. The aim of this study was to characterize the geographic origin of Asian sesame oils using a combination of methods to analyze different types of oil components such as fatty acids, lignans, triacylglycerols, and carbon, hydrogen, and oxygen stable isotopes. The analytical data were obtained from 84 roasted oil samples that were prepared from 51 Korean, 19 Chinese, and 14 Indian sesame seeds harvested during 2010-2011 and distributed in Korea during the same period. Canonical discriminant analysis, a type of multivariate statistical analysis method was used for analyzing multiple outcome variables. By applying two canonical discriminant functions, >90% of the sesame oil samples were correctly classified by their geographic origin, indicating that multiple analytical approach is a useful tool for the traceability of the oils.

Identification and Quantification of Tetraacylglycerols in Lesquerella Oil by HPLC and MS. J.T. Lin*, United States Department of Agriculture, Albany, CA, USA.

Tetraacylglycerol (an acylglycerol estolide) contains an acyl chain attached to the hydroxyl group of another acyl chain attached to the glycerol backbone. Lesquerolic acid (Ls) is the main fatty acid in lesquerella oil and may be used industrially for the manufacture of biodegradable industrial products. Electrospray ionization mass spectrometry of the lithium adducts of acylglycerols in the HPLC fractions of lesquerella oil was used to identify thirteen tetraacylglycerols. They were LsLsLsLn, LsLsLsL, LsLs-OH20:2-O, LsLsLsO, LsLsLnLn, LsLsLLn, LsLsOLn, LsLsLL, LsLsOL, LsLsOP, LsLsOO, LsLsLS and LsLsOS. For the four tetraacylglycerols containing one normal fatty acid (non-hydroxy fatty acid), LsLsLsLn, LsLsLsL, LsLs-OH20:2-O and LsLsLsO, the normal fatty acids were all directly attached to the glycerol backbone, not to the hydroxyl group of fatty acids. We propose that the biosynthetic precursors (triacylglycerol acyltransferase) of these four tetraacylglycerols were LsLsLn, LsLsL, LsLsO (Ls-OH20:2-O) and LsLsO individually. Quantitations of these tetraacylglycerols were by HPLC with evaporative light scattering detector and MS of HPLC fractions (comparing the ion signal intensities). The highest content of these tetraacylglycerols was LsLsLsO at about 0.3% in lesquerella oil.

Characterization of TAGs in Edible Oils with UltraPerformance Convergence Chromatography. J. Yang* and G. Isaac, Waters Corporation, Milford, MA, USA.

Natural oils and fats are complex mixtures consisting primarily of triacylglycerols (TAGs). Chromatographic separation of TAGs is a challenging task since a large number of TAG species may exist in oils and fats. TAG profile can be used to assess the quality and the authenticity of oil and fat products. UltraPerformance Convergence Chromatography™ (UPC^2) leverages the unique properties of compressed CO_2 at or near its supercritical state, such as low viscosity and high diffusivity, and sub-two micron particle packed columns to improve separation efficiency, speed, and selectivity. The low polarity of compressed CO_2 also makes UPC^2 suitable for TAG analysis. Time-of-flight (Tof) mass spectrometry with MS^E simultaneously collects the exact-mass of precursor ions and their corresponding fragment ions, which is convenient for identification and structural elucidation. TAGs in three common edible oils, peanut, sunflower seed, and soybean oils, were separated on a UPC^2 C18 column using the ACQUITY UPC^2 system with a gradient elution. All TAGs eluted within 15 minutes and showed baseline separation for all the major TAGs. TAG peaks were identified using the accurate mass spectra collected by QTOF MS with MS^E. The method optimization, peak assignment using accurate mass data, and TAG elution order under UPC^2 conditions will be discussed.

ANA 4: Rapid and Real Time Analysis

Chairs: H. Adams, Archer Daniels Midland Co., USA; and G. Sekosan, Bunge, USA

The Role of Full-automated NMR in the Screening of Edible Oils Using Targeted and Non-targeted Methods. M. Link*, M. Spraul, H. Schäfer, B. Schütz, and F. Fang, Bruker BioSpin GmbH, Rheinstetten, BW, Germany.

NMR has found its way into the quality control of food and beverages over the last years. The advantage of NMR is its absolute reproducibility and transferability for laboratory to laboratory, which is not equaled by other methods currently used in food analysis. NMR reproducibility allows statistical investigations e.g. for detection of variety, mixing of varieties, production methods and geographical origin, where smallest changes of many ingredients at the same time must be recorded. Preparation, measurement and processing are based on strict standard operation procedures which are substantial for this fully automated solution.

The non-targeted approach to the data allows detecting even unknown deviations, if they are visible in the proton NMR spectra of edible oils. In this approach not only the highly concentrated lipids can be used, but also Polyphenols, Flavonoids and other compounds, which are important for example in detecting the geographical origin.

The same data acquired in high throughput mode are also subjected to quantification of multiple compounds. The fully automated NMR methodology will shortly be introduced and then results on various types of edible oils will be presented and the advantages of the NMR method shown.

Determination of Supplement Identity and Quality by Using DART-Mass Spectrometry for Rapid Triglyceride Analysis. B. Musselman*, J. Lapointe, and R. Goguen, IonSense, Inc., Saugus, MA, USA.

Nutritional supplements incorporate a wide variety of plant materials. Some supplements are single plant but often different tea leaves and even chemicals are added to produce products with the desired composition. As the quality control of these materials must improve with new regulations the need for relevant chemical test increases the burden on small manufacturers and exporters.

We have completed analysis of a wide variety of medicinal supplements utilizing Direct Ionization in Real Time ambient ionization mass spectrometry. The method provides an easy means to complete chemical analysis with little sample prep. During these investigations we have been able to generate lipid profiles for the triglycerides in just seconds per sample. Statistical analysis of the data can be used to determine the presence / absence of different plant materials. The method affords a rapid means to check for sample composition and even quality as detection of irregular lipid profiles is made possible even when small concentrations of lipids are present as in the case of dried leaves.

Identification of Lipid Oxidation Products Using SPME-DART-QTOF. S. Seegers* and T. West, Bunge North America, Bradley, IL, USA.

Typically, a variety of gas chromatography (GC) methods have been used to evaluate oxidation products in edible oils. There are many different ways to introduce the sample into the GC such as cold-trapping, gas sampling, and more recently, solid-phase microextraction (SPME). Coupled to a mass spectrometer, these methods can concentrate, separate, and identify oxidation products. Direct analysis in real time (DART) is an ambient ionization technology used to introduce samples into a mass spectrometer. When used in conjunction with a quadrupole time of flight (QTOF) mass spectrometer, samples can be quickly analyzed with high mass accuracy. This methodology was applied to oxidation products in edible oils. Because these oxidation products are in low concentrations, SPME was also employed to concentrate the volatiles and used in conjunction with the DART. The SPME fiber was then directly desorbed in the DART stream and the oxidation products introduced into the QTOF.

Application of a Portable Mid-infrared Detector to the Rapid Determination of Total *trans* Fatty Acids in Representative Fast Food Samples. M.M. Mossoba*[1], C. Srigley[1], S. Farris[1], J.K.G. Kramer[2], and J.I. Rader[1], [1]Food and Drug Administration, College Park, MD, USA, [2]Guelph Food Research Center, Agriculture and Agri Food Canada, Guelph, ON, Canada.

Gas chromatography (GC) is time consuming and requires expertise to carefully identify and accurately determine individual fatty acid methyl ester (FAME) peaks attributed to *trans* FA and their positional isomers. In this study, the total content of *trans* fat extracted from 22 representative fast foods was rapidly (<5 min) quantified as FAME, in a single

measurement, by using a portable Fourier transform infrared (FTIR) detector in the transmission mode and a benchtop attenuated total reflection (ATR)-FTIR spectrometer for comparison. The total *trans* FA content obtained by FTIR fell in the range from 0.1 – 3.1 grams/serving. Quantitative FTIR results of total *trans*-unsaturated fat proved comparable to those determined by the Official ATR-FTIR Method AOCS Cd 14e-09 and Official GC Method AOCS Ce 1j-07. These results suggest that portable FTIR detectors are potentially suitable for the rapid and routine quantification of the total *trans* FA content in food extracts.

Mathematical Relationship Between IV and RI for Single Vegetable Oil or Vegetable Oils in Blends. S. Mukhopadhyay*, Emami Biotech Limited, Kolkata, West Bengal, India.

Most of the explored vegetable oils have definite standard ' Iodine Value' range and 'refractive index (in a specific temperature) range'. The IV and RI largely depends upon the property of constituents fatty acid chain ... attached to the glycerol in esterifies form. The relationship may further extend to cyanolipids and other lipids of vegetable oil sources. A 'mathematical relationship' between IV & RI has been established by me on a group of vegetable oils as individual and also in blends.

Determination of Peroxide Value in Edible Oils by FTIR Spectroscopy Using Disposable Polyethylene Films. X. Yu*[1,2], Q. Li[2], D. Sun[2], X. Chen[2], and T. Wang[1], [1]Iowa State University, Ames, IA, USA, [2]Northwest A&F University, Yangling, Shaanxi, China.

A new technique was developed to facilitate mid-FTIR transmission analysis of viscous edible oil samples using disposable polyethylene (PE) films as a sample handling accessory for the determination of peroxide value (PV) of edible oils,. The basis of the PV determination was the rapid reaction of triphenylphosphine (TPP) with the hydroperoxides present in an oil to produce triphenylphosphine oxide (TPPO), which exhibited a measurable absorption band at 542 cm^{-1} Calibration standards, prepared by the gravimetric addition of TPPO to a peroxide-free oil, were handled in the same manner, and a linear calibration equation was obtained to relate the concentration of TPPO (expressed as the equivalent PV) to the absorbance of TPPO at 542 cm^{-1} relative to a baseline at 530 cm^{-1} in the normalized spectra, with a regression standard deviation (SD) of ±0.134 mmol/kg oil. PV determinations on two sets of validation samples, spanning PV ranges of 0~25 and 0~2 mmol/kg oil, were carried out in parallel using the American Oil Chemists' Society (AOCS) titrimetric and our PE-based FTIR procedures. A comparison of the results of these duplicate analyses by the two methods indicated that the latter is more reproducible and slightly more sensitive.

Direct Quantification of Fatty Acids in Biological Samples by Fast Gas Chromatography. C. Cruz-Hernandez*, S.K. Thakkar, W. Buosi, and F. Giuffrida, Nestlé Research Center, Lausanne, Switzerland.

Quantification of fatty acid (FA) composition in biological fluids such as, plasma and erythrocytes may serve as biomarker to fat intake/absorption, quantitatively as well as qualitatively. Nevertheless, due to the heterogeneous nature of lipid classes in such complex biological matrices it is imperative to have a validated methodology. Therefore, the aim of current work was to select, optimize and fully validate a Fast Gas Chromatography (GC) method for quantification of FAs in plasma and erythrocytes. We realized sample preparation is key; therefore we employed the use of lysis buffer for erythrocytes and ethanol for plasma to address matrix differences. FA profile was determined by acid methylation and GC equipped with a flame ionization detector. The triacylglycerols 11:0 or 13:0 and methyl esters 21:0 or 23:0 were used as internal standards. Within the linearity of the calibration, the ratio of the peak area of each FA over the peak area of the IS was constant (coefficient of variation £ 2.5). Satisfactory repeatability [CV(r) < 20%] and intermediate reproducibility [CV(r) < 20%] were observed. Finally this validated method was applied to a pre-clinical trial that investigated impact of various dietary fats on accretion of specific FAs in plasma and erythrocytes.

Effect of Different Emulsifiers on Water Droplets Measured by Novel Light Scattering Technique Under Dynamic Conditions. K. Bhattacharya* and F. Moller, DuPont Nutrition Bioscience ApS, Brabrand, Denmark.

When an emulsifier reaches an interface it reduces the interfacial tension causing droplet size reduction of the added phase under shear. The present work compares rate of migration of emulsifiers having different fatty acid composition under dynamic conditions by monitoring the change in water droplet size distribution as a function of time using sub-surface laser scattering. Emulsions comprising of 1:1 liquid oil:water were produced

with different emulsifiers under constant shear and a cooling rate of 0,5° C per minute. Laser light were pointed through the emulsion vessel and sub-surface laser scattering were recorded using camera and scattering halos were analyzed. The number of water droplets increase and size decrease during the cooling and agitation. The light scattering is significantly changed by the forming water droplets, which can be seen as a change in laser halo. As emulsification continues, depending on the type of emulsifiers there is a change in water droplet size. Emulsifiers chosen for the study were fully saturated monopalmitate/monostearate blend, unsaturated mono-oleates and polyglycerol ester all of which showed different effects on emulsification. The presented methodology can in a simple and noninvasive way be applied to characterize food emulsions, during production and to predict stability.

FT-NIR as a Rapid Method for Non-targeted Screening of Edible Oils. D.E. Roberts*, Bruker Optics Inc., Madison, WI, USA.

Economically Motivated Adulteration (EMA) of food ingredients poses a significant problem in the global food chain. EMA causes concern for health risks, as evidenced by recent Melamine contamination in Pet foods and infant formula, but is also of concern in maintaining brand integrity. Dilution of Extra Virgin Olive Oil with lower cost vegetable oils degrades the value of the oil and has a negative impact on brand reputation. Changes in the constituent oils in custom blends can have significant impact on oil performance for frying applications, or as ingredients in prepared mixes. The wavelength accuracy and high resolution capability of FT-NIR measurements in edible oils have been applied to determination of Iodine Value and other parameters in edible oils. New qualitative characterization methods can be employed to quickly determine if an incoming raw material lot is consistent with previous material lots having been determined to be suitable for the process and product. Merging qualitative and quantitative methods using FT-NIR spectroscopy provides even smaller manufacturers the ability to rapidly determine if an incoming raw material is fit for purpose. Examples of rapid, non-targeted screening using different qualitative and quantitative tests will be presented for olive oil and other vegetable oils.

ANA 5: General Analytical

Chairs: Y. Itabashi, Hokudai University, Japan; and S. Bhandari, Silliker Laboratories, USA

New Enzymatic Method to Determine FA Composition at *sn*-2 of Triglycerides: Results of the Collaborative Study. Y. Watanabe*[1], M. Asada[2], T. Arishima[3], Y. Iida[4], J. Imagi[5], R. Sasaki[6], S. Sato[7], C. Sato[8], T. Sano[5], T. Shibuya[2], T. Nagai[9], T. Fukazawa[4], R. Hori[5], R. Homma[10], Y. Miyazaki[11], [1]Osaka Municipal Technical Research Institute, Osaka, Japan, [2]Showa Sangyo Co. Ltd., Tokyo, Japan, [3]Fuji Oil Co, Osaka, Japan, [4]Japan Institute of Oil & Fats, Other Foods Inspection, Tokyo, Japan, [5]J-Oil Mills, Inc., Tokyo, Japan, [6]Miyoshi Oil & Fat Co. Ltd, Tokyo, Japan, [7]Japan Food Research Laboratories, Nagoya, Japan, [8]The Nisshin OilliO Group, Ltd, Tokyo, Japan, [9]Tsukishima Foods Industry Co Ltd, Tokyo, Japan, [10]Kao Co. Ltd., Tokyo, Japan, [11]NOF Co. Ltd., Tokyo, Japan, [12]ADEKA Co., Tokyo, Japan.

New enzymatic method to determine FA composition at *sn*-2 of TAGs is currently under the collaborative evaluation for JOCS Standard Methods. The method consists of the 1(3)-specific transesterification of target TAGs with 10 wt parts of ethanol to oil, using immobilized *Candida antarctica* lipase. Commercially, Novozym 435 (Novozymes) or Chirazyme L-2, C4 (Roche Diagnostics) are available. The lipid mixture (0.1 mL) obtained from the 3-h transesterification was subjected to Sep-pak silica cartridge (0.65 g, Waters), after the removal of ethanol. FAEE was eluted by 10 mL of solvent mixture (hexane:diethyl ether =8:2, v/v). DAG was eluted by 20 mL of the solvent. 2-MAG was then recovered by 10 mL of diethyl ether, methylated and brought to GC analysis. RSDs of the data from 12 labs in n=2 were below 5% for major FAs in soybean and palm oil, and below 7% for major FAs in sardine oils. The method is advantageous that it is applicable to TAGs consists of C4-C24 and PUFAs. The recovery of 2-MAGs from TAGs is highest compared to the chemical method and to conventional enzymatic method based on the 1(3)-specific hydrolysis of TAGs, which might be contributed to the low RSDs of the method.

Crotonaldehyde in Fats and Oils: Analysis and Formation Pathways. M. Granvogl*, Technical University of Munich, Freising, Bavaria, Germany.

Three stable isotope dilution assays (SIDAs) were developed for the quantitation of 2-butenal (crotonaldehyde) in heat-processed edible oils and in food using synthesized [13C4]-crotonaldehyde as internal standard. First, a direct GC-MS headspace method, followed by two indirect methods on the basis of derivatization with either pentafluorophenylhydrazine (GC-MS) or 2,4-dinitrophenylhydrazine (LC-MS/MS), was developed. Applying these three methods on five different types of oils varying in their fatty acid compositions revealed significant differences in the concentrations of crotonaldehyde, which were formed after heating at different temperatures and times, e.g., 0.32 mg/kg of coconut oil or 34.4 mg/kg of linseed oil after heat-processing for 24 h at 180 °C. Comparison of the results showed that the concentration of formed crotonaldehyde seemed to be correlated with the amount of linolenic acid in the oils. Further, the formation of crotonaldehyde was compared to its homologue acrolein demonstrating that always acrolein was present in higher amounts in heat-processed oils. In addition, crotonaldehyde was quantitated in fried foods, e.g., potato chips and donuts.

Finally, stable isotopically labeled precursors were synthesized to prove the formation pathways of crotonaldehyde in oils.

Vitamin K2-7 Analysis by a HPLC Method in Different Matrices. S.D. Bhandari* and T. Gallegos-Peretz, Silliker Laboratories, Crete, IL, USA.

Vitamin K2-7 has been implicated in a variety of health benefit functions including protection from osteoporosis and pathological calcification. It has acquired self-affirmed GRAS status from FDA and is considered a safe food ingredient by European Union. Methods of the vitamin K2-7 extraction and HPLC analysis from raw ingredient blends, dietary supplements and selected food matrices were evaluated in this study. The method employs solvent extraction of the vitamin and reverse-phase HPLC and fluorescence detection after post-column reduction. The chromatography of the vitamin in calibration standard solutions and investigated sample extracts was good with no interferences. The relationship between vitamin concentration and corresponding response was found to be quite linear. The precision of the analysis in terms of % relative standard deviation in the evaluated matrices usually ranged between 0.7% to 6.2%. The analyzed values obtained in the ingredient blends and supplements compared favorably to the expected values. % Recovery of the vitamin in orange juice, liquid dietary supplements and breakfast cereals at different concentrations of the spike was found to be in the range of 95% -112%. Recovery at 0.1-0.9 ppm in whole fluid milk was found to be in the range of 74%-79%. LOD of the method was calculated to be 0.02 ppm and LOQ of 0.07 ppm.

A Free Radical Mediated Mechanism for Formation of 3-monochloro-1,2-panediol (3-MCPD) Fatty Acid Esters. X. Zhang*[1] and L. Yu[1,2], [1]Institute of Food and Nutraceutical Science, School of Agricultural and Biology, Shanghai Jiao Tong University, Shanghai, China, [2]Department of Nutrition and Food Science, University of Maryland, College Park, College Park, MD, USA.

The formation mechanism of 3-monochloro-1,2-panediol (3-MCPD) fatty acid esters, a group of new process-induced food toxicants, was investigated under high temperature and low moisture conditions. In the first part of this research, a free radical mechanism, including the formation of cyclic acyloxonium free radical (CAFR) from diacylglycerol (DAG) at high temperature followed by the consequential reaction with the chlorine radical to form 3-MCPD diesters, was proposed and confirmed for the first time using ESR, FT-IR and Q-TOF-MS. These results led to the second study on the formation mechanism of 3-MCPD esters from triacylglycerol (TAG). The results suggested that iron ion might catalyze the formation of 3-MCPD esters from TAG under the experimental conditions. In addition, the formation of 3-MCPD esters from TAG might associate with lipid peroxidation. The results of this study might be used to reduce the level of 3-MCPD esters during oil deodorization to improve food safety.

Detection, Isolation and Characterization of *Camelina sativa* Glucosinolates. D. Yuan*[1], J. Shen[2], and M. Reaney[2], [1]Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Science, University of Saskatchewan, Saskatoon, SK, Canada.

Three glucosinolates with similar structures have previously been reported to occur in seed meal of *Camelina sativa* (L. Crantz), an oilseed crop from the Brassicaceae family. These three compounds,

glucoarabin (9-(methylsulfinyl)nonylglucosinolate – GS9), glucocamelinin (10-(methylsulfinyl)decylglucosinolate – GS10), and camelinin (11-(methylsulfinyl)undecylglucosinolate - GS11), may possess significant health promoting benefits due to their structural similarity to glucoraphanin (4-methylsulfinyl-3-butenyl), another methylsulfinylglucosinolates that is the precursor for the isothiocyanate sulforaphane, a compound with potent antioxidant activity. In this presentation we demonstrate improved processing methods for isolation and purification of *Camelina* glucosinolates. High concentrations of *Camelina* glucosinolates were detected and identified in seed fractions by HPLC-MS/MS. Standards of GS9, GS10, and GS11 were prepared from Camelina extracts using reversed phase HPLC, purified based on ion exchange chromatography, and their structures characterized using HPLC–MS/MS, 1D and 2D NMR. Quantification was performed on proton NMR, which allowed efficient measurements of glucosinolates during processing of *Camelina sativa*.

Phytochemicals, Benzyl-Isothiocyanate (BITC) Level and Antioxidant Activity of Ultrasound-and Solvent-Extracted Papaya Seed Oil from Different Malaysian Papaya Varieties: Functional Properties. S. Samaram[1], H. Mirhosseini*[1], H. Mohd Ghazali[1], C.P. Tan[1], S. Kostadinovic[2], and S. Bordbar[1], [1]University Putra Malaysia, Serdang, SE, Malaysia, [2]Faculty of Agriculture, Goce Delcev, Štip, Macedonia.

The main objective of the present study was to investigate the effect of different Malaysian papaya varieties (i.e. *Sekaki* and *Eksotik*), extraction conditions and methods (i.e., Soxhlet (SXE), solvent extraction (SE) and ultrasound-assisted extraction technique (UAE)) on the level of benzyl-isothiocyanate (BITC) and radical-scavenging antioxidant activity of papaya seed oil. Results revealed that, extraction temperature had considerable effect on the recovery of BITC in papaya seed oil. In general, ultrasound-assisted extraction (UAE) and solvent extraction at the elevated temperature (50 ºC) resulted in high levels of BITC in papaya seed oil. *Eksotik* papaya seed oil had the strongest antioxidant activity among all samples. Identification of phytochemicals indicated that papaya seed oil could be a source of functional compounds and natural antioxidants like BITC, squalene and l-(+)-ascorbic acid 2,6 dihexadecanoate. In addition, other phytochemicals (i.e., mesitylene, Dithiothreitol, Phthalic acid butyl tetradecyl ester, 18,19-Secoyohimban-19-oic acid,16,17,20,21-tetradehydro-16-(hydroxymethyl)-) were also identified in papaya seed oil.

Cypermethrin Residue in Soil and Leaf in an Oil Palm Plantation. C.B. Yeoh*, N. Sulaiman, N.S.K. Khairuddin, F.K. Ahmad Bustamam, and H. Muhamad, Malaysian Palm Oil Board, Selangor, Malaysia.

A field trial to study the fate of cypermethrin in an oil palm agro ecosystem was conducted at the Yuwang Estate, Sepang, Selangor. Experimental plots in the estate were sprayed with the insecticide cypermethrin, while the control plot was sprayed with water. The concentrations of the insecticide used were at the recommended manufacturer's dosage and at double the recommended dosage. Composited soil samples were collected randomly from five sampling points at different depth (0-10cm, 10-20cm, 20-30cm, 30-40cm and 40-50cm) whereas leaf samples were the composite of three different portions (Top, Middle and Bottom) from different palms. Cypermethrin residue in soil and palm leaves was monitored after spraying. All samples were taken at intervals of -1, 0, 1-13, 15, 21 and 30 day(s) after treatment.

Cypermethrin was not detected in any of soil samples at any sampling days for both treatment dosages. For leaf samples, cypermethrin was detected in leaf sampled from Day-0 up to 8 days after treatment. For the palms treated at double recommended dosage, cypermethrin was detected up to 10 days after treatment.

Analysis of Oil – Biodiesel Samples by HPLC Using the Normal Phase Column of New Generation and the Evaporative Light Scattering Detector. S.N. Fedosov*[1], N.A. Fernandes[2], and M.Y. Firdaus[1], [1]Dept. Engineering Science, Aarhus University, Aarhus, Denmark, [2]Universidade do Estado de Santa Catarina, Departamento de Engenharia Ambiental, Lages, SC, Brazil.

Conversion of vegetable oil to biodiesel is usually monitored by gas chromatography, which requires an elaborate derivatization of the samples. HPLC methods are apparently more convenient, but none of the described variants had won a wide recognition so far. Here we report an HPLC procedure suitable for separation of biodiesel, free fatty acids, tri-, di- and monoglycerides. The normal phase column of new generation (Poroshell 120 HILIC) and the novel gradient were used. The method was tested on both the artificial mixtures and the crude reaction samples. Elution of the

analytes was monitored on an evaporative light scattering detector. This method is usually confined to a very limited range of masses, because of a complex shape of the calibration curves. We have analyzed the light scattering signal within a very broad range of masses, whereupon the data were approximated by the appropriate equations. An experimental conversion of rapeseed oil to biodiesel was performed by a liquid lipase formulation, and this process was monitored by HPLC to illustrate advantages of the suggested registration method.

Novel Approaches for Determination of Steryl Glycoside Profiles in Foods without Artifact Formation. L.H. Münger* and L. Nyström, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland.

Steryl glycosides (SG) are bioactive phytosterol conjugates, which occur in foods and could be used for food fortification. Their biological activity may differ between different sterols, which highlights the need for analytical techniques that reveal true sterol profile. Current analytical methods fail to reflect correct SG profiles because the acetyl bond is usually cleaved via acid hydrolysis before analysis with GC. Some abundant and labile sterols isomerize under acidic conditions resulting in the production of artifacts, which underlines the importance for alternative approaches to SG analysis. Therefore, we developed an enzymatic procedure to replace acid hydrolysis prior to GC-FID analysis. Under these milder conditions, we have determined correct SG profile of various foods, and demonstrated that the sterol profile is falsified by the acid treatment, especially in foods containing d7-sterols and other labile sterols (e.g. d5-avenasterol). In some cases, half of the peaks detected after acid hydrolysis were artifacts. Furthermore, we have developed a highly sensitive UPLC-QTOF/MS method using ESI for the analysis of intact SG, as well as a MS/MS method to identify sterols and their fragmentation behavior. These methods can be applied for the analysis of true sterol profiles in plant foods, when evaluating their bioactivity.

ANA-P: Analytical Poster Session

Chair: S. Bhandari, Silliker Laboratories, USA

1. **Comparison of the Ability of Rheology and Turbiscan Methods for Determining Gelification Times.** J.M. Montes de Oca Avalos[1], J.A. Rincon Cardona[1,3], C. Huck Iriart[2], R.J. Candal[2,3], and M.L. Herrera*[1], [1]Instituto de Tecnologia de Polimeros y Nanotecnologia, UBA-CONICET, Buenos Aires, Argentina, [2]INQUIMAE, UBA-CONICET, Buenos Aires, Argentina, [3]Instituto de Investigaciones e Ingenieria Ambiental, San Martin, Buenos Aires, Argentina.

Chemical and epidemiological research has shown that there is a positive relationship between *trans* fatty acid intake and cholesterol levels. An alternative to eliminate *trans* fat in foods is formation of hydrogels that contain liquid oils. In this way, food may be formulated with less saturated and *trans* fats. There are some techniques usually used to determine gelification times. Among them rheology is a classical approach. The aim of the present study was to compare the ability of two methods, rheology and analysis by Turbiscan, for determining gelification times. Emulsions were formulated with 10 wt.% sunflower oil, 0.5, 2.0 or 5.0 wt.% sodium caseinate, and 0, 5, 10, 15, 20, or 30 wt.% sucrose. Gels were prepared from emulsions lowering the pH with Glucono Delta Lactone acid. Two different ratios protein/acid were used. They were 5:1 and 5:2. Although values calculated from both methods had significant differences for some of the conditions, tendencies were similar. Gelification times diminished with protein and acid concentration. Addition of sucrose had no effect on gelification times.

2. **Characterization of Volatile Compounds of Virgin Olive Oil Produced in the United States.** H. Zhu*[1], S. Tang[1], C. Shoemaker[1], and S. Wang[2], [1]Department of Food Science and Technology, University of California Davis, Davis, CA, USA, [2]University of California Davis Olive Center, Davis, CA, USA.

The unique and desirable flavors of virgin olive oil are mainly contributed by volatile compounds, which are highly affected by the olive variety, olive ripening stage and geographical origin. Several studies have been done to classify virgin olive oils according to their geographical origins by volatile profile, however, such information has yet to be collected for olive oils that are produced in the United States. In this study, major volatiles in single variety virgin olive oils from different producing regions in the United States are characterized and quantified by solid phase microextraction-gas chromatography/mass spectrometer (SPME-GC/MS). The volatile compositions are compared with those from previous studies on the European olive oils. Statistical analyses are applied to determine the key volatile compounds that are able to discriminate the oils from varieties, ripeness, and cultivation regions.

3. **Assessment of Cretan Wines Maturation Using NMR Spectroscopy, Sensory Testing, and Chemometrics.** P. Dais*, M. Amargianitaki, and A. Spyros, NMR Laboratory, Department of Chemistry, University of Crete, Heraklion, Crete, Greece.

Wine maturation is one of the fundamental and most important winemaking processes. Understanding and controlling wine maturation is a prerequisite in order to obtain high quality wines with increased market value.
We have been studied the maturation of wines obtained from four local Cretan grape varieties (the red Kotsifali and Mantilari, and the white Vilana and Dafni) by using NMR spectroscopy in conjunction with sensory evaluation, and chemometrics. NMR analyses have been performed periodically at defined intervals (every three months for a total time of nine months) for wine samples being matured in four different types of wooden barrels (French and American oak, acacia, chestnut), and in stainless steel barrels using oak chips. The NMR spectra of wine samples obtained from barrels have been used for metabonomic analysis to construct classification models for wines according to grape variety, type of maturation, maturation level and type of wooden barrel. Application of partial least squares (PLS) to sensory and chemical evaluation attributes and the physicochemical parameters obtained from NMR analyses have been performed in efforts to pre-standardize wine maturation and establish novel indices characterizing the progress of maturation under different conditions

4. **Liquid and Gas Chromatographic Analyses of Triacylglycerols for Asian Sesame Oil Traceability.** H.J. Bang*[1], C.T. Kim[2], and B.H. Kim[1], [1]Chung-Ang University, Anseong, Gyeonggi-Do, Republic of Korea, [2]Nongshim Co Ltd, Seoul, Republic of Korea.

This study aimed to investigate the triacylglycerol (TAG) composition of Korean, Chinese,

and Indian roasted sesame oils that were distributed in Korea and to differentiate the geographic origins of the oils using the analytical data in combination with canonical discriminant analysis (CDA). The TAG compositions of 51 Korean, 19 Chinese, and 14 Indian sesame oil samples were analyzed by liquid chromatography-evaporative light scattering detector (LC-ELSD) and gas chromatography-flame ionization detector (GC-FID), separately. The content values of seven major TAG species determined by LC-ELSD showed strong positive linear correlation (Pearson's coefficients > 0.9) to those measured by GC-FID. Both chromatographic analyses showed that the mean LLL and OLL contents of the Korean sesame oils were greater than those of the Chinese sesame oils, although their mean PLL, POL, and POO contents were lower than those of the Chinese products; meanwhile, the mean OOL and OOO contents of the Korean sesame oils were higher than those of the Indian products. The CDA results showed that good discrimination (hit ratio, ~92%) between the oil samples could be achieved by applying the respective canonical variables generated from the LC-ELSD and GC-FID data.

5. **Hydrolysis of Lipoprotein PtdCho by Human Group IIA sPLA2 in Presence of Nanomolar Amounts of PtdCho Isoprostanes.** A. Kuksis*[1] and W. Pruzanski[2], [1]Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, Canada, [2]Department of Medicine, University of Toronto, Toronto, ON, Canada.

Previous work has demonstrated that lipoprotein PtdCho is a poor substrate for group IIA sPLA2 and requires increased enzyme concentration and prolonged incubation times for significant hydrolysis (Pruzanski et al., 1998). We now wish to report that the hydrolysis of the PtdCho of plasma lipoporoteins by group IIA sPLA2 is associated with the presence of small amounts of PtdCho-IPs and PtdCho-OOHs. Lipoprotein samples that contained low nanomolar or high picomolar amounts of PtdCho-IPs and, to a lesser extent, PtdCho-OOHs gave significant hydrolysis of PtdCho of LDL, HDL, acute phase HDL (APHDL) and HDL3 in presence of adequate amounts of enzyme (2.5 ug/ml). The demonstration used an LC/ESI-MS assay of PtdCho-IPs and PtdCho-OOHs over a period of 0 to 24 h of digestion at 37 oC of each lipoprotein with group IIA sPLA2 (0.1 to 2.5 ug/ml). A domain-induced enzyme activation resulting from a local change in monolayer composition and loosening of lipid packing order is proposed as an explanation for the results. The findings support previous reports on the effect of minimal oxidation and storage of LDL on phospholipid hydrolysis by group IIA sPLA2 demonstrated by chemical (TBARS) and radioactive assays.

6. **Electronic Storage of LC-ESI-MS Total Ion Current Profiles of Native and Peroxidized Plasma Lipoprotein Lipids for Subsequent Analytical Work-up of Known and Unknown Molecular Species.** A. Kuksis* and W. Pruzanski, University of Toronto, Toronto, ON, Canada.

Analyses of complex plasma lipids are time consuming and require sample storage, which leads to lipid degradation. Storage of oxidized samples is particularly difficult. A preparation of a new set of samples is inconvenient, expensive or impossible when dealing with large numbers of individual specimens. An unlimited length of storage, however, is possible of the electronic records of the total ion current recordings obtained by LC/ESI-MS in presence of internal standards. Thus, total ion current scans of total lipid extracts of plasma lipoproteins may be stored on computer discs and quantitative single ion mass chromatograms obtained at any time by targeting particular lipid compounds. Other lipid molecules not targeted at the time may be assessed later when other more recent studies prompt to do it. We have utilized the Hewlett-Packard technology to obtain quantitative profiles of molecular species of various natural and peroxidized phospholipids before and after digestion of total lipid samples with secretory PLA_2s. A comparison of the lipid profiles obtained under identical analytical conditions showed differences, which were well suited for biochemical and physico-chemical assessment of enzyme activity.

7. **Effect of the Extraction Method of the Free Fatty Content of the Oil.** V.J. Barthet* and A. Puvirajha, Canadian Grain Commission, Winnipeg, MB, Canada.

Several samples of canola with various free fatty acid (FFA) contents were extracted by three different methods to assess the effect of the method on the FFA content of the recovered oil. Statistical analysis (paired t-test) showed that hot extraction (immersion followed by 30 min reflux) extracted more FFA than a simple cold extraction. It seems that a two steps extraction method did not show any statistical difference with the cold extraction.

8. **Determination of the Gelation Mechanism of Frozen-Thawed Hen's Egg Yolk.** C. Au*, N. Acevedo, and T. Wang, Iowa State University, Ames, IA, USA.

Food processors use hen's egg yolk as a highly nutritional and functional ingredient in many products. The food industry freezes bulk yolk to increase shelf life and practices the addition of 2-10% salt, sugar, or corn syrup to reduce gelation during freezing. Gelation, the loss of fluidity induced by freezing and thawing of egg yolk, is an unfavorable phenomenon because of reduction in yolk functionality and ability to disperse in other ingredients. The purpose of this research is to determine the mechanism of gelation in hen's egg yolk. Knowledge gained in determining the mechanism of this undesirable effect can be used in food industry applications in which low-sodium, low-sugar, sodium-free, or sugar-free products using bulk yolk are made. In this study, whole yolk, yolk components, and their behavior pre- and post-gelation were observed. Fractionation results showed that water is better retained in gelled than in fluid yolk, confirming changes in the matrix following gelation. Rheological analysis revealed a positive correlation between yolk gel strength and frozen storage time of yolk. The measured stress yield of yolks frozen 20 h was approximately 2.3 kPa. Yolks frozen 7 d and 14 d yielded stress values 470% and 830% greater than yolks frozen 20 h, respectively.

9. **Analysis of Retinal Gangliosides by Hydrophilic Interaction Liquid Chromatography/Mass Spectrometry.** O. Berdeaux*, E. Sibille, S. Cabaret, L. Bretillon, and E.A.Y. Masson, CSGA, Dijon, France.

Gangliosides (GG) are sialic acid-containing glycosphingolipids exhibiting a wide variety of structures depending on the oligosaccharidic chain as well as the ceramide moiety. Particularly abundant in the central nervous system, including the retina, their profile is specific to the organ and its differentiation or pathophysiological status. An efficient method of identification and quantification is therefore crucial to apprehend the huge heterogeneity of GG and highlight changes.

We report a powerful analytical method based on HILIC/ESI-MS. Under Hilic conditions, the different GG of a highly heterogeneous mixture were effectively separated. A structural characterization of each GG molecular species was obtained using a LTQ-Orbitrap™, providing information on the nature of the ceramide moiety and the oligosaccharidic chain. The semi-quantitative analysis of GG molecular species was performed using QqQ mass spectrometer by single reaction monitoring thanks to a characteristic sialic acid fragment (m/z 290) produced in the collision cell. This glycolipidomic approach offers an efficient, sensitive, straight-forward, highly accurate and reliable tool to describe the precise GG composition of the retina and investigate the role of GG in retina's function and pathologies. Particularly, it will help decipher the role of the ceramide variability, which is so far not well understood.

10. **Improved Fatty Acid Analysis of Each CLA Egg Yolk TAG and PL Species as Determined by ESI-MS.** S. Shinn*, A. Proctor, R. Liyange, and J. Lay, University of Arkansas, Fayetteville, AR, USA.

A novel CLA-rich soy oil (CLARSO) has been produced by isomerization of soy oil linoleic acid, which contains 20% CLA. This oil was used to supplement poultry feed to produce eggs with 120 mg of CLA. Previous studies reported increased saturated fatty acids and decrease monounsaturated fatty acids in total yolk lipids. However, there are no credible reports of separation and fatty acid analysis of either triacylglyceride (TAG) or phospholipid (PL) species. Previous MALDI TOF/MS studies suffered from suppression of TAG peaks, thus requiring an alternative ESI-MS approach. The study determined fatty acid composition of total lipids in 120mg CLA rich egg yolk produced with CLARSO using GC-FID; determined the total fatty acid composition of yolk TAGS and PL by MALDI-TOF AND ESI-MS; and isolated each TAG and PL species in CLA rich egg yolk and determined the fatty acid composition by ESI-MS. Sixty percent of the CLA was found in TAGs and occurred predominantly in C52:5 and C52:4 TAG species. The fatty acid composition of PL species were not significantly different among egg types, but CLA eggs contained more lysophosphatidyl choline than the other eggs. This novel ESI-MS method produced more detailed data showing peaks that were suppressed by MALDI-TOF, and is the most comprehensive analysis of egg yolk lipids to date.

11. **Peroxide Value Determination in Olive Oil.** A. Ross* and L. Sheng, EPL Bio Analytical Services, Niantic, IL, USA.

AOCS Official Method Cd 8b-90 describes the determination of peroxide value of fats and oils. The peroxide value provides a quantitative measure of the degree to which fats and oils have been oxidized. The peroxide value test was identified by International Olive Council as one of the critical

measures to grade olive oil. In 2010, USDA adopted a voluntary olive oil standard similar to the International Olive Council. However when we conducted the AOCS official method for peroxide value on olive oil, we found for olive oil samples the starting color upon the addition of the starch indicator as well as the color change at the end point were inconsistent with the description in the AOCS official method. Here we present a detailed titration procedure and a series of photographs of the sample color change during the titration. Following this procedure, we have achieved satisfactory results in the AOCS olive oil proficiency program.

12. ***trans* Fatty Acid Determination and Fatty Acid Profile of Oils Including Olive Oil.** L. Sheng*, EPL Bio Analytical Services, Niantic, IL, USA.

According to the National Academy of Sciences, *trans* fatty acids are not essential and provide no known benefit to human health whether of animal or plant origin. Dietary trans fatty acids are more deleterious with respect to coronary heart disease than saturated fatty acids. In November 2013, FDA stated that hydrogenated vegetable oils containing *trans* fats are no longer generally recognized as safe (GRAS). We present a testing procedure for *trans* fats and fatty acid composition based on AOCS Official Methods Ce 2-66, Ce 1f-96, and Ce 1h-05. The method was validated using a certified reference material (CRM) BCR-162R, an oil standard from IRMM and recommended in Ce 1f-96. Composition values of *trans* and the other fatty acids comparable to the certified values were acquired with excellent precision. Using this method fatty acid profiles were tested for several brands of olive oil, and also for different types of oils and fats. With demonstrated proficiency and reliability, the olive oils were graded following the fatty acids and *trans* fats purity criteria in the USDA Standards for Grades of Olive Oil and Olive-Pomace Oil. The fatty acid composition test was sufficient to differentiate olive oil from various vegetable oils, which confirmed fatty acid profile can be used as a critical discriminating factor in the detection of olive oil adulteration.

13. **Determination of Nutritional and Cyclopropenoid Fatty Acids in Cottonseed by a Single GC Analysis.** B. Mitchell*, D. Michalica, J. Sabbatini, and B. Rozema, Covance Laboratories, Inc., Madison, WI, USA.

Historically, a complete analysis of cottonseed fatty acids, including the cyclopropenoid fatty acids (CPEFA) typically found in cottonseed oil, required two separate procedures. The CPEFA were analyzed as phenacyl derivatives by high pressure liquid chromatography (HPLC) and the nutritional fatty acids were analyzed as methyl esters by gas chromatography (GC). HPLC analysis of CPEFA is labor intensive and requires lengthy run times, and the resulting chromatography is unacceptable for nutritional fatty acid analysis. GC applications for CPEFA were not feasible using packed columns, due to adverse column interactions with the propene ring structures of CPEFA. The advent of capillary columns, however, eliminated this, and numerous articles have since been written describing GC methods for CPEFA. Covance has now developed a method that analyzes both CPEFA and nutritional fatty acids in a single GC procedure. This method uses a simple and relatively fast alkaline trans-esterification to protect the propene ring structure and optimizes the chromatographic conditions for the resolution of all fatty acids of interest. This study presents the linearity, accuracy, specificity and precision of the new method.

14. **Measuring Hydroperoxides Evolution in Biodiesel by Ambient Mass Spectrometry.** G.G. Pereira*[1], L.L. Ferreira[1], R.M. Alberici[2], M.N. Eberlin[2], and D. Barrera-Arellano[1], [1]Fats and Oils Laboratory, Faculty of Food Engineering, University of Campinas-UNICAMP, Campinas, São Paulo, Brazil, [2]Fats and Oils Laboratory, Faculty of Food Engineering, University of Campinas-UNICAMP, Campinas, São Paulo, Brazil, [3]ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas-UNICAMP, Campinas, São Paulo, Brazil, [4]ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas-UNICAMP, Campinas, São Paulo, Brazil, [5]Fats and Oils Laboratory, Faculty of Food Engineering, University of Campinas-UNICAMP, Campinas, São Paulo, Brazil.

Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is a technique that allows direct and fast analysis in open atmosphere with minimal or no sample preparation. In this work we have evaluated the ability of EASI-MS to monitor hydroperoxides evolution in biodiesel in the earlier stages of oxidation. For comparison, classical procedure was performed. For that, we used as a model case, soybean biodiesel that was oxidized in the Rancimat apparatus (10 g, 110°C, and air flow rate of 10 L h^{-1}). Samples were analyzed at different time intervals during the induction period (IP) by peroxide value and EASI-MS. 2 µL of sample was added directly onto a paper surface and mass

spectra were acquired over a *m/z* range of 100-1000 using a singlequadrupole mass spectrometer (Shimadzu). We used the ion of *m/z* 349 as a marker of oxidation because it is the major hydroperoxide present in soybean biodiesel. The intensity of this ion increased moderately during the IP following a zero-order reaction kinetics that was highly correlated with the results for peroxide value (r = 0.9). In conclusion, EASI-MS proved to be a fast technique to monitor the primary oxidation products formed in biodiesel. Supported by FAPESP and CAPES.

15. **Microwave-assisted Saponification Method to the Simultaneous Determination of Cholesterol and Cholesterol Oxides.** H.A.L. Souza and N. Bragagnolo*, UNICAMP, Campinas, São Paulo, Brazil.

Free or esterified cholesterol and cholesterol oxides can be found in food, therefore a saponification step is necessary before chromatographic analysis. Although there is no microwave-assisted saponification method for cholesterol oxides described in the literature, the use of microwave energy has been outstanding among several modern techniques to accelerate organic chemical reactions because of its high efficiency to saponify and extract sterols. The aim of this study was to develop a microwave-assisted saponification method for the simultaneous determination of cholesterol and cholesterol oxides in salted dried shrimp. Method development was carried out by a central composite design with 3 variables: concentration and volume of an ethanolic KOH solution and temperature. Time of reaction (15 min), maximum microwave power (200 W) and magnetic stirring rate (120 rpm) were kept constant. After the saponification step, the samples were extracted with hexane. The best condition for the microwave-assisted saponification method was achieved using 20 mL of 1 mol/L ethanolic KOH solution and 45°C. The main cholesterol oxide found in salted dried shrimp was 7-ketocholesterol, representing more than 40% of total cholesterol oxides.

16. **Validation of Reagent-free GC Lipid Derivatization Method Using an Enzymatic Microreactor.** Y. Zhang*[1], S.M. Mugo[2], and J.M. Curtis[1], [1]University of Alberta, Edmonton, AB, Canada, [2]Grant MacEwan University, Edmonton, AB, Canada.

Derivatization of fatty acids to produce volatile methyl or ethyl esters (FAME or FAEE) prior to GC analysis is an indispensable procedure in lipid analysis. A lipase immobilized porous polymer monolith microreactor was developed and shown to achieve online and quantitative conversion of triglycerides to FAEE. When in use, a low flow of oil in ethanol is passed through the 15cm long microreactor. 100% conversion to FAEE will be completed during the passage of the solution through the enzymatic microreactor, so that the products can be collected for direct GC analysis. Here we describe a validation of the first generation microreactor for reagent-free derivatization of natural oil samples with varied fatty acid distributions. Results will be presented in order to demonstrate (i) artifact-free quantitative FAEE formation giving equivalent overall accuracy compared to AOCS method Ce 1k-09 for FAME; (ii) microreactor reproducibility and stability; (iii) finding the optimum microreactor length and flow rate in order to achieve conversion in the shortest time. These attributes are required for a future automated system.

17. **Simultaneous Determination of Fat-soluble Vitamins in Food Matrices Using Solid Phase Extraction and Liquid Chromatography-mass Spectrometry.** P. Mathur* and C. King, Department of Nutrition and Food Sciences, Texas Woman's University, Denton, TX, USA.

An analytical method was developed for simultaneous determination of vitamin A (Retinol, beta-carotene, retinyl-palmitate, and retinyl-acetate), vitamin D (D2, and D3), Vitamin E (Vitamin E (a,ß,δ,γ tocopherols, and tocopheryl-acetate), and vitamin K (K1, K2, and K3). The polarities of all the vitamin forms were determined, and a solid-phase extraction method was developed to separate these vitamin forms from different food matrices. Quantification was conducted using spectrophotometry and liquid chromatography-mass spectrometry. Beta-carotene was determined to be the most non-polar vitamin, followed by vitamin esters (retinyl-palmitate, retinyl-acetate, tocopheryl-acetate), ketones (Vitamin K1, K2, K3), and alcohols (tocopherols, vitamins D2, D3, and retinol). Methanol and water were found to be the most suitable solvents for conditioning of the solid-phase column, and hexane was used for elution. This method was then used to determine the recoveries of vitamin standards, which were found to range between 95-100%. This methodology will be further applied to separate fat-soluble vitamins from food matrices such as meal replacement shakes, vitamin

premixes, supplements, and infant formulas.

18. **Olive Oil and Other Food Type Provenance Verification by Advanced, Multiple Analytical Instrument, Data Set Analytics.** R. Packer* and C. Stacey, PerkinElmer Inc., Shelton, CT, USA.

The verification of provenance is very important in establishing food authenticity. For high value foods, the country of origin has a dramatic effect on the price, as such; falsifying the provenance of foods is an attractive proposition to fraudsters. A prime example is marking North African olive oil as Italian. Whilst various techniques such as trace metal analysis or isotopic ratios have been shown to separate different geographical classes, other factors such as year on year crop variations and changing weather conditions have caused these separations to be less conclusive. As such using more than one technique to differentiate can be more conclusive. This work uses advanced analytics and visualization software to separate the geographical origin of olive oil and Whiskey using ICPMS and LCMS data.

19. **Detecting the Adulteration of Extra Virgin Olive Oil by Differential Scanning Calorimetry.** K. Menard, B. Menard, C. Stacey, and R. Packer*, PerkinElmer Inc., Shelton, CT, USA.

Food adulteration normally makes the news with cases like melamine in milk. However, high-value products are often subjected to adulteration by lower-value materials and this can be difficult to detect. As a high-priced produce, a pint of extra virgin olive oil (EVOO) is close in cost to that of a half gallon of food-grade olive pomace oil. University of California at Davis has reported that the majority of the extra virgin olive oils sold in California fail the tests for the same (EVOO), using a variety of techniques (ultraviolet and visible spectroscopy [UV/Vis], gas chromatography [GC], liquid chromatography [LC]), and wet methods. However, considering the way EVOO is made, one would expect a relationship to its thermal properties.

Differential scanning calorimetry (DSC) is commonly used to analyze foods in both quality control and research labs. DSC is often used to compare materials on heating, but cooling studies often give more information as materials can respond more thermodynamically under controlled cooling.

In this study, we looked at the thermograms of various grades of olive oils as well as blends of EVOO and lesser grades. Intergration of the cooling curves over veriosu temperature regions was able to detect both the adulteration of the oil as well as see differences between varietals.

20. **Rapid and Inexpensive Method for Olive Oil Adulteration and Identification.** H. Harma*, University of Turku, Turku, Finland.

Olive oil is the most adulterated food product. Recent reports show that fraudulent activities are obvious as olive oil is sold at a higher price than other vegetable oils. To preserve its quality and authenticity new analytic methods are required. We have developed a label array fingerprinting technique to detect subtle changes in the content of vegetable oils. The core of the array technique bases on Time Resolved Luminescence (TRL), label chemistries and a ”fuzzy” fingerprint that provides a unique sample-specific signature of virtually any liquid or liquidized sample. TRL chelating structures are highly unstable, and the sample interacts non-specifically with the chelating structures modulating the TRL luminescence signal. The array is created through multiple different chelating agents as samples interact differently with each chelating structures. In the current protocol the sample is simply mixed with the chelates and signal is measured within five minutes. Until now the technology platform has been shown to be powerful in a number of food applications. Here olive oil adulteration (spiked) with canola oil down to 1% is measured with a large dynamic range as the signal-to-background ratio of assay was more than 500. Identification of vegetable oils is also presented.

21. **Novel Mass Spectrometric Methods for Analysis of Sterols in Biological Samples.** I. Hailat* and R.J. Helleur, Memorial University of Newfoundland, St. John's, NL, Canada.

Free sterols are neutral molecules that are difficult to ionize by MALDI or ESI and their molecular ions are difficult to observe since they easily fragment by losing H_2O. Therefore, in order to increase their ionization efficiency as well as their sensitivity, and selectivity; number of selected sterols were derivatized by different reagents to give picolinyl esters, N-methylpyridyl ethers and sulphated esters. The derivatives were optimized for MALDI-TOFMS analysis through proper selection of the matrix; those screened were DHB, THAP, p-nitroaniline and dithranol. Sterol picolinyl esters were identified as sodiated adducts $[M + Na]^+$ and their signal enhanced after addition sodium acetate (20 mM). Sterol N-methylpyridyl ethers which are positively charged ions, were easily detected as $[M]^+$.

The sulphated sterols were best detected as $[M-H]^-$. Also, these derivatives were investigated using ESI-quadruple ion trap (QIT) mass spectrometry with good sensitivity. Our MALDI approach was tested on sterols which were lipid extracted from blue mussels. The most abundant sterols in mussels were cholesterol, desmosterol, brassicasterol, and 24-methylenecholesterol. It was found that sulphated esters derivatives have the best MS sensitivity, about 5 times higher than picolinyl ester derivative and about 75 times more sensitive than free sterols.

22. **Separation and Analysis of Cottonseed Oil Deodorizer Distillate (CODD) Using Molecular Distillation.** A. Jafri*[1], C. King[1], R. Vowell[2], and D. Casper[3], [1]Texas Woman's University, Denton, TX, USA, [2]Doc Hale Food Ingredients, Highland Village, TX, USA, [3]Control Plus Inc., Elmhurst, IL, USA.

A molecular distillation method was developed to separate the unsaponifiables from cottonseed oil deodorizer distillate (CODD). The CODD contains free fatty acids (FFA), mono/diglycerols, and healthy ingredients including tocopherols and sterols. Using a model KDL6 glass still molecular distillation still with 0.06 m^2 evaporator, five cuts were conducted using different evaporator temperatures of 135°C, 145°C, 155°C, 165°C and 175°C and with the condenser temperature held at 60°C and vacuum at 0.22 mmHg. With the feed rate of 0.2 kg/hr, at a temperature of 60°C, samples of the CODD was separated into the distillate and residue portion. Using a gas chromatography silylation method, quantification of the components of both phases was determined. The cut-4 distillate was composed of 90.8% of FFA and only 4.3% of unsaponifiables. The residual had a very small amount of FFA's, <0.4% and 63% of tocopherols and sterols. Then this residual portion was then used as feed for stage-2 distillation. In one of the various trials, tocopherols and sterols were 84.5% of the distillate while the residual had 97.14% diglycerols and 2.6% unsaponifiables. Thus, this laboratory method using a 2-stage process successfully distilled the tocopherols and sterols from FFA's and other components of CODD.

23. **Detection and Quantification of Furan Fatty Acids in Sea Urchins by GC-FID and GC-MS.** Y. Itabashi*[1], S. Yamashina[1], A. MacKenzie[2], and M. Vyssotski[2], [1]Hokkaido University, Hakodate, Hokkaido, Japan, [2]Callaghan Innovation, Lower Hutt, New Zealand.

Detailed fatty acid analysis was carried out for lipid extracts of sea urchins obtained in Japan (*Pseudocentroutus depressus*, *Strongylocentrotus nudus*, *S. intermedius*) and New Zealand (*Evechinus chloroticus*). Urea fractionation, Ag+-SPE, GC-FID, and GC-MS techniques revealed the occurrence of a series of furan fatty acids in all the species examined (4.3-42 mg/g lipid). The most predominant furan acid in four species was F4, accounting for 42-67% of total furan acids. This was followed by F6 (15-37%), F5 (8-15%), F2' (2-11%), F1 (1-5%), F3 and F6'(1-3%), and F2 (0-3%). Very small amount of F5' and F8 were also detected in *S. nudus* and *S. intermedius*. A lipid extract of the New Zealand green-lipped mussel (*Perna canaliculus*) has reportedly displayed anti-inflammatory effects in animal models and in human controlled studies. Until recently, the active components were unknown, but now have been found in furan acids (Wakimoto et al., 2011). We have found that the furan acid composition of sea urchins is similar to that of *P. canaliculus*, except that F6 is the most predominant furan acid of the mussel. The present study suggests that the lipids from Japanese and New Zealand sea urchins may have potential anti-inflammatory activity

24. **Fast, Efficient, and Convenient Extraction of Polynuclear Aromatic Hydrocarbons from Olive Oil Using a Dual-Layer SPE Cartridge.** K. Stenerson, O. Shimelis, K. Espenschied, M. Ye, and L. Sidisky*, Sigma-Aldrich, Bellefonte, PA, USA.

Olive oil can become contaminated with polynuclear aromatic hydrocarbons (PAHs) through exposure of the olives to pollution in the environment. Concern over exposure to these compounds has caused some countries within the European Union to set limits on PAH content in olive oil. Analysis of oily/fatty samples presents an analytical challenge due to the heavy matrix effects often encountered. In the case of GC-MS, fatty matrix can cause contamination of the GC inlet, column and detector.

An SPE cartridge containing two different sorbent layers was evaluated in the simultaneous extraction and cleanup of PAHs from olive oil. The layers consist of Florisil and a mix of zirconia-coated silica/C18. Olive oil sample was loaded directly onto the SPE cartridge, followed by elution of the PAHs with acetonitrile while fatty matrix remained bound to the sorbents. The resulting extract was concentrated, and analyzed by GC-MS. The dual-layer SPE cartridge was evaluated with olive oil samples spiked with 28 different PAHs, and found to yield recoveries of >70% for most compounds. In

addition to being fast and convenient, the SPE method was found rugged and applicable to the GC-MS analysis of PAHs from edible oil.

25. **Highly Reproducible Fatty Acid Analysis with Automatic Calculation of Multiple Key Factors.** M. Sasser*, G. Jackoway, and K. Kunitsky, MIDI, Inc., Newark, DE, USA.

With intent to submit an "AOCS Standard Procedure" for computer software creating fully automated analysis of fatty acids, a method has been created that incorporates multiple features designed to enhance inter-lab reproducibility of FAME analysis. Use of an external calibration mixture allows adjustment of the GC for minor differences in oven temperature sensors and column polarity variations enhancing inter-lab reproducibility. A quantitative calibration mixture provides interpolated ECL values within 19 divisions of an analysis, thus providing ECL values at four decimal place precision. Response factor (RF) corrections are calculated based on either the theoretical RF or a more precise RF based on the precision calibration mixture. The basic method provides analyses covering all key FAMEs from 4:0 through 24:0 with sub-methods for more rapid analysis of components of the FAME groups (*e.g.* cis-trans, MUFA-PUFA, etc.). A detector correction factor (RF) is calculated, based on either a theoretical RF or on the more precise external calibration mixture. Post-analysis calculations such as iodine value, TAG equivalents, EPA-DHA, etc. are accessed through "check box" turn on. The software enhances the ability of laboratory personnel to obtain highly reproducible results with ease and a minimal chance of error.

26. **Two Dimensional Gas Chromatographic Separation of Fatty Acids.** A.R. Fardin-Kia, D.G. Hayward, and P. Delmonte*, US Food and Drug Administration, College Park, MD, USA.

Recent improvements in the GC separation of fatty acid methyl esters (FAME) using long highly polar capillary columns increased the number of FAMEs that could be quantified in a single analysis. Comprehensive two-dimensional gas chromatography (GC x GC) provides improved separation of FAMEs by combining the selectivity of two GC separation columns. The selectivity of a highly polar capillary column as SLB-IL111 can be coupled with the selectivity of a lower polarity column, resolving in the second dimension of separation the co-elutions of FAMEs not separated by the first column. Alternatively, a capillary reducer (consisting of a capillary tube coated with palladium) can be added between the two dimensions of chromatographic separation, allowing the use for the two separations of two capillary columns coated with the same phase. The application of this platform (termed GC x online reduction x GC) provides in the second dimension of separation the resolution of FAMEs with different chain lengths, eliminating the overlap between FAMEs differing simultaneously for the number of carbons and number of double bonds. FAMEs are identified by GC x GC-TOF after their conversion to dimethyl oxazoline derivatives (DMOX).

27. **Enzyme Linked Lectin Assay.** M.L. Breeze* and E. Leyva-Guerrero, Monsanto Company, St. Louis, MO, USA.

Soybean lectin otherwise known as soybean agglutinin (SBA) is regarded as an anti-nutrient in soybean due to the negative effect feeding raw soybean has on the ability of monogastric animals to gain weight. SBA has been measured in the evaluation of new soybean varieties to establish compositional equivalence using a haemagglutination procedure established by Liener et al (1955). Due to the time-consuming nature of the haemagglutination procedure and the need to screen a large number of samples, we investigated the published literature for a methodology that could more efficiently be applied to large number of samples. The method of Wang et al (2009) that takes advantage of the carbohydrate binding specificity of specific lectins for specific carbohydrates using a sandwich ELISA-like format was identified as a candidate methodology. Following the single lab validation of this methodology, an extensive global collaborative study of this method's performance was initiated to fully characterize the expected variability of the candidate test method. The validated methodology, termed Enzyme Linked Lectin Assay (ELLA) was applied to a population of soybeans with commercial launch dates from 1972 to 2008.

28. **Analysis of Emulsifiers in Foods by High Pressure Liquid Chromatography and Charged Aerosol Detection.** M.A. Plante*, B. Bailey, and I.N. Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Emulsifiers are used to maintain a uniform suspension of immiscible materials. These compounds are typically surfactants, and can be

designed for use in specific applications and products. Lecithin (mainly phosphatidylcholine) is commonly found in chocolate and spray oils; acid esters of monoglycerides are used as dough conditioners in the baking industry; and hydroxypropylmethyl cellulose (HPMC) is used to thicken dairy products and help improve flavor characteristics. HPMC is also an important emulsifier used in the pharmaceutical industry.

The analysis of emulsifiers is becoming increasingly important, for both purity and stability properties. High pressure liquid chromatography is one of the more prevalent methods for analyzing these compounds. However, the majority of analytes do not contain a chromophore, which then requires the use of a universal detector, such as evaporative light scattering, refractive index, or charged aerosol detection. The charged aerosol detector was used in the analyses of lecithin, by normal phase HPLC, and HPMC, by reversed-phase HPLC, in food products, and results are provided. Linear calibration curves were generated for both analytes over four orders of dynamic range. Limits of quantitation were 20 ng o.c. for lecithin and 10 ng o.c. for HPMC.

29. **Analysis of Free Phytosterols in Natural Products by HPLC-ECD.** B. Bailey, M.A. Plante*, and I.N. Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Phytosterols are a group of naturally occurring steroid alcohols found in plants. They are key structural components of plant cell membranes, assuming the role that cholesterol plays in mammalian cells. There is considerable interest in phytosterols as dietary supplements as they are reported to lower cholesterol levels and also have a positive impact on cardiovascular diseases. Recent research suggests that phytosterol supplementation may aggravate atherosclerosis and lead to aortic valve stenosis. Phytosterols are typically measured by gas chromatography (GC). This approach is time-consuming since it requires saponification of the sample, several extractions, and followed by derivatization. Here, a simplified method is presented using reversed phase, high-performance liquid chromatography (HPLC) and electrochemical detection using a boron doped diamond electrode.

Samples were prepared for HPLC analysis by simple dilution. Five standards campesterol, cholesterol, stigmasterol, beta-sitosterol, and stigmastanol were eluted in < 10 min. The LOD was = 1 ng on column for all analytes. The method presented here was used to determine free cholesterol content in whole blood. The HPLC method with electrochemical detection is simple to implement, has good linearity and sensitivity, and is capable of measuring numerous phytosterols in plant and animal extracts.

30. **Characterization of Used Cooking Oils by HPLC-MS and Corona Charged Aerosol Detection.** M.A. Plante*, B. Bailey, and I.N. Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Cooking oils are critical to food preparation, and are used in the production of many of the foods enjoyed today. Being one of the significant components in cooking, cooking oil quality has an important role in flavor and in health. As cooking oil is re-used, its chemical composition changes, with many of the volatile components decrease in quantity, whereas fatty acids and their oxidation products appear, and other novel compounds are created. Some changes, such as the loss of volatile components are relatively harmless and do not affect the oil quality. However, oxidation of fatty acids creates rancidity in oils and the resulting oxidation products are purported to cause health issues.

Samples of soybean oils were characterized by high performance liquid chromatography, using charged aerosol detector to provide details about the quality of the oil, and mass spectrometry was used for identification of some aldehydes. Oils were characterized using a universal lipids method on whole oils, and a free fatty acid method for hydrolyzed cooking oils, as well as a normal phase method. The use of HPLC-charged aerosol detection with the universal lipids method provided a facile means of determining cooking oil quality, without the need for any sample derivatization.

31. **Analysis of Phospholipids in Natural Samples by Normal Phase HPLC and Charged Aerosol Detection.** M.A. Plante*, B. Bailey, and I.N. Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Phospholipids are a broad class of lipids that can be divided into glycerophospholipids (GPLs) and sphingolipids (SLs). Both groups show great structural diversity. Phospholipids are amphiphilic molecules, having a hydrophilic head group, and a lipophilic fatty acid tail. Several families of GPLs exist biologically, differing in the type of polar head group present, and include: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), cardiolipin, and phosphatidylinositol (PI). Each compound contains

many species resulting from differences in their fatty acid composition. Normal phase liquid chromatography (NP-HPLC) uses differences of polar moieties to separate analytes, and use of NP-HPLC will provide more quantitative data with less effort. Other diacyl phospholipids would exhibit similar properties. Lysophosphatides (e.g., lysophophatidylcholine (LPC)) are produced from the action of phospholipase enzymes, which removes the C2 fatty acyl side-chain.

The derivatization-free HPLC-CAD method, enables the direct measurement of PE, PS, PI, PC, and LPC, down to limits of detection < 25 ng o.c. with linear calibration curves spanning four orders of magnitude. Measurements of phospholipids in egg yolk, lecithin, and krill oil are provided as examples.

32. **Determination of Olive Oil Adulteration by Principal Component Analysis with HPLC - Charged Aerosol Detector Data.** M.A. Plante*, B. Bailey, and I.N. Acworth, Thermo Fisher Scientific, Chelmsford, MA, USA.

Adulteration is a common problem typically found with high-value products: less costly materials are often added to high-cost materials for sale. Adulteration of food has occurred for hundreds of years, and analytical techniques are always improving reliability in detecting such adulteration. Some recent examples include the adulteration of orange juice with other juices, peel and pulp wash, the use of marjoram and thyme as additions to oregano, and the use of a variety of vegetable oils (lampante grade, canola oil (up to 70%), as well as avocado, palm, and sunflower oils), as substitutes for olive oil.

With the anticipated, future shortages of olive oil, combined with the anticipated increases in value, it is likely that adulteration will become an escalating issue for olive oil in the market. Reliable and accurate determinations of olive oil quality are required to maintain the integrity of olive oil products, and analytical methods are continuously improving to address this need. Principal component analysis of data obtained using HPLC with charged aerosol detection for either triglyceride analysis of whole oils, or free fatty acid analysis from hydrolyzed oil samples was used to evaluate the adulteration by different oils, including corn, hazelnut, and pomace oils.