# 2013 Annual Meeting Abstracts

# Analytical

## MONDAY

### AFTERNOON

**ANA 1/LOQ 1.1: Marine Oils - Analytical and Stability**
Chair(s): J. Reuther, Eurofins Central Analytical Labs, USA; V. Barthet, Canadian Grain Commission, Canada

#### Quantitation of Fatty Acids in Marine Oils by Comprehensive gc-online Hydrogenation x Gc.

P. Delmonte[(1)], A. Fardin Kia[(2)], J. Rader[(3)]
[(1)]US Food and Drug Administration, United States of America [(2)]US Food and Drug Administration, United States of America [(3)]US Food and Drug Administration, United States of America

In this study, the separation of fatty acid methyl esters (FAME) prepared from marine oils provided by an SLB-IL111 capillary column is enhanced by adding a second dimension of separation in a comprehensive GCxGC design. After elution from the first column, the FAME are reduced to their fully saturated form by passing through a capillary tube coated with palladium in the presence of hydrogen carrier gas. The products of reduction are then separated by the secondary high polarity capillary column. The two dimensional separations obtained using this technique can be easily interpreted based on the principle that all the saturated FAME lie on a straight line bisecting the separation plane, while the FAME with the same carbon skeleton but differing in the number, geometric configuration or position of double bonds lie on lines parallel to the D1 time axis. This methodology provides the quantitation of the FAME with different chain lengths that are not separated by mono-dimensional chromatography and it also provides valuable structural information without use of a mass spectrometer. The ease of interpretation of the two dimensional chromatograms and the higher separation capability make this technique far superior to the most refined mono-dimensional separations of FAME.

#### Determination of Trans Polyunsaturated Fatty Acid Content in Fish oil Supplements Available in the U.S. Market

C. Tyburczy[(1)], J. Rader[(2)]
[(1)]U.S. Food and Drug Administration, United States of America [(2)]U.S. Food and Drug Administration, United States of America

The health effects of dietary trans polyunsaturated fatty acids (PUFA), specifically those of the trans isomers of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), remain to be fully determined. The present study evaluated the content of trans PUFA in fish oil (FO) supplements available in the U.S. market. FA methyl esters (FAME) from 48 FO supplements were prepared according to Official Method Ce 2-66 of the American Oil Chemists? Society and separated by gas chromatography on a 200 m SLB-IL111 ionic liquid column using a combined ramped temperature and flow program. FAME standards for the trans isomers of EPA and DHA were prepared by isomerization with p-toluenesulfinic acid and fractionated by silver ion thin layer chromatography according to the number of trans double bonds. Across all FO samples, the combined content of trans EPA and trans

DHA (0.1 ? 1.3% of total fat) was unrelated to the total content of all-cis EPA and DHA. Additionally, the content of total trans EPA (0.3 ? 4.8% of all-cis EPA; i.e., the degree of isomerization) was highly correlated with that for DHA (R2 = 0.95). Taken together, these results suggest that processing is the major source of trans PUFA in FO supplements. Our findings demonstrate that the production of FO supplements with low levels of trans PUFA (~1% of total fat) is possible and that the 200 m SLB-IL111 column serves as an important analytical tool for the quantitation of trans PUFA in samples of marine origin.

**Microalgae: a Potential Source to Enrich Eggs With Omega-3 Fatty Acids**

C. Lemahieu[(1)], C. Bruneel[(2)], R. Termote-Verhalle[(3)], K. Muylaert[(4)], J. Buyse[(5)], I. Foubert[(6)]
[(1)]KU Leuven University Kulak, Belgium [(2)]KU Leuven University Kulak, Belgium [(3)]KU Leuven University Kulak, Belgium [(4)]KU Leuven University Kulak, Belgium [(5)]KU Leuven University, Belgium [(6)]KU Leuven University Kulak, Belgium

The long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA), EPA and DHA, are associated with several health benefits. Unfortunately, the average daily intake of n-3 LC-PUFA is below the recommended level, raising interest in food enrichment. To that end, the objective of this study was to increase the level of these n-3 LC-PUFA in eggs by feed adaptation using microalgae. Laying hens were fed with four different n-3 PUFA rich autotrophic microalgae (Phaeodactylum tricornutum, Nannochloropsis oculata, Isochrysis galbana and Chlorella fusca). Depending on the amount of algae used and on the algae species, egg yolk could be enriched with different levels of n-3 LC-PUFA, ranging from 50 to 120 mg per egg. This experiment pointed out that Isochrysis was the most appropriate alga to use for further research. In a second experiment, a dose response study was performed in which nine different n-3 LC-PUFA doses of Isochrysis were fed to laying hens. Taking into account the n-3 LC-PUFA enrichment and the efficiency of n-3 LC-PUFA incorporation, supplementation of 120 mg algal ALA+SDA+EPA+DPA+DHA / 100 g feed was the most optimal dose. This dose gave rise to the highest efficiency of n-3 LC-PUFA incorporation (60%) and an enrichment of 84.5 mg n-3 LC-PUFA/egg.

**Analysis of Poly Aromatic Hydrocarbons in Seafood and Fish Oil by GC-MS and GC-MS/MS**

A. Pradhan[(1)]
[(1)]Eurofins Central Analytical Laboratories, United States of America

Many Polycyclic Aromatic Hydrocarbons (PAHs) are known to be toxic to humans, and some are carcinogenic. PAHs persist in the environment for long periods of time, increasing the chances of exposure to humans through the food chain. Our laboratory has developed fast and accurate testing methods for PAHs and aliphatic hydrocarbon (AHCs) residues in various types of seafood matrices (e.g. crab, fish, oyster, shrimp) and fish byproducts like fish oil for government agencies and commercial buyers and sellers. We play a major role in implementing high-throughput analysis of PAHs using a saponification/alumina cleanup method for fish oil as well as an ASE method for edible seafood. GC-MS and GC-MSMS quantitative methods has been developed to analyze both PAHs and AHCs at the same time. Discussion of proper quality control measures that ensure the reliability of data will be presented.

**Rapid Analysis of In-process Marine Oil by Quality Trait Analysis (QTA) Infrared Spectroscopy**

K. Ma[(1)], K. Kramer[(2)], C. Teng[(3)]
[(1)]Eurofins QTA, Inc., United States of America [(2)]Eurofins QTA, Inc., United States of America [(3)]Eurofins QTA, Inc., United States of America

Recent studies have indicated that consumption of omega 3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), can result in numerous health benefits. EPA and DHA are most abundantly found in the oils of fatty fish. To provide consumers with EPA/DHA dietary supplements, fish oils are refined to increase the EPA and DHA content. It is desirable to have a rapid test to monitor the process in real time. The current method of analysis, gas chromatography, is slow, expensive, and resource-intensive. We present a rapid and simple test for in-process fish oil that can be performed by an operator with no science background or technical expertise. To ensure product safety and accuracy in labeling, EPA/DHA supplements are subject to the US Food and Drug Administration? s current Good Manufacturing Practices. Therefore, an accurate test for EPA, DHA, and Total Omega 3 is important for both in-process and finished product oils. QTA patented IR technology provides easy-to-use, reliable and accurate measurement of EPA, DHA, Total Omega 3, Mono- Di-, and Tri- glyceride, Ethyl Ester, and Oligomer for both in-process and finished product marine oils.

**Issues in Fortification and Analysis of Omega 3s in Foods**

E. Hernandez[(1)], M. Rusli[(2)]
[(1)]Omega Protein, United States of America [(2)]Omega Protein, United States of America

There is an increasing awareness of the health benefits of omega-3 fatty acids which is reflected by the growth in consumption of omega-3 fats either through dietary supplements or fortified foods. Omega-3 fatty acids, particularly the longer chain fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been extensively studied for their health promotion and disease prevention properties. When fortifying foods and beverages omega-3s fats are usually added in relatively small amounts (mg per serving) and these fatty acids can become imbedded in the matrix in the food components (i.e., complexes of protein, carbohydrates and other fats) which makes the analysis of EPA and DHA makes analysis ever more difficult. Sometimes this can result in appreciable differences between the calculated levels of EPA and DHA in the products formulated and the experimental results from the lab. Fortified foods can have as little as 32mg of EPA and DHA in a 250g serving. This presentation will include examples of foods fortified with omega 3s, techniques of sample preparation, methods of analysis and reporting for ALA, EPA and DHA for certificates of analysis, product validation, and for labeling.

**Bioimprinting And/or Immobilization of Lipases for Selective Ethanolysis of Fish Oil**

D. Kahveci[(1)], X. Xu[(2)]
[(1)]Yeditepe University, Food Engineering Department, Turkey [(2)]Aarhus University, Denmark

Lipases are unique enzymes with their hydrophobic active site buried under an amphiphilic peptidic lid, which goes under a conformational rearrangement upon contact with a water-lipid interface, leading to the exposure of the active site. This phenomenon, called interfacial activation, is the key to bioimprinting strategy, which involves the incubation of lipase with a substrate analogue in aqueous medium, followed by lyophilization. The lipase is activated since it gets ?caught in action? and unable to adopt its former conformation due to its rigid structure in organic solvent. Bioimprinting of lipases not only improve their activity and stability in anhydrous medium, but also can help to modify their selectivity. If the process is designed properly, immobilization has been revealed as a powerful tool to improve enzyme properties, such as stability, activity, specificity and selectivity. Combination of bioimprinting and immobilization has been employed to several lipases in order to improve activity and stability. The present lecture aims to give a brief overview of the use of bioimprinting w/o immobilization to improve activity and selectivity of lipases, followed by the authors? work on enhancement of those of Candida rugosa lipase (CRL) and Candida antarctica lipase A (CALA) for selective ethanolysis of fish oil. CRL bioimprinted with fatty acids exhibited 8-fold enhanced transesterification activity in hexane. Fatty acid selectivity of CALA was improved by immobilization

combined with bioimprinting, resulting in 5.5-fold lower omega-3 PUFA in ethyl esters fraction.

**Accelerated Solvent Extraction of Lipids: A Highly Efficient Method Preferred for Lipid Oxidation Studies**

L. Yao[(1)], K. Schaich[(2)]
[(1)]Rutgers University, United States of America [(2)]Rutgers University, United States of America

The handling required in extraction of lipids for oxidation analyses always poses problems with adventitious oxidation. In previous research, we found that Accelerated Solvent Extraction (ASE) extracts lipids from complex matrices such as processed cheese, biological tissues, and extruded food products with high efficiency. This study focused on optimizing ASE extractions from extruded pet foods (mixed meat and grain base) observed that ASE extractions also minimize degradation during extraction. Extruded kibbles ground to 250 micron particle size were ASE extracted with chloroform, chloroform-methanol 2:1, hexane, and hexane:methanol 2:1. Extraction static time and numbers of extraction cycles were varied to optimize lipid yields. Extracts dried under vacuum were analyzed for lipid composition by thin layer chromatography and for oxidation by conjugated dienes (AOCS Ti 1a-64) and hydroperoxides (PeroxySafeTM assay). ASE extraction at 40 °C provided comparable or higher yields than manual or Soxhlet extraction in shorter extraction times (10-40 minutes depending on the matrix) while inducing less oxidation than manual extraction and less thermal degradation than Soxhlet. Oxidation increased with extraction temperature (60 °C max), but remained lower than other methods. Results also demonstrated that ASE can also utilize normally immiscible solvents to advantage. Hexane and methanol injected into extraction cells separately were able to nearly duplicate extractions of chloroform:methanol, providing an option for replacing chlorinated hydrocarbon solvents. However, differences in oxidation for the two solvents were observed, probably resulting from stabilizers in the chloroform. Overall, ASE appears to provide a superior method for lipid oxidation studies.

**Oxidative stability of krill (Euphasia superba) oil**

I. Bruheim[(1)], A. Oterhals[(2)], B. Haugsgjerd[(3)], G. Vogt[(4)], M. Griinari[(5)], B. Thomsen[(6)], H. Lu Fung Sieng[(7)], C. Jacobsen[(8)]
[(1)]Olympic Seafood AS, Norway [(2)]Nofima, Norway [(3)]Nofima, Norway [(4)]Nofima, Norway [(5)]Clanet, Finland [(6)]Technical University of Denmark, Denmark [(7)]Technical University of Denmark, Denmark [(8)]Technical University of Denmark, Denmark

Krill oil is a unique source of omega-3 LCPUFA where the omega-3 fatty acids are bound predominantly to phospholipids. Data in the public domain suggests that the oxidative stability of krill oil assessed by peroxide and anisidine values (PV and AV) is extremely good. However, data from controlled experiments using a broad range of analytical methods to study oxidative stability are lacking. We set out to perform an experiment where oxidation of food grade krill oil and fish oil was studied under accelerated conditions (samples incubated at 40 C, exposed to air and stirred). Sampling was performed on day 0, 7 and 21 and the oxidative status was assessed by determination of PV, AV, thiobarbituric acid reactive substances (TBARS), conjugated dienes, volatile secondary oxidation products and volatile Strecker degration products (both by dynamic headspace GC-MS analysis), tocopherols, astaxanthin, and pyrroles. Increases in PV, conjugated dienes and TBARS as well as decrease in tocopherols were observed in fish oil samples whereas there were no changes in krill oil. AV increased in fish oil and a less pronounced and variable increase was observed for krill oil. This observation was consistent with pronounced increase in volatile secondary oxidation products in fish oil samples and less pronounced response in krill oil samples. Strecker degradation products and hydrophobic pyrroles increased only in krill oil samples and slight decrease in krill oil astaxanthin was also observed. These findings suggest that PV and AV may have limited use in assessment of the oxidative changes in krill oil.

# TUESDAY

## MORNING

**ANA 2: Review of Old Analytical Methods - Challenges, Solutions**
Chair(s): R. Della Porta, Frito-Lay Inc., USA; T. Mason West, Bunge Oils Inc., USA

### A Flow-through Microreactor for the Direct Ethylation of Oils and Fats - a new Alternative for Lipid Derivitization Prior to Conventional gc Analysis.

J. Curtis[(1)], S. Tuan Anuar[(2)], S. Mugo[(3)]
[(1)]University of Alberta, Canada [(2)]University of Alberta, Canada [(3)]Grant MacEwan University, Canada

A simple, direct method for the conversion of oils and fats into fatty acid ethyl esters (FAEE) has been developed using a prototype enzymatic silica monolith microreactor. The microreactor contains lipase from Candida antarctica immobilized onto the large surface area of a silica monolithic network housed within a 480 µm (OD) fused silica capillary. The quantitative conversion of triacylglycerols from a variety of animal and vegetable oils into FAEE was achieved at room temperature using the lipase-loaded flow-through microreactor under optimized conditions, as demonstrated by LC/ELSD, GC/FID and GC/MS. This flow-through ethylation method is proposed as an alternative procedure to the conventional methylation/ethylation procedures that are performed prior to GC analyses of lipids. There are many advantages over standard procedures such as the avoidance of toxic chemicals, mild conditions, the reusability of the microreactor, and the production of derivatives directly from small lipid sample sizes in amounts compatible with GC analyses. In this presentation we evaluate the potential of the microreactor derivitisation technology to replace the standard methods for converting oils and fats into FAEE or FAME for analytical purposes.

### Droplet size distribution, NMR vs. Microscopy.

G. Sekosan[(1)], T. West[(2)], K. Reihel[(3)]
[(1)]Bunge Oil, United States of America [(2)]Bunge Oil, United States of America [(3)]Bunge Oil, United States of America

A method using Pulsed Field Gradient Nuclear Magnetic Resonance for water in oil emulsion droplet size determination has been optimized and compared with optical microscopy. The correlation between the NMR and microscope was good (0.99 and 0.98) in the emulsions with a water content of 10 to 20% for the droplets in the 50 and 97.5 % volume interval. The precision for the determination of the D3,3 (mean diameter of the volume distribution) value by microscope is worse than that of NMR for emulsions with more than 20% water even when four images were used to calculate this parameter (8% and 3% RSD, respectively).

### Separation of Fatty Acids in Marine Oils With Highly Polar Ionic Liquid gas Chromatographic Columns.

A. Fardin-Kia[(1)], P. Delmonte[(2)], J. Rader[(3)]
[(1)]U.S. FDA, United States of America [(2)]U.S. FDA, United States of America [(3)]U.S. FDA, United States of America

The fatty acids (FA) contained in marine oils and fats are traditionally analyzed by gas chromatography after their

conversion to fatty acid methyl esters (FAME) using capillary columns coated with polyethylene glycol (PEG) phases. PEG columns provide simple elution patterns of PUFAs based on their chain length and number of double bonds, but fail to separate most of their geometric and positional isomers. The SLB-IL111 capillary column, characterized by higher polarity, provides separation of more complex samples than PEG columns, and it allows the separation of geometric and positional isomers of PUFAs which are not separated by PEG columns. In this study, we separate the FAME prepared from menhaden oil using a 200 m x 0.25 mm SLB-IL111 column. Identification of FAMEs is facilitated by applying Ag+-HPLC fractionation and GC-MS in chemical ionization mode. The proposed method can simultaneously separate short chain FAs, mono-unsaturated trans fatty acids, and PUFAs. In addition to providing the quantitation of the FAs contained in pure marine fats and oils, this procedure also allows the quantitation of the FAs contained in mixed fats and oils.

**Determination of Slip Melting Point, Iodine Value and Moisture% in Refined Palm Oil Using FT-NIR**

P. Chabot[(1)]
[(1)]ABB Analytical Measurements, Canada

FT-NIR spectroscopy provides a simple, secure and time saving solution to analyze multiple properties at once while minimizing considerably operator errors for enhanced precision. Refined, Bleached and Deodorized palm olein is obtained from fractionating refined palm oil to separate liquid parts (olein) from solid parts (stearin). Palm olein is a clear yellow liquid at room temperature used as cooking oil and frying oil for food industries. It is also used as raw material for margarine and shortening. The melting point of fats is an important parameter for many specifications used in trade and, in some countries is an element of the legal definition of food products. Fats consist in a complex mixture of glycerides and therefore do not have sharp melting points, unlike pure chemical substances. The slip melting point of a fat is defined as the temperature at which a column of fat in an open capillary tube moves up the tube when it is subjected to control heating in a hot water bath. Because of their polymorphic behavior, the slip point of some fats is dependent upon the previous treatment of the samples. SMP is widely used to characterize the melting and solidification properties of oils and fats. It changes with the chain length of fatty acids, unsaturation ratios, trans fatty acid content and the position of the fatty acids in the glycerol backbone. Other properties such as Iodine Value, Moisture% and Free Fatty Acids are used to characterize the RBD palm oil for a tight quality assessment.

**Dilute-and-shoot GC/MS Analysis of Olive Oils for the Determination of Quality and Authenticity**

P. Wylie[(1)]
[(1)]Agilent Technologies, United States of America

Recent reports from the UC Davis Olive Center indicate that more than 60% of "extra virgin" olive oils do not qualify for that quality distinction. Another report by Moore, et al. reveals that olive oil is the most commonly adulterated food or food ingredient. Extra virgin olive oil may be adulterated with cheaper olive pumice oil or with seed oils. Olive oil quality can degrade with time and with exposure to light and oxygen. The objective of this study was to develop a ? dilute-and-shoot? GC/MS method for the analysis of olive oils, thus avoiding tedious sample preparation steps. Olive oils were diluted 10:1 in solvent and were analyzed by GC/MS with no further sample preparation. Several approaches were tried: 1) high temperature chromatography in an attempt to elute all of the olive oil constituents, 2) elution of the free fatty acids and volatiles through the sterol fraction with backflushing of the heavier components, and 3) use of a Deans switch to divert the heavy components through an uncoated restrictor to waste with post-run backflushing. The latter approach proved to be very rugged and was applied to 31 different olive oil samples that were purchased locally. The data were analyzed using Principle Component Analysis which showed clear differences among olive oils of varying quality.

**Identification of Tocopherols and Tocotrienols, and Their Fatty Acid Esters in Residues and Distillates of Structured Lipids Purified by Short Path Distillation**

L. Zou[(1)], C. Akoh[(2)]
[(1)]University of Georgia, United States of America [(2)]University of Georgia, United States of America

The fate of endogenous vitamin E isomers during production and purification of structured lipids (SLs) was investigated. Two SLs involving tripalmitin, stearidonic acid soybean oil, and docosahexaenoic acid were synthesized by transesterification catalyzed by Novozym 435 (NSL) and acidolysis by Lipozyme TL IM (LDHA), and purified by short path distillation (SPD). The electron impact and chemical ionization mass spectra of tocopheryl and tocotrienyl fatty acid esters in the distillates measured by gas chromatography mass spectrometry (GC-MS) in synchronous scan/selected ion monitoring (SIM) mode, demonstrated that these esters were formed during acidolysis as well as transesterification. The predominant esters were tocopheryl palmitate, tocopheryl oleate, and tocopheryl linoleate homologues and no tocopheryl or tocotrienyl linolenate, stearidonate, and docosahexaenoate was found. Meanwhile, none of these esters were detected in the residues for both NSL and LDHA. Less than 50% of vitamin E isomers were present in residues after SPD. This loss played a major role in the rapid oxidative deterioration of SLs from previous studies with less contribution from the formation of tocopheryl and tocotrienyl esters. The lost tocopherols and tocotrienols present at high concentration in the distillates may be recovered and used to improve the oxidative stability of SLs.

**The Effect of Different Extraction Solvents on the Polyphenol Content and Antioxidant Activity of Limnophila Aromatica Extracts**

Q. Do[(1)], Y. Ju[(2)]
[(1)]National Taiwan University of Science and Technology, Taiwan [(2)]National Taiwan Univesity of Science and Technology, Taiwan

Limnophila aromatica is commonly used as a spice and a medicinal herb in Southeast Asia. In this study, total polyphenolic content, and antioxidant capacity of the freeze-dried L. aromtica extracts were studied by using various in vitro assay. Different solvents, including reverse osmosis (RO) water and various concentrations of methanol, ethanol and acetone (50%, 75 % and 100%) in RO water, were used to perform the extraction. The extract obtained by 100% ethanol showed the highest total antioxidant activity, reducing power and DPPH radial scavenging activity. The extract obtained by 100% ethanol also exhibited the highest phenolic content of 405 mg GAE/g DFLA (defatted Limnophila aromatica) and the highest flavonoid content of 311.1 mg QCE mg/g DFLA. These results indicate that L. aromatica can be used in dietary applications with potential to reduce oxidative stress.

**Evaluation of thiosulfate, xylenol orange, ferric thiocyanate, and triphenyl phosphine assays for quantitating lipid hydroperoxides**

K. Schaich[(1)], E. Steltzer[(2)]
[(1)]Rutgers University, United States of America [(2)]Rutgers University, United States of America

Given the current focus on reformulating foods with polyunsaturated fatty acids for health, sensitive analysis of oxidation products has become critical for accurately measuring lipid degradation and stability. Thus, four assays for hydroperoxides were re-evaluated to compare linearity of response, accuracy, limits of detection, active concentration range, reproducibility, and required conditions and handling, with cumene and tert-butyl hydroperoxides as test

standards; optimized procedures were then applied to oxidized methyl linoleate. Thiosulfate titration was most accurate and the only method providing absolute quantitation of hydroperoxides. It was stoichiometric, linear, and useful for high peroxide concentrations, but unclear endpoints limit sensitivity and handling must be rigorously controlled to provide reproducible results. PeroxySafeTM and PeroxoQuantTM xylenol orange (XO) assays detected nanomolar hydroperoxides, hence require extensive dilution of most samples before analysis. The ferric thiocyanate method (FeSCN, chemical reaction or Cayman LPOTM kit) detected as low as 5 nanomoles, but reaction stoichiometry varied with solvent as well as hydroperoxide structure and concentration. Both XO and FeSCN assays exhibited distinct disadvantages in variation of reaction response with hydroperoxide structure plus bleaching of detection complexes at high hydroperoxide concentrations, causing underestimation of peroxide values. Neither optical assay can determine absolute hydroperoxide concentrations in mixed systems. Triphenylphosphine selectively and stoichiometrically detected as low as 5 picomoles hydroperoxides, and multiple species could be separated by HPLC. The reaction has promise, but needs further development to become quantitative. Results for all methods highlight the importance of excluding oxygen during assays and understanding the correct concentration range for each assay.

### FAME Analyisis with Ionic Liquid Capilary Columns

L. Sidisky[(1)], G. Baney[(2)], J. Desorcie[(3)], D. Shollenberger[(4)], G. Serrano[(5)]
[(1)]Supelco, United States of America [(2)]Supelco, United States of America [(3)]Supelco, United States of America [(4)]Supelco, United States of America [(5)]Supelco, United States of America

Analyses of fatty acid methyl esters (FAMEs) are continuing to gain importance as more research is focusing on their biomedical impacts. This includes the analysis of saturated and polyunsaturated FAMEs along with the positional geometric (cis and trans) FAME isomers. Traditionally, FAME analyses have been performed using silicone polymer or polyethylene glycol based stationary phases that yield typical elution patterns. Analysts performing the task of analyzing the fatty acid composition of food have a wide variety of capillary column selectivity?s available for resolving the fatty acids as FAMEs depending upon the information they require from their analyses. Nonpolar methylsilicone columns provide a boiling point separation of the FAME isomers with limited resolution of polyunsaturated isomers. Polar polyethylene glycol (PEG) columns resolve the isomers by degree of unsaturation with minimal overlap of the carbon chain lengths. The highly polar cyanosilicone columns will resolve cis and trans isomers along with possibly providing positional geometric isomer separations depending upon the column type. New classes of stationary phases based on Ionic Liquid technology have been developed and have demonstrated to provide unique elution patterns for FAME isomers compared to the traditional silicone or polyethylene glycol based stationary phases. The two new phases are SLB-IL60 with a PEG like selectivity and the SLB-IL111 with highly polar selectivity. We will compare and contrast the selectivity of the ionic liquid phases with polymeric based phases for various FAME samples. Window diagramming of combinations of various ionic liquid phases will also be examined.

## AFTERNOON

### ANA 3: Analysis of Trace Contaminants in Vegetable Oil and By-Products

Chair(s): M. Collison, Archer Daniels Midland Co., USA; S. MacMahon, FDA, USA

### Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Detection of Glycidyl Esters and MCPD Esters in Edible Oils

S. MacMahon[(1)], T. Begley[(2)], G. Diachenko[(3)]
[(1)]U.S. Food and Drug Administration, United States of America [(2)]U.S. Food and Drug Administration, United States of America [(3)]U.S. Food and Drug Administration, United States of America

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), 2-monochloro-1,3-propanediol (2-MCPD), and glycidol are potentially, through ester hydrolysis, carcinogenic chemical contaminants formed during the processing of edible oils. The contaminants are found in food products containing refined oils and have proven challenging to reliably detect and quantitate. The most widely used approaches are indirect detection techniques using transesterification to cleave the esters of 3-MCPD and glycidol, followed by derivatization and detection by GC-MS. While the reliability of indirect methods has improved, direct detection of intact esters of 2-MCPD, 3-MCPD and glycidol remains the preferred approach for the collection of occurrence data. The LC-MS/MS methods described in this presentation have been validated for the direct detection and quantitation of intact 2-MCPD, 3-MCPD and glycidyl esters. The development of SPE and LC conditions, the importance of MS/MS quantitation ion selection, method validation, and occurrence data results will be discussed. This direct approach is rugged, sensitive, specific and allows for the determination of fatty acid esters of 2-MCPD, 3-MCPD and glycidol using methodology suitable for regulatory analysis.

**Analysis of Intact Fatty Acid Esters of Glycidol in Vegetable Oils Using Gas Chromatography - Mass Spectrometry**

K. Hrncirik[(1)], H. Steenbergen[(2)], H. Janssen[(3)]
[(1)]Unilever, Netherlands [(2)]Unilever, Netherlands [(3)]Unilever, Netherlands

The presence of glycidyl esters (GE) in refined vegetable oils attracts significant attention of chemists, food technologists, toxicologists and other professionals. The development of a reliable analytical methodology presents a particular challenge in the research around these processing contaminants. To date several analytical methods for the determination of GE in oils and fats have been published. In the direct methods intact glycidyl esters (GE) are measured using LC/MS after extensive sample clean-up. In the indirect methods GE are converted to a halogenated derivative, either 3-chloropropanediol (3-MCPD) or 3-bromopropanediol, which is derivatized and quantified by using GC/MS. In this speech a GC-MS based method for the analysis of intact GE in oils and fats is presented. The method consists of a simple extraction step of GE from the lipid matrix, purification (LLE) of the extract and isolation of GE by normal phase LC. Individual GE are separated and quantified by standard GC/MS operated in the setup that prevents thermal degradation of GE. Over the period of several months of extensive use the method showed an excellent performance in all aspects. The comparison of experimental values with spiked levels and with the results obtained by another method indicates a good trueness.

**Progress in the Analysis of 2-/3-mcpd Esters and Glycidyl Esters: From Refined Oils to Oil-based Foodstuffs**

A. Ermacora[(1)], K. Hrncirik[(2)]
[(1)]Technical University of Berlin, Germany [(2)]Unilever R&D Vlaardingen, Netherlands

Fatty acid esters of 2- and 3-monochloropropanediol (2-/3-MCPD) and glycidol are food-borne contaminants formed during high temperature processing of fat-based matrices, particularly during refining of vegetable oils. Their full toxicological significance and risk assessment is still under evaluation, although free 3-MCPD and glycidol were shown to exhibit several harmful effects in laboratory animals. Substantial research effort is currently spent on this issue across food industry, Academia and food safety authorities. The availability of a reliable methodology for the quantification of both classes of these contaminants is essential for further progress of the research, namely for the estimation of the dietary exposure, understanding their mechanism of formation, but also for evaluating the efficiency of proposed mitigation strategies. While several analytical methods for the determination of MCPD esters in bulk oils have already been developed and evaluated, only very few reliable procedures are currently available for the analysis

of glycidyl esters. Furthermore, no validated procedure for the quantification of any of these contaminants in final food products has been presented yet. In this speech, a method for the simultaneous determination of MCPD esters and glycidyl esters that we recently developed in our laboratory is presented. The results show that the method can be successfully applied to the analysis of both bulk oils/fats and, for the first time, also various fat-based products.

**Interconversion Between Monochloropropanediols and Glycidol in the Course of DGF Standard Method C-VI 18 (10)**

H. Sato[(1)], N. Kaze[(2)], H. Yamamoto[(3)], D. Ono[(4)], M. Shizuma[(5)], Y. Watanabe[(6)]
[(1)]Osaka Municipal Technical Research Institute, Japan, Japan [(2)]UEDA OILS and FATS MFG CO., LTD. Japan, Japan [(3)]UEDA OILS and FATS MFG CO., LTD. Japan, Japan [(4)]Osaka Municipal Technical Research Institute, Japan [(5)]Osaka Municipal Technical Research Institute, Japan [(6)]Osaka Municipal Technical Research Institute, Japan

Fatty acids esters of monochloropropanediols (MCPDs) and glycidol are undesirable contaminants in oil processing. The amount of 3-MCPD and its esters and glycidol and its esters are undirectly measured by the DGF standard method C-VI 18 (10), assay A and B, respectively. We recently reported that 3-MCPD and glycidol interconverted each other at the transesterification and extraction step in the course of process. 2-MCPD was ignored at that stage. In this study, the dynamics of 2-MCPD, 3-MCPD and glycidol, and their esters during DGF standard methods C-VI 18 (10) were directly analyzed using NMR and indirectly using GC/MS. 2-MCPD partly converted to glycidol under basic conditions of the transesterification step, and the glycidol reconverted to the original 2-MCPD under acidic conditions of the extraction step. Interestingly, 3-MCPD was also converted to glycidol at the transesterification step, the glycidol reconverted to the original 3-MCPD at the extraction step. 2-MCPD spiked in soybean oil was detected as 98% 2-MCPD and 2% 3-MCPD from the area ratio in GC/MS. From these results, 2- and 3-MCPD almost recovered to the original isomer by way of glycidol in the course of DGF standard method C-VI 18 (10).

**Formation of the Toxicologically Relevant 2-alkenals Acrolein and Crotonaldehyde in Comparison to Aroma-active Compounds During Deep-frying of Food**

M. Granvogl[(1)], P. Shieberle[(2)]
[(1)]German Research Center for Food Chemistry, Germany [(2)]German Research Center for Food Chemistry, Germany

In the past, many studies have been undertaken to elucidate the key odorants of food and to identify formation pathways of the so-called ?food-borne toxicants?, e.g., acrylamide, furan, etc. But, up to now, analytical approaches including the quantitation of desirable aroma-active compounds in combination with undesirable toxicological relevant substances by sensitive methods are scarcely available. Therefore, the aim of this study was to combine the analysis of important aroma compounds and of selected food-borne toxicants formed during deep-frying, exemplified for potato chips and donuts in different edible oils. Odorants were identified by gas chromatography-olfactometry as well as GC-MS and quantitated by stable isotope dilution analysis (SIDA). For quantitation of both 2-alkenals, two new analytical approaches (direct method via headspace GC-MS; indirect method via GC-MS after derivatization) using isotopically labeled [13C3]-acrolein and synthesized [13C4]-crotonaldehyde as internal standards were developed. The lecture will highlight the influence of the fatty acid composition of oils on the formation of aroma-active compounds and of 2-alkenals. For example, a frying fat mainly based on mono-unsaturated or saturated fatty acids significant revealed much lower amounts of acrolein (30 mg/kg) in contrast to oils containing linolenic acid (200 mg of acrolein/kg). In summary, the study showed that the appropriate choice of the frying medium significantly influences the formation of odorants as well as toxicologically relevant compounds. Thus, lowering the amounts of undesirable compounds in combination with the maintenance of an overall aroma well accepted by the consumers is a challenging task.

**Development and Validation of Hexaconzole Residue in Crude Palm oil by gas Chromatograph With Electron Capture Detector and Confirmed With Mass Spectrometer**

H. Muhamad[(1)]
[(1)]Malaysian Palm Oil Board, Malaysia

This paper describes the development and validation of an analytical method for determination of hexaconazole in crude palm oil (CPO). This method was based on low temperature precipitation extraction with acetonitrile, followed by clean-up using graphitized carbon black solid phase extraction for extraction of hexaconazole residue in CPO. Determination of hexaconazole was performed using gas chromatograph (GC) with electron capture detector (ECD). The identity of hexaconazole recovered from CPO samples was further confirmed by GC-MSD. The calibration curves for hexaconazole using GC-ECD showed good linearity with coefficient correlation of 0.9995 for six points calibration from 0.1 to 5 µg/mL. Calculated LOD and LOQ for hexaconazole using GC-ECD were 0.2 and 1.0 µg/L, respectively. The precision was performed by analyzing six replicates of the same concentration on two different days and with two different analysts and the RSD did not exceed 1%. Meanwhile, the accuracy of the method was carried out by spiking a known amount of hexaconazole standard solution into hexaconazole-free CPO sample. Recoveries of hexaconazole in CPO spiked with different levels of hexaconazole at (0.01 to 0.05 µg/g) were performed. The recovery of hexaconazole from spiked crude palm oil sample ranged from 82.4% to 91.3% with RSD between 1.2% to 9.2%. The method was successfully applied to the analysis of hexaconazol in crude palm oil samples obtained from local palm oil mills throughout Malaysia. No hexaconazole residue was found in the 30 samples analysed.

**Stability and reactivity of MCPD esters as well as glycidyl esters in model systems**

M. Granvogl[(1)], A. Ewert[(2)], P. Shieberle[(3)]
[(1)]German Research Center for Food Chemistry, Germany [(2)]German Research Center for Food Chemistry, Germany [(3)]German Research Center for Food Chemistry, Germany

After 3-MCPD esters and glycidyl esters have been analyzed in food, especially in refined edible oils and fats, a lot of efforts have been undertaken by industry as well as by research institutes to minimize their concentrations due to the fact that after consumption a cleavage of the esters to harmful free 3-MCPD and glycidol is possible and has already been shown under certain conditions. Thus, beside the demand for robust, quick, and sensitive quantitation methods, it is also of high interest to get more information about stability and reactivity of the esters. The lecture will provide deeper insights into the stability of glycidyl esters as well as 2- and 3-MCPD esters affected by the presence of enzymes and by the influence of heating temperature and heating time. Further, the possibility of adduct formation was investigated and some possible structures will be discussed. Both stability and reactivity of the esters will be compared to free glycidol as well as of free 2- and 3-MCPD.

**Do results for glycidyl ester and MCPD ester contents obtained by different analysis methods agree?**

T. Wenzl[(1)], L. Karasek[(2)]
[(1)]Institute for Reference Materials and Measurements, Belgium [(2)]Institute for Reference Materials and Measurements, Belgium

Many analysis procedures for the determination of glycidyl esters and MCPD esters in edible oils and fats were proposed in the last years. The scope of these methods is as different as the proposed analytical approaches. Some of these methods were initially considered fit-for-purpose, but had to be retracted at a later stage after the formation of artefacts was identified. A lack of trust in the reliability of the proposed analysis procedures and the produced data was

the consequence. The Joint Research Centre organised in 2012 an interlaboratory comparison study on the determination of glycidyl esters and MCPD esters in edible oils, with the purpose to remediate the situation by evaluating whether different direct and indirect analysis methods for the determination of glycidyl esters and MCPD esters provide comparable results. Only expert laboratories from all over the world, covering official food control, academia and industry, were invited to participate in this study. They applied both indirect and direct analysis procedures for the determination of the glycidyl ester and MCPD ester content of the seven test samples. The presentation will provide the outcome of the study. The comparability and the trueness of the results gained by the different analysis procedures will be discussed and the question whether agreement on a particular method would be required will be answered.

### Approaches to Analysis of Foods for Glycidol and 2- & 3-MCPD Esters at Health Canada

A. Becalski[(1)]
[(1)]Bureau of Chemical Safety, Health, Canada

Two direct stable isotope dilution analysis methods of glycidol fatty acid esters in foods based on positive ion atmospheric pressure chemical ionization LC-MS/MS will be discussed. The first method targets five analytes: glycidol esters of palmitic, stearic, oleic, linoleic and linolenic acid, the second one is used for four glycidol esters: lauric, myristic, eicosapentaenoic and docosahexaenoic. Lipids are extracted with 10% diethyl ether in hexane or, for milk containing products, with a mixture of DCM/MeOH (2:1) For the analysis, 10 mg sample of lipids is dissolved in acetone, spiked with deuterium labelled analogs of glycidol esters, and purified by two-step chromatography on C18 and normal silica solid phase extraction cartridges using methanol and 5% ethyl acetate in hexane respectively. If the concentrations of analytes are expected to be below 0.5 mg/kg, 0.5 g sample of oil is pre-concentrated first using a silica column. The dried extract is re-dissolved in 250 µL of a mixture of methanol/isopropanol (1:1, v/v), 15 µL is injected on the analytical C18 LC column, and analytes are eluted with 100% methanol. A method suitable for determination of both free and bound 2- and 3-MCPD using a single extraction combined with a new derivatization protocol employing cyclohexanone / fluorinated sulfonated resin (Nafion) will be outlined as well. These methods were applied to the pilot survey of glycidol and 2- & 3-MCPD fatty acid esters in food products on the Canadian market. Results of this survey for edible oils and fats as well as cookies will be presented.

## WEDNESDAY

MORNING

**ANA 4: Rapid and Real Time Analysis**
Chair(s): M. Mossoba, FDA, USA; H. Zhao, Missouri University of Science & Technology, USA

### In-vivo and Rapid Monitoring of Obesity Using FT-NIR Spectroscopy

H. Azizian[(1)], J. Kramer[(2)], S. Winsborough[(3)]
[(1)]NIR Technologies Inc., Canada [(2)]Retired, Canada [(3)]NIR Technologies Inc., Canada

Obesity has more than doubled since 1980 as reported by World Health Organization (WHO). At least 2.8 million adults die each year as a result of being overweight or obese. Obesity is a leading preventable cause of death worldwide. Body Mass Index (BMI) is the most common method for assessing obesity but BMI does not measure

body fat instead assumes that body mass is closely associated with body fat and that has been shown to be a questionable assumption. Chronic conditions associated with body fat include heart disease, osteoarthritis, diabetes, cancer, sleep apnea, fatty liver, etc. There is a need for a rapid, reliable, non-invasive and accurate analytical method to determine body fat and assist in monitoring and reversing the obesity trend. A non-invasive method using FT-NIR has been developed to measure the body fat content in one minute. The body fat determinations were compared to established reference methods such as MRI for individuals (indirectly) and DXA for young overweight and obese women (directly). In both cases a statistically significant relationship was found between FT-NIR and MRI or DXA. Data will be presented to show how the body fat content can vary significantly with exercise and how fat loss affects sleep apnea.

**Choice of Calibration Standard for the Quantitation of Trans fat in Edible Oils by Attenuated Total Reflection-fourier Transform Infrared Spectroscopy**

C. Tyburczy[(1)], M. Mossoba[(2)], A. Fardin-Kia[(3)], J. Rader[(4)]
[(1)]U.S. Food and Drug Administration, United States of America [(2)]U.S. Food and Drug Administration, United States of America [(3)]U.S. Food and Drug Administration, United States of America [(4)]U.S. Food and Drug Administration, United States of America

The present study evaluated the use of trielaidin as a calibration standard for quantifying trans fat in edible oils by attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR). ATR-FTIR analysis is based on the response at 966 cm-1 in the FTIR spectrum, attributed to the C=C-H out-of-plane deformation band that is quantitatively characteristic of total isolated trans double bonds. The content of trans fat in a sample is calculated from the calibration standard curve linear regression function, generated using neat triglyceride standards for trielaidin (trans-9 18:1) added to tripalmitin or triolein. Deodorization is known to cause the cis-to-trans isomerization of double bonds in polyunsaturated fatty acids (FA). The majority of the trans FA in deodorized oils are mono-trans dienoic (MTD) and mono-trans trienoic (MTT) FA isomers. Negligible levels of mono-trans monoenoic (MTM) and di-trans dienoic (DTD) FA may also be present. The responses of the MTD, MTT and DTD FA isomers at 966 cm-1 in the FTIR spectrum, relative to those of the MTM FA isomers, were investigated in the present study and found to differ based on degree of unsaturation and number of trans double bonds per molecule. Our findings suggest that the quantitation of trans fat in deodorized edible oils by ATR-FTIR analysis may be biased when trielaidin is used for calibration.

**Rapid (<5 Min) FT-NIR Screening of Edible Oils for the Determination of the Total SFA, Trans FA, MUFA and PUFA Contents and Comparison to GC and Declared Values on Nutrition Facts Panels**

M. Mossoba[(1)], H. Azizian[(2)], C. Tyburczy[(3)], J. Kramer[(4)], P. Delmonte[(5)], A. Fardin Kia[(6)], J. Rader[(7)]
[(1)]FDA, United States of America [(2)]NIR Technologies, Inc, Canada [(3)]FDA, United States of America [(4)]Guelph Food Research Center, Agri-food Canada, Canada [(5)]FDA, United States of America [(6)]FDA, United States of America [(7)]FDA, United States of America

Labeling requirements for total trans FA and total SFA are mandatory, and GC has been the method of choice to provide FA composition. However, GC is time consuming, requires conversion of fats and oils to their FAME, and accurate identification of GC peaks is challenging. In the present study, FT-NIR spectroscopy was applied to the rapid (<5 min) determination the total SFA, trans FA, MUFA, and PUFA contents of neat fats and oils. No significant difference was observed between GC and FT-NIR for all the oils examined. The trans FA content declared on all products were found to be in compliance with the US regulations regarding the declaration of 0 grams trans FA per serving (0.5 g or <3.6% of total FA per serving). However, many of the oils examined were not found to be in compliance regarding their SFA, MUFA and PUFA contents, mainly due to the presence of high oleic acid varieties that are not specifically identified, and some oils that did not appear to be accurately analyzed. While there was agreement in the total trans FA content above 2% of total FA between the GC and FT-NIR results, there were

significant differences between these two methods in the determination of very low (<2% of total FA) trans FA content. In general, GC results underestimated the total trans FA content, while FT-NIR overestimated it. FT-NIR can be used as a rapid alternative screening method for regulatory compliance verification of fats and oils.

### Quality assessment of olive paste, pomace and oil by NIR

H. Li[(1)], C. Larkin[(2)]
[(1)]Bruker Optics, Inc., United States of America [(2)]Bruker Optics, Inc., United States of America

Near Infrared spectroscopy (NIR) has gained more and more attention from the olive oil industry as a rapid quality assessment tool due to its versatile nature. Compared to traditional chemical and sensory methods used, NIR analysis is rapid (measurements take seconds) with little-to-no sample preparation, while allowing for multiple components to be predicted with only a single measurement. Furthermore, it is a green technique, producing no waste or pollution, thus making it even more appealing. This presentation will cover the use of European-derived methods for the quality assessment of olive paste, pomace and oil from the USA (California). The performance of each calibration will be evaluated in order to determine if there are obvious differences between the European and Californian products, while providing insight into the underlying causes.

### Application of portable FT-IR spectrometers to authenticate raw materials

L. Rodriguez-Saona[(1)]
[(1)]The Ohio State University, United States of America

Optical technology is rapidly developing and instruments are available commercially as portable, hand-held, and micro- devices that can be used when it is not practical or economical to use the more sophisticated and costly instruments used in research laboratories. We will present information on the feasibility of handheld and portable infrared systems in applications relevant to the food industry. We have evaluated their performance against benchtop systems directed at developing fingerprinting strategies for rapid and specific analysis of high-risk foods (i.e. cocoa butter, olive oil, milk), providing reliable tools for assessment of quality and safety. Food applications have been targeted at authentication, detection of economic adulteration and detection of chemical food contaminants through development of spectral signature profiles permitting the chemically authentication of raw materials. This technology can enable the food manufacturer for real-time and field-based measurements to control the raw material stream, addressing safety and brand equity. Implementation of rapid testing by the industry and regulatory agencies would help to streamline food safety and quality assurance and will prevent the growing danger to consumers from adulterated or substituted products as evidenced by the melamine incident associated with milk-derived products.

### Strategies for Rapid Characterization of Supplements Using Simple Extraction Protocol Followed by DART MS of Intact Triglycerides and Fatty Acids

B. Musselman[(1)], J. LaPointe[(2)]
[(1)]IonSense, United States of America [(2)]IonSense, Inc., United States of America

Recent changes in FDA regulations call for more through characterization of so called dietary supplements. Rapid methods for quality assessment of these materials are needed to make meaningful measurements of their quality however those methods are often time consuming requiring GC, LC or GC/MS. Direct Analysis in Real Time (DART)

technology has been demonstrated to provide a rapid means to determine both the mass of intact triglycerides and free fatty acids in plant materials at a rate of seconds per sample. We demonstrate the utility of the DART analysis of a rapid extraction of oil from supplement materials which are often provided in leafy or ground plant material format as a means to determine quality and type of supplement. Chemometric methods are applied to various extracts in order to develop a more comprehensive quality assessment tool for product characterization.

### A Simple Analytical Method for Detailing the Composition and Oxidation Levels of Marine Omega-3 Supplements

R. Freeman[(1)], I. Iwai[(2)], D. Randle[(3)], C. Watanabe[(4)]

[(1)]Frontier Laboratories, United States of America [(2)]Frontier Laboratory, United States of America [(3)]Frontier Laboratory, United States of America [(4)]Frontier Laboratories, Japan

The health benefits of increasing the dietary levels of polyunsaturated fats (PUFA) have been well documented. Omega-3 fatty acids are rapidly becoming an essential element of diets, the world over. However, PUFA are easily oxidized and little is known about the health effects of rapidly increasing the intake of oxidized lipids. Analytical methods being used to characterize marine oils are cumbersome and often unreliable. This work describes a simple, one step GC/MS method for profiling the fatty acids present in both oils and supplements. Oxidation, based on the level of 4-hydroxy-2-nonenal (HNE), is quantitated using thermal desorption (TD) ? GC/MS. The fatty acid profile is obtained by combining an organic alkali (m-triflourophenyl trimethyl ammonium hydroxide) and the sample at 250?C. This is often referred to as reactive pyrolysis. The sudden exposure to heat results in the thermal hydrolysis and methylation of fatty acids. The fatty acid methyl esters (FAMEs) are subsequently swept directly onto the GC column. The sample is analyzed ?as is?; no sample prep is necessary. The precision of this method is approximately 2% (e.g., DHA = 1.43 %RSD). Accuracy is ±5% (e.g., % Difference DHA = 5.0). HNE is determined using thermal desorption (TD) ? GC/MS. The sample is analyzed ?as is?. Based on the evolved gas thermogram and extracted ion profiles, the thermal desorption zone for HNE was determined to be 100 to 250 ?C at 10 ?C/min.. Fatty acid profiles and and HNE levels will be reported for 4 supplements and 2 fish oils.

### minispec Time-Domain NMR Applications in the Oil / Food Industry: Applications Overview and Updates as well as newly added Efficiency Improvement Capabilities

H. Todt[(1)]

[(1)]Bruker BioSpin GmbH, Germany

Time-Domain NMR is widely used in oil / food industries for fast and accurate determination of crucial product parameters like - Solid Fat Content Determination in Fat Compositions and other food samples like Chocolate - Total Fat and Moisture Content Analysis in various Food Products, like milk powders, cocoa powders, crisps, pretzel or any types of nuts - Oil and Moisture Content in oil seeds, presscakes and residues - Oil and Moisture Content in single seeds or single maize kernals for seed breading purposes - Oil / Water Droplet Size Analysis in Emulsions Many of the above mentioned applications are International (AOCS) Standard Methods since already a couple of years. TD-NMR is a fast and accurate technology that in many cases requires no or only minimum calibration efforts. Sample preparation is on a minimum level, too. TD-NMR instruments require only electricity for operation; cryogenic liquids are not in use at all. The analyzers are simple to operate and require only a minimum of maintenance. NMR investigates the entire sample; especially large and non-homogenous objects are analyzed completely. Sample color has no impact on the NMR data. Despite those advantages, laboratories always have a need for an improvement in efficiency and in improved reliability. This can be realized by applying modern and intelligent automated approaches. Some examples will be presented and a few solutions will be described.

**ANA 4.1/H&N 4: Advances in Analytical Aspects of Lipid Nutrition**
Chair(s): S. Bhandari, Silliker Inc., USA; B. Ward, Utah State University, USA

### Assessing Stability of Fingertip Prick and Venous Whole Blood During Long-term Storage and Associated Mechanisms of Degradation

A. Metherel[(1)], K. Stark[(2)]
[(1)]University of Waterloo, Canada [(2)]University of Waterloo, Canada

High-throughput omega-3 profiling can be enabled by rapid blood collection techniques such as fingertip prick (FTP) blood sampling. However, improved ease of collection increases the number of samples collected and places pressure on storage capacities. In the past, fatty acid oxidation of highly unsaturated fatty acids (HUFA) in erythrocytes has been observed at storage temperatures of -20°C. The tendency for oxidation of HUFA in FTP and venous whole blood has not been well characterized. Presently, FTP and venous whole blood samples were stored for up to 6 months under multiple temperature (room temperature (RT), 4°C, -20°C, -75°C), antioxidant (butylated hydroxytoluene(BHT), no BHT) and omega-3 content (low vs. high) conditions. Significant reductions in HUFA levels were observed starting after 1 day at -20°C, 30 days at 4°C and 60 days at RT. No degradation was observed at -75°C. Mechanisms involved in the expedited -20°C degradation were further assessed by storing samples with glycerol, a cryoprotection agent, and/or deferoxamine, an iron chelator. Cryoprotection of FTP whole blood with glycerol prevents haemolysis and HUFA degradation as compared with controls. In addition, iron chelation significantly reduced HUFA degradation to 10% after 7 days of storage at -20°C compared to 72% in untreated controls. In conclusion, BHT treatment protects against FTP and venous whole blood HUFA degradation and high omega-3 blood is more resistant to degradation, and degradation during storage at -20°C appears to be due to an iron-mediated peroxidation mechanism as a result of haemolysis during the freeze-thaw process.

### Improved Lipid Profile in Hyperbilirubinemic Subjects Contributes to Cardiovascular Protection

K. Wagner[(1)]
[(1)]University of Vienna, Austria

Gilbert's syndrome (GS) is characterized by a benign, mildly elevated bilirubin concentration in the blood and affects up to 10% of the population. Recent reports show clear protection from cardiovascular disease in this population. Protection of lipids, proteins and other macromolecules from oxidation by bilirubin represents the most commonly accepted mechanism contributing to protection in this group. However, a recent meta-analysis estimated that bilirubin only accounts for ~34% of the cardioprotective effects within analysed studies. To reveal the additional contributing variables we have explored circulating cholesterol and triglyceride concentrations, which appear to be decreased in hyperbilirubinemic individuals and animals, and are accompanied by lower body mass index in highly powered studies. These results suggest that bilirubin could be responsible for the development of a lean and hypolipidemic state in GS. Within the presenation it will also be discussed the possible contributing mechanisms that might reduce circulating cholesterol and triglyceride concentrations, proinflammatory cytokines or LDL subfractions in individuals with syndromes affecting bilirubin metabolism/excretion. Further the antioxidative potential of bilirubin will be addressed.

### Plasma, Erythrocytes and Whole Blood Fatty Acids: Translating Compositional Data

K. Stark[(1)], J. Aristizabal Henao[(2)], A. Metherel[(3)], L. Pilote[(4)], f. GENESIS PRAXY investigators[(5)]

[1]University of Waterloo, Canada [2]University of Waterloo, Canada [3]University of Waterloo, Canada [4]McGill University Health Centre, Canada [5]McGill University Health Centre, Canada

Fatty acid composition can be determined from a variety of blood fractions with erythrocytes recommended for determining eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) status. Plasma and whole blood samples are often used and can be difficult to compare with studies using erythrocytes. The fatty acid composition of total lipids of plasma (PTLE), erythrocytes (RBC) and whole blood (WB) from participants of the GENESIS PRAXY study (n = 789) were determined by gas chromatography. The fatty acid composition of plasma phospholipids (PPL), plasma triacylglycerols (PTAG), plasma cholesteryl esters (PCE) and plasma free fatty acids (PFFA) were also examined for a subset of 40 participants. The relative percentage of eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) was 3.82 ± 1.20% in RBC, 2.91 ± 0.97% in WB, and 2.67 ± 0.97% in PTLE (n= 789). RBC EPA+DHA was significantly correlated to PTLE and WB EPA+DHA ($r > 0.70$, $P < 0.001$, for both, n = 789) with RBC EPA+DHA=0.93(PTLE EPA+DHA)+1.34 and RBC EPA+DHA=1.11(WB EPA+DHA)+0.6. PPL, PCE and PTAG EPA+DHA were very strongly correlated to RBC EPA+DHA ($r > 0.80$, $P < 0.001$, for all) with RBC EPA+DHA=0.93(PPL EPA+DHA)+0.55, RBC EPA+DHA=1.59(PCE EPA+DHA)+2.05, and RBC EPA+DHA=2.71(PTAG EPA+DHA)+2.56. PFFA EPA+DHA was also correlated to RBC EPA+DHA ($r=0.67$, $P < 0.001$). In conclusion, EPA+DHA determined from various blood fractions including whole blood can be used to estimate the EPA+DHA in erythrocytes.

**Relative Role of Dietary fat Amount and Structure in the Meal on the Secretion of Chylomicrons and Associated Endotoxin Transport in Lean and Obese Humans**

C. Vors[1], F. Laugerette[2], G. Pineau[3], H. Vidal[4], M. Laville[5], M. Michalski[6]
[1]INRA USC1362, CarMeN Laboratory, France [2]INRA USC1362, CarMeN laboratory, France [3]INRA USC1362, CarMeN laboratory, France [4]INSERM U1060, CarMeN laboratory, France [5]CENS, France [6]INRA USC1362, CarMeN laboratory, France

Postprandial hyperlipemia and low-grade inflammation are major metabolic features in obesity that increase the risk of cardiovascular diseases. Moreover, we recently reported the role of gut endotoxin absorption during the digestion of lipids, partly due to endotoxin transport by chylomicrons during their secretion. Our new objective was to test the impact of different lipid amounts and structures in the meal on both chylomicronemia and associated endotoxin transport in humans. Therefore, 8 lean and 8 obese volunteers digested 10g or 40g of unemulsified dairy fat (spread on bread + drink) or 40 g emulsified in a drink (+ bread). Plasma and chylomicrons were collected during 8h of digestion. Chylomicron triglycerides and endotoxemia were analyzed. Increasing fat amount from 10g to 40g resulted in increased postprandial chylomicronemia both in lean and obese subjects, as expected. However, in obese subjects, this was associated with an increased postprandial accumulation of endotoxins in plasma, which was not observed in lean subjects ($P<0.05$). When 40g fat was emulsified, chylomicron triglycerides increased sooner and sharper than with 40g of unemulsified fat, in both groups. In lean subjects, this did not affect endotoxin transport by chylomicron, while in obese subjects, this enhanced early endotoxemia of the chylomicron fraction 60 min after meal ($P<0.05$). Altogether, our results show that (i) the amount and structure of fat in the meal modify the kinetics of chylomicronemia and (ii) this has an impact on the properties of postprandial endotoxemia in obese subjects. Long-term consequences on low-grade inflammation thus deserve to be elucidated.

**LCMS Analysis of Vitamins D2, D3, 25(OH)D2 and 25(OH)D3 in Food Samples**

S. Bhandari[1], H. Wu[2], T. Gallegos-Peretz[3]
[1]Silliker Laboratories, United States of America [2]Silliker Laboratories, United States of America [3]Silliker Laboratories, United States of America

Studies were performed to validate a LC-MS/MS method to quantify vitamins D2, D3, 25(OH)D2 and 25(OH)D3 in various food matrices. The method gave a linear response in relation to analyte concentration in tested range for all the four analytes. The method was found to be accurate for vitamin D3 analysis by testing the certified and in house reference materials and by comparing results with that of a reference method. The accuracy of the method for all the analytes was also demonstrated by a satisfactory spike recovery of the respective analyte in a variety of matrices. The replicate analysis of analytes in different matrices, spiked and unspiked demonstrated a satisfactory precision of the method. Identity of the analytes in the tested samples was established by a satisfactory ratio of the quantifier to qualifier transitions for the analyte in the tested matrices. The study also determined the LOD and LOQ of the method for all the analytes. The method differs in extraction from the conventional methods in the use of a solid- supported liquid-liquid extraction (SLE) instead of liquid-liquid extraction (LLE). The SLE method is about 4 times more efficient than the one reported earlier using LLE.

**Effect of dairy product consumption on cognitive performance among elderly participants of the Cache County Study on Memory, Health and Aging.**

R. Ward[(1)]
[(1)]Utah State University, United States of America

The objective of this study was to investigate any role dairy product consumption may have in protection against cognitive decline among elderly participants in the Cache County Study on Memory, Health and Aging. This entailed analysis of the fatty acid methyl esters (FAMEs) obtained from approximately 2,000 red blood samples with a focus on those associated with dairy fat, C15:0, C16:1n7t and C17:0. Data from the Modified-Mini Mental State Exam (3MS) was available from three waves of the study. Among the 3,364 subjects evaluated at baseline, eleven categories of dairy products were estimated: skim milk, 2% milk and higher fat, chocolate milk, cream, sour cream, ice cream, yogurt, cottage cheese, cream cheese, other cheese, and butter. Intakes were estimated from the Food Frequency Questionnaires. Mean baseline intake was 2.7 cups per day. Approximately 9% of the respondents abstained from dairy. Plasma phospholipid C16:1n7t content was below the detection limit in RBC samples while 15:0 and 17:0 were 0.2% and 0.4% of RBC FAMES, respectively. Baseline 3MS scores were compared between groups separated by dairy intake and found to be highly significant. 2.5 units of dairy was associated with the highest 3MS scores at baseline. The association remained at wave 3 when there was a FFQ administered and it is from this time point that the RBCs were collected. In sum, our data indicate that intake of 2.5 units of dairy per day was associated with the highest 3MS scores.

**Generating Meaningful Results with Lipidomics**

S. Watkins[(1)]
[(1)]Metabolon, United States of America

Metabolomics has progressed over the last ten years to become a routine analytical technology for discovery applications, monitoring bioprocessing reactions and for clinical and diagnostic discovery and development. The quality of most metabolomic approaches can be assessed using the conventional measures of quantitation, throughput, breadth and cost. Data sets are often simply lists of metabolite identities and quantities within a given biological matrix. These data sets are largely sufficient to map the results to biological pathways and to begin to make interpretations of study results. Lipids, however, are special cases among metabolites and require unique handling. This is because the pathways that regulate lipids operate at multiple and distinct levels including, at least, the levels of: intermediary (fatty acids, sterols), complex (triacylglycerol, phospholipids) and aggregate (membranes, lipoproteins) lipid metabolism. Thus, there are more dimensions of relationships among lipid metabolites than are described in a

standard biochemical pathway map, and changes in lipid metabolism occurring at one level of metabolism have an impact on the entire lipid profile. The techniques for measuring and reporting lipids in an interpretable way must therefore be capable of identifying not only the changes but the causes of the change. As an example, one should probably not interpret a global drop in plasma triglyceride levels as an independent decrease in the concentration of hundreds of individual triglyceride species. This talk will identify some of the major challenges facing building a lipidomics platform that yields interpretable results, and provide both real world examples and potential solutions to these problems.

### Targeted Lipidomics Of Signaling Sphingolipids In Health And Disease

E. Berdyshev[(1)]
[(1)]University of Illinois at Chicago, United States of America

Our knowledge about distribution, qualitative and quantitative composition of lipid molecules in natural systems is experiencing a tremendous rise during last fifteen years. This would not be possible without recent progress in analytics of non-volatile lipid molecules. This progress was achieved through the invention of ion sources capable of handling solvent flows of ordinary HPLC systems with a resulting expansion in LC/MS-based methods for direct analysis of complex lipid mixtures without their preliminary chemical processing. In case of signaling sphingolipids, the progress in their analytics was supported by relative stability of analytes and by great attention given to these molecules playing critical role in such fundamental processes as cell survival, proliferation, and death. Great examples of such bioactive sphingolipids are sphingosine-1-phosphate and ceramides often playing opposite role in physiological responses. We are still learning about their physiological functions and deciphering the complexity of their interaction with other signaling systems. Our better understanding of signaling pathways regulated by these sphingolipids now offers novel avenues for the treatment of multiple diseases and pathological conditions including cardiac arrest, pulmonary inflammation and fibrosis, and cancer.

### Lipidomic Analysis of Essential Fatty Acids and their Metabolites in the Fat-1 Transgenic Mouse

J. McKenzie[(1)], G. Astarita[(2)], J. Kang[(3)]
[(1)]Massachusetts General Hospital and Harvard Medical School, United States of America [(2)]Georgetown University, United States of America
[(3)]Discovery and Life Science, Waters Corporation, United States of America

Essential fats, such as omega-3 and omega-6 fatty acids, must be obtained through the diet and cannot be synthesized de novo in mammals. In the modern Western diet, n-6 consumption is dramatically higher than n-3 consumption, and many research studies demonstrate that this imbalance may impact many chronic diseases through differential modulation of inflammation. The fat-1 transgenic mouse model can endogenously convert omega-6 to omega-3 fatty acids to achieve a balanced omega-6/omega-3 tissue ratio. Studies have revealed that the fat-1 mouse is protected against a wide variety of chronic diseases including inflammatory diseases, atherosclerosis, diabetes, and cancer. However, a comprehensive characterization of lipid profiles in the fat-1 mouse using sensitive analytical techniques remains to be explored. In this study, we employed state-of-the-art, high-throughput assays (such as LC-MS/MS) to analyze bioactive lipid species in plasma and liver samples from fat-1 and wild-type mice, and revealed significant differences in lipid metabolites resulting from alteration of the omega-6/omega-3 tissue ratio. Our study has yielded valuable new clues about the underlying pathways and mechanisms involved in the health benefits associated with a balanced omega-6/omega-3 tissue ratio.

### Dietary Fat Metabolism in Humans Using Deuterated Fatty Acids: Perceptions, Realities, Questions

E. Emken[(1)]
[(1)]Midwest Research Consultants, United States of America

Perceptions, realities and questions related to the metabolism and health effects of nearly all the fatty acids in US diets were addressed by a multiple-labeled stable isotope experimental protocol that has significant advantages over single-isotope studies. For example, a series of human studies with $^{2}$H-18:1 positional isomers dispelled allegations that *trans* isomers were poorly absorbed, accumulate in phospholipids, and adversely competed with oleic acid. The reality is that negative physiological properties reported for trans 18:1 isomers are inconsistent with their metabolic properties. The perception that humans were unable to convert 18:3n-3 to 20:5n-3 and 22:6n-3 but can readily converted 18:2n-6 to 20:4n-6 was investigated by feeding subjects a mixture of $^{2}$H-18:3n-3 and $^{2}$H-18:2n-6. The reality was that 18:3n-3 is converted to 20:5n-3 and 22:6n-3 and conversion of 18:2n-6 to 20:4n-6 is not greater than 18:3n-3 to 22:6n-3 conversion. The unresolved question: why is there a large variability between results from various studies and individuals for conversion of 18:3n-3 to 22:6n-3 and 18:2n-6 to 20:4n-6? The belief that interesterified fats may have negative health effects was addressed by dosing subjects with triglycerides labeled with $^{2}$H-16:0 and 2H-18:2n-6 at specific *sn*-1(3) and *sn*-2 positions. The reality is that chylomicron TAG structures are rearranged after absorption and the *sn*-1(3) and *sn*-2 positions of dietary TAG does not influence fatty acid accretion but conversion of 18:2n-6 to 20:4n-6 was influenced. The questions: How is the rearrangement of chylomicron TAG structures accomplished? Why does the acyl TAG position influence conversion but not accretion?

AFTERNOON

**ANA 5: General Analytical**
Chair(s): V. Jain, Bunge, USA; A. Tang, University of California - Davis, USA

**The Direct Determination of Double Bond Positions in Lipid Mixtures by Liquid Chromatography/ In-line Ozonolysis/ Mass Spectrometry (lc/o3-ms).**

C. Sun[(1)], Y. Zhao[(2)], J. Curtis[(3)]
[(1)]University of Alberta, Canada [(2)]University of Alberta, Canada [(3)]University of Alberta, Canada

This presentation will describe the direct determination of double bond positions in unsaturated lipids using a new method for in-line ozonolysis-mass spectrometry (O3-MS). In this experiment, ozone penetrates through the semi-permeable Teflon AF-2400 tubing containing a flow of a solution of fatty acid methyl esters (FAME). Unsaturated FAME are thus oxidized by the ozone and cleaved at the double bond positions. The ozonolysis products then flow directly into the atmospheric pressure photoionization (APPI) source of a mass spectrometer for analysis. Aldehyde products retaining the methyl ester group are indicative of the double bond positions in unsaturated FAME. For the first time, O3-MS is able to couple directly to high performance liquid chromatography (HPLC), making the double bond localization in lipid mixtures possible. LC/O3-MS was applied to a sample of bovine fat. A total of 9 unsaturated FAME including 6 positional isomers were identified unambiguously, without comparison to standards. The in-line ozonolysis reaction apparatus is applicable to most mass spectrometers without instrumental modification; it is also directly compatible with various LC columns. The LC/O3-MS method described here is thus a practical, versatile and easy to use new approach to the direct determination of double bond positions in lipids, even in complex mixtures.

**Odorant Synergy Effects as the Cause of Fishy Malodors in Algal Marine Oils R.T. Marsili and C.R.**

**Laskonis, Marsili Consulting Group, Rockford College, Rockford, IL 61108 USA**

R. Marsili[(1)], C. Laskonis[(2)]
[(1)]Marsili Consulting Group, United States of America [(2)]Marsili Consulting Group, United States of America

While oxidation reactions of DHA and EPA are responsible for fishy off-flavors in marine oils, gas chromatography-olfactometry (GC-O) and other types of analytical studies have failed to reveal which specific oxidation products are involved. The aim of this study is to determine which chemicals cause fishy malodors. Initial GC-MS-O studies of marine oils with fishy malodors revealed numerous oxidation products, but none were characterized as fishy. Samples were analyzed by two dimensional GC-MS. When all sample volatiles were captured and then desorbed simultaneously in GC-O experiments, the fishy malodor was evident, indicating odorant synergy effects were responsible. A simple, novel method was developed using an olfactometry detector as a fraction collector to trap various peaks in marine oil chromatograms. The nose cone of the olfactometry detector was replaced with a PDMS foam absorption tube at various times during GC analysis. Combinations of GC peaks were trapped on PDMS tubes, desorbed in a Gerstel Thermal Extractor (off-line) at 260°C and sniffed. The combination of two analytes were found to cause fishy malodors: heptanal and 3,5-octadien-2-one (isomer undetermined). Purge-and-trap, SPME and headspace stir bar sorptive extraction (HSSE) sample preparation methods prior to GC-MS were investigated. All methods confirmed the combination of heptanal and 3,5-octadien-2-one as the cause of fishy odor.

**A Rapid Method to Determine Sterol, Erythrodiol, and Uvaol Concentrations in Olive Oil**

B. Mathison[(1)], D. Holstege[(2)]
[(1)]United States Military Academy, United States of America [(2)]University of California, Davis, United States of America

A rapid, accurate, and efficient method for determining the sterol, uvaol, and erythrodiol concentrations was developed to meet International Olive Council (IOC) certification criteria for Extra Virgin Olive Oil (EVOO). The unsaponifiable fraction of sample (0.2g) was separated with a diatomaceous earth column, and the sterol and triterpenic dialcohols were isolated with a novel base-activated silica solid phase extraction (SPE) cartridge cleanup protocol. The improved method and the IOC method provided identical pass/fail results (n=34) for each of the six sterol and erythrodiol/uvaol IOC criteria used to assess olive oil. This method was validated and recoveries of stigmasterol (88%) and ?-sitosterol (84%) were greater than previously published values using the IOC method. This method requires approximately one-third the time required to complete the IOC method and has great utility for the rapid screening of EVOO to detect adulteration, false labeling, and inferior product.

**Regiospecific Determination of Conjugated Linoleic Acids (cla) and Trans-vaccenic Acid (va) in Triacylglycerol of Regular and High cla Milk-fat**

D. Zope[(1)], P. Angers[(2)], J. Arul[(3)]
[(1)]Laval University, Canada [(2)]Laval University, Canada [(3)]Laval University, Canada

The beneficial health effects like anti-obesity, anti-atherosclerosis, anti-cancer and immune system modifications, of conjugated isomers of linoleic acid (CLA) have become the focus of an increasing amount of interest. Human body is not able to produce / isomerize CLAs from linoleic acid; but ~25% of trans-vaccenic acid (VA) is converted into CLAs in human body. We analyzed two kinds of dairy fats, differing in their contents of cis9,trans11-conjugated linoleic acid (cis9,trans11-CLA or rumenic acid) - one was commercially available regular milk fat and another was previously prepared by dietary modification of dairy cattle that was enriched in CLAs (2% compared with 0.5-0.8% in regular milk fat) and trans-VA (6% compared with 0.5-1% in regular milk fat) - for fatty acid composition and their

regiospecific distribution by Grignard degradation method by taking into account the response factor and minor acyl migration from secondary to primary positions on the glycerol moiety. The two fats differed mainly with regards to their contents in the palmitic acid, isomers of C18:1, and CLA (cis-9,trans-11). About 75% of trans-vaccenic acid in CLA enriched milk fat is distributed at sn-2 position, while not able to detect in the regular milk fat as it is present in small amount. About 59 to 65% of the total CLAs are located at the sn-1(3) positions in both the fats. The regiospecific distribution indicates that the positions of CLAs are preserved in both types of fats.

### CANCELLED-Microtitration of free fatty acids in oil and biodiesel samples using absorbance and/or fluorescence of pyranine.

S. Fedosov[(1)], J. Brask[(2)], X. Xu[(3)]
[(1)]Dept. of Engineering Science, Aarhus University, Denmark [(2)]Novozymes A/S, Denmark [(3)]Dept. of Engineering Science, Aarhus University, Denmark

### Regiospecific Analysis of Solid Fats by a Novel Enzymatic Method

Y. Watanabe[(1)], T. Nagai[(2)], S. Sato[(3)], C. Sato[(4)], S. Sera[(5)], R. Sato[(6)], T. Aki[(7)]
[(1)]Osaka Municipal Technical Research Institute, Japan [(2)]Tsukishima Foods Industry Co., Ltd, Japan [(3)]Japan Food Research Laboratories, Japan [(4)]The Nisshin OilliO Group, Japan [(5)]The Nisshin OilliO Group, Japan [(6)]Hiroshima University, Japan [(7)]Hiroshima University, Japan

A novel enzymatic method for FA analysis at sn-2 of TAGs has been modified to fit for solid fats. Previously, transesterification of oil was conducted with ethanol (1:10 by weight) at 30oC for 3 h by CalB and specifically accumulated ca. 30 mol% 2-MAGs. The method is applicable to oils containing saturated and unsaturated C4-C24 FAs including PUFAs, and solved the problem of the conventional method using 1,3-specific lipase (Provisional 7_2003. In Standard Methods for the Analysis of Fats, Oils and Related Materials. Japan Oil Chemists Society), which was not applicable to oils with PUFAs and short chain FAs. However, the novel method might not be suitable for solid fats, which required high temperature (>50oC) to liquefy fats to be reacted by lipases. Actually, transesterification at 50oC increased acyl migration and decreased the regiospecificity. In contrast, transesterification at 50oC for 0.2 h followed by 30 oC for 2.8 h liquefied palm oil and minimized the acyl transfer as well. FA analysis of the resulting 2-MAGs gave 16:0, 34%; 18:1, 1%; 18:1, 64%; 18:2, 20%, which was in a good agreement with that reported in the literature. The modified method was also applicable to other solid fats, such as sheabutter and DHA rich oil from Aurantiochytrium sp., and is currently under the evaluation of collaborative studies by the technical committee by JOCS.

## ANA 5.1/S&D 5: Emerging Test Methods for Surfactants and Detergents

Chair(s): M. Tsumadori, Kao Corp., Japan; H. Li, Bruker Optics Inc., USA

### Characterization of Surfactant Iron Oxide Nanoparticle Interactions Using Isothermal Titration Calorimetry (itc)

Z. Wang[(1)], S. Xu[(2)], E. Acosta[(3)]
[(1)]University of Toronto, Canada [(2)]University of Toronto, Canada [(3)]University of Toronto, Canada

Formation of stable nanoparticle suspensions is important for their potential use in cosmetic, pharmaceutical, environmental, and medical applications. One method to produce stable aqueous nanoparticle suspensions is to adsorb surfactants on the surface of the nanoparticle to create a steric and electrostatic barrier against particle aggregation. However, depending on the structure of the surfactant tail, the charge of the surfactant head group, and the binding of the surfactant to the surface of the particle, one obtains substantially different stabilities. Therefore, a better understanding of surfactant-nanoparticle binding is necessary to design stable suspensions. In this work, the binding between sodium oleate, sodium laurate, sodium dodecyl sulfate, and sodium dodecyl phosphonate and iron oxide nanoparticles was systematically investigated using isothermal titration calorimetry (ITC). Comparing the ITC results and the adsorption isotherm obtained for these systems, in the cases of sodium oleate and dodecyl phosphonate, a strong chemical binding ? beyond a simple physisorption ? takes place in the presence of low surfactant concentrations. However, the formation of higher order structures (e.g. surfactant bilayers) cannot be accurately reflected in the ITC experiments. Those surfactants that exhibited strong chemical binding also produced the more stable suspensions.

**Using Inflection Points in Surfactant Blend Properties as a Guide to System Synergies**

R. Theiner[(1)], R. Bennett[(2)]
[(1)]Air Products and Chemicals, Inc., United States of America [(2)]Air Products and Chemicals, Inc., United States of America

Neat surfactant behavior is well understood thanks to established models, but blended systems continue to yield synergies that are sometimes unexpected. A process of examining deviation from the anticipated model may be used to simplify the search for such synergies. This paper presents determined critical micelle concentrations for blends of lauryldimethylamine oxide with a common linear alcohol ethoxylate and shows how inflection points outside of linear behavior reveal performance synergies. Although this study is provided as an example, the technique is particularly highlighted.

**Quantification of oil extraction from microalgae using conventional solvents and microemulsions**

E. Acosta[(1)], J. Chan[(2)], R. Xu[(3)], L. Diosady[(4)]
[(1)]University of Toronto, Department of Chemical Eng. and Appl. Chemistry, Canada [(2)]University of Toronto, Canada [(3)]University of Toronto, Canada [(4)]University of Toronto, Canada

This work describes the process of extraction of lipids from Scenedesmus obliquus microalgae using hexane (a conventional solvent) and three lecithin-linker microemulsions, one continuous in water (Type I), one continuous in the oil phase (Type II) and one bicontinuous in oil and water (Type IV). The fraction of lipids was determined by measuring the lipid content in lyophilized microalgae before and after extraction. To this end, the Folch?s chloroform/methanol extraction method was applied in both cases, and the extracted lipid underwent transesterification followed by GC chromatography to quantify the fatty acid methyl esters (FAMEs) in the sample. We will describe our initial challenges in producing a reliable measurement of the oil content in the microalgae as well as the adjustments we needed to introduce in the Folch extraction methodology. Since the Folch method did not capture the extraction of carotenoids from the microalgae, we adapted a liquid chromatography method to determine the extraction of carotenoids from the samples. We will discuss the relative extraction efficiency for lipids and carotenoids of the solvents explored and the potential advantages and disadvantages of those extraction methods.

**Use of acoustic spectrometry to determine drop size distribution of water-in-bitumen emulsions**

E. Acosta[(1)], W. Ren[(2)], S. Ng[(3)]
[(1)]University of Toronto, Department of Chemical Eng. and Appl. Chemistry, Canada [(2)]University of Toronto, Canada [(3)]Syncrude Canada Ltd., Canada

One of the biggest issues when trying to characterize emulsions and dispersions is that most of the technologies available require taking a sample of the suspension or emulsion and placing it in a microscope or diluting it for laser scattering techniques. These procedures may affect the morphology of the emulsion or the dispersion. The advantage of acoustic spectroscopy is that it can be used in large concentrated samples, thus minimizing sampling issues. The first half of this presentation will review the principles of acoustic spectroscopy and its use in characterizing emulsions and suspensions. The second half will describe literature examples on the use of this technique as well as our own experience with this technique for analyzing coexisting emulsions and microemulsions, samples of bitumen emulsions and other turbid emulsions. We will describe the potentially powerful use of the technique to access information that is not available from other techniques, but the importance of running controls and complementary techniques to chose the right model to analyze the sound absorption spectra, which is the primary set of data obtained from the instrument.

## Analytical Poster Session

Chair(s): K. Ma, Cognis Corp., USA

### Chemistry and Utilisation of Madhuca Insignis (radlk.) H.j. lam (sapotaceae), Lesser Known new Tree Borne Oilseeds of Western Ghats, India

M. Srinivasa[(1)]
[(1)]Institute of Wood Science & Technology, Malleshwaram, Bangalore,India, India

Inadequate oilseed production and increased demand for oil in India due to rise in population has necessitated the investigation of more and more tree species to meet the internal consumption. Madhuca insignis (Radlk.) H.J. Lam which bears edible fruits is an evergreen tree growing in riverine soils in coastal Karnataka of Western Ghats in India. So far no chemical work has been carried out on this species with regards to fruit value or the oil content in seeds. This paper deals with the oil content, fatty acid composition and utilization of the species. Its seeds contain 42.5% fatty oil on kernel weight basis. The seed weight is 0.42 gm or 1.0 kg contains 2300 number of seeds with seed coat. Fruit to seed ratio is 3:1. Analysis of the fatty acid present in the fatty oil revealed the presence two major unsaturated fatty acids namely Oleic acid (40.8%) and Linoleic acid (18.0 %), two saturated fatty acids namely Palmitic acid (23.6%) and Stearic acid (12.64 %) and other minor acids (~ 1% each). Physico-chemical properties of the oil showed Acid value- 9.43, Saponification value- 299.4, RI- 1.4635, Iodine value 59.2, Peroxide value 4.0 and Specific gravity-0.9116 which compares well with edible oils. Local people use the oil for culinary preparations. High oleic acid content makes it good oil for heart patients.

### Secretory Phospholipases A2(spla2s)release Free F2-isoprostanes From Lipoprotein Phospholipids

A. Kuksis[(1)], W. Pruzanski[(2)]
[(1)]University of Toronto, Canada [(2)]University of Toronto, Canada

The objective of the study was the demonstration of hydrolysis of F2-isoprostanes of plasma lipoproteins by group IIA, V and X secretory phospholipases (sPLA2). Auto-oxidized preparations of high (HDL) and low (LDL) density lipoproteins containing 10-100 nanomoles/g protein of isomeric phospholipid-bound F2-isoprostanes were used. Total HDL, HDL3 and LDL were digested separately using varying substrate/enzyme ratios. Digestions were performed for 1-4 h at 37 oC in a total volume of 1 ml Tris/HCl buffer, pH 7.5, containing 10 mM CaCl2 and 0.01% BSA, using 0.1-2.5 µg sPLA2/mg protein. The reactions were stopped by adding the extracting solvents (CHCl3/MeOH, 2:1 v/v). Normal-phase LC/ESI-MS analyses were performed as previously described. It was shown that PtdCho isoprostanes in HDL and LDL were completely hydrolysed in 1 h by group V and X human sPLA2s using 1 µg sPLA2/mg protein. In

contrast, group IIA sPLA2 required 4 h and 2.5 µg enzyme/mg protein for a comparable hydrolysis. It is concluded that human sPLA2s, especially those of groups V and X, posses effective F2-isoprostane releasing capability comparable to or greater than the PAF acetylhydrolases, which until now have been considered the major phospholipases involved in the release of esterified isoprostanes.

**PTAD (4-phenyl-1,2,4-triazoline-3,5-dione) as a Novel Reagent in the GC-MS Identification of Conjugated Fatty Acid Positional Isomerism**

U. Shah[(1)], A. Proctor[(2)], J. Lay[(3)]

[(1)]University of Arkansas, United States of America [(2)]University of Arkansas, United States of America [(3)]University of Arkansas, United States of America

The objective of this study was to find a rapid, simple means of determining positional isomerism of trans,trans CLA isomers in CLA-rich oil. Positional isomer determination in conjugated diene fatty acids by MS is vital in bioactive compounds. However, the mass spectra of underivatized diene fatty acids are often unreliable due to the migration of the double bonds during MS analysis. The reagent 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) is used to form stable conjugated fatty acid Diels-Alder reaction products because it produces larger mass fragments for diagnosing the conjugated diene position by MS analysis than carboxyl derivatives. However, MTAD is expensive, not readily available and undesirable due to toxic intermediates formed during its synthesis. An alternative Diels-Alder reagent, phenyl derivative, 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) was investigated as a safe, readily available and cheaper reagent alternative to MTAD. CLA-rich oil FAMEs were reacted with PTAD reagent and analyzed by GC-MS. The PTAD-CLA adducts were readily volatilized at 325 oC. The total ion chromatogram showed only two PTAD-CLA adduct peaks (9,11 and 10,12 CLA positional isomers) in CLA-rich oil, without prior purification of the crude FAMEs preparation. This method could be applied for isomer identification of all oils with conjugated dienes as FAMEs.

**Pulp oil of Acrocomia Aculeata: the Variability in Fruits From Pantanal and Cerrados Biomes (Brazil)**

S. Favaro[(1)], G. Ciconini[(2)], C. Miranda[(3)], C. Souza[(4)], L. Martins[(5)]

[(1)]Brazilian Agricultural Research Corporation (EMBRAPA), Brazil [(2)]Catholic University Dom Bosco, Brazil [(3)]Brazilian Agricultural Research Corporation (EMBRAPA), Brazil [(4)]Catholic University Dom Bosco, Brazil [(5)]Catholic University Dom Bosco, United States of America

The aim of this study was to investigate the variability of physical chemical characteristics and carotenoid content of fruit pulp oil of macauba from the Cerrado and Pantanal biomes in the of state of Mato Grosso do Sul (Brazil), to recollect information for the future establishment of commercial crops, breeding programs and industrial uses. Oil obtained from macauba fruit pulp showed excellent physical and chemical quality. Free fatty acid content was lower than 1%, and no peroxides were detected. Iodine values indicates a high degree of unsaturation in the oil. Total carotenoids showed a great variability, had a positive correlation with raw oil red color, and reached a maximum of 739.15 ?g/g in plants from the Aquidauana region. Plants from this region also showed the highest concentration of ?-carotene and retinol equivalent. It is concluded that around 1 ½ spoons (soup) of macauba pulp oil may supply A vitamin RDI of for adults.

**Rapid Lipid Extraction From egg Yolks**

S. Shinn[(1)], A. Proctor[(2)]

[(1)]University of Arkansas Dept of Food Science, United States of America [(2)]University of Arkansas, United States of America

The objective of this study was to develop a rapid chloroform-free egg yolk lipid extraction method, that would obtain the same amount of lipid as the classical Folch method and have the same fatty acid profile. Six egg yolks were combined and yolk samples were diluted with distilled water to obtain 100%, 75%, 50% and 25% dilutions. Duplicate extractions of each dilution were made by both the Folch method and a 5 min extraction with hexane/isopropanol (1:1, v/v), using a solvent/sample ratio of 20:1, v/v. GC-FID FAMEs fatty acids analysis were performed in duplicate. The correlation coefficient (R2) between the two extraction methods was 0.997 with a gradient of 1. There was no significant differences in the fatty acid content of extracted obtained by Folch and the rapid hexane/isopropanol extraction method as shown by one-way ANOVA. The rapid extraction method was an effective alternative to the Folch method producing a similar extraction yields and fatty acid profile of the extract.

**Discrimination of Geographic Origin of Asian Sesame Oils by Carbon, Hydrogen, and Oxygen Stable Isotope Analyses**

H. Jeon[(1)], S. Lee[(2)], Y. Cho[(3)], J. Oh[(4)], K. Kwon[(5)], B. Kim[(6)]

[(1)]Chung-Ang University, Korea, Republic of [(2)]Korea Apicultural Association, Korea, Republic of [(3)]Korea Food and Drug Administration, Korea, Republic of [(4)]Korea Food and Drug Administration, Korea, Republic of [(5)]Busan Regional Korea Food and Drug Administration, Korea, Republic of [(6)]Chung-Ang University, Korea, Republic of

The aims of this study were to investigate the effects of geographic location and climatic characteristics of the sesame producing sites on the carbon, hydrogen, and oxygen stable isotope ratios of Korean sesame oil and to differentiate Korean sesame oil from Chinese and Indian sesame oils that are distributed in Korea using isotopic data in combination with canonical discriminant analysis. The isotopic data were obtained from 84 roasted oil samples that were prepared from 51 Korean, 19 Chinese, and 14 Indian sesame seeds harvested during 2010 and 2011. The ?13C, ?D and ?18O values of Korean sesame oil were significantly ($p < 0.01$) negatively correlated with latitude, distance from the sea, and precipitation (May-September), respectively. A good discrimination between sesame oils from Korea, China, and India was achieved by applying two canonical discriminant functions, with 89.3% of the samples correctly classified into the geographic origin.

**Discrimination of Origin of Sesame Oils Using Fatty Acid and Lignan Profiles in Combination With Canonical Discriminant Analysis**

H. Jeon[(1)], I. Kim[(2)], H. Choi[(3)], C. Lee[(4)], C. Akoh[(5)], B. Kim[(6)]

[(1)]Chung-Ang University, Korea, Republic of [(2)]Korea University, Korea, Republic of [(3)]Korea Food Research Institute, Korea, Republic of [(4)]Chung-Ang University, Korea, Republic of [(5)]The University of Georgia, United States of America [(6)]Chung-Ang University, Korea, Republic of

The aims of this study were to investigate total fatty acid composition and lignan contents of Korean, Chinese and Indian roasted sesame oils and to differentiate the geographic origins of the oils using analytical data in combination with canonical discriminant analysis. The analytical data were obtained from 84 oil samples that were prepared from 51 Korean, 19 Chinese, and 14 Indian white sesame seeds harvested during 2010 and 2011 and distributed in Korea during the same period. Six variables selected for the discriminant analysis were the contents of three fatty acids (linoleic, oleic, and palmitic) and three lignans (sesamin, sesamolin, and sesamol). A good discrimination between sesame oils from Korea, China, and India was achieved by applying two canonical discriminant functions, with 97.6 % of the samples correctly classified into the geographic origin. When the origins of five commercial oil samples (one was prepared from Korean sesame seeds and the other four were made from imported sesame seeds) were predicted using discriminant functions, the Korean sesame oil was accurately distinguished from the others.

**Edible oil Adulteration Testing by Ft-nir**

C. Heil[(1)], D. Drapcho[(2)], H. He[(3)]

[(1)]Thermo Fisher Scientific, United States of America [(2)]Thermo Fisher Scientific, United States of America [(3)]Thermo Fisher Scientific, United States of America

Intentional adulteration of pure edible oils by lower quality oils is an economically advantageous practice. These adulterated oils are a threat to product authenticity and in some cases a serious consumer health issue. The melamine in baby formula discovery in 2008 brings to light the serious health consequences of adulterants in food products. Companies that utilize edible oils are taking a significant financial liability if they cannot positively authenticate the purity of oils that they are using in the manufacturing of food products. For successful authenticate testing, food companies need a fast and rugged analytical technique with a high degree of sensitivity. Also the technique must be rugged enough for operates to use in a production plant receiving environments. FT-NIR is an ideal analysis technique that meets food companies needs for analysis in the matter of seconds and ease of use without sacrificing the sensitivity needed to discriminate between pure versus slightly adulterated oil samples. FT-NIR also has the flexibility to non-destructively analyze bulk samples in their original container without sample preparation.

**CANCELLED-Acid Number Determination of Vegetable Oils by Different Titration Methods**

F. Ribeiro Bürgel[(1)], A. dos Santos Vieira[(2)], C. de Mello Alves Portella[(3)], M. Jorge Vinhoza[(4)], S. Cabral de Menezes[(5)]

[(1)]PUC-Rio, Brazil [(2)]PUC-Rio, Brazil [(3)]PUC-Rio, Brazil [(4)]PUC-Rio, Brazil [(5)]Petrobras, Brazil

Acid number determination is an important issue in oil quality assessment, as it is related to its functionality and processability. Usually this determination is carried out by titration techniques where the equivalent point can be determined by using different techniques and solvents. The ASTM D664 standard Test Method is a known method that covers procedures to determine acidic constituents in petroleum products, lubricants, biodiesel and blends of biodiesel by potentiometric titration. The main aim of this study is to investigate alternative methods to measure acidity, other than the adopted and compare the results for different vegetable oils trying to evaluate their advantages and precision. Two typical methods for acid number determination in vegetable oils were chosen to be used as reference, the AOCS Cd 3d-63 and the EN ISO 660, which are based on colorimetric and potentiometric titration techniques respectively. The matrices studied were refined soybean and canola oil, carried out through seven replicates for each sample, for each method. Statistical tests were applied to the data and the values obtained compared.

**Quantitative Comparison of Direct in Situ Transesterification of Plasma, Red Blood Cells and Brain Tissues With and Without Prior Lipid Isolations**

K. Ramirez[(1)], K. Wynalda Camozzi[(2)]

[(1)]DSM Nutritional Products, United States of America [(2)]DSM, United States of America

The analysis of fatty acids as components of animal tissue is important in studying lipid metabolism. Analysis of mammalian tissues has historically used a liquid-liquid extraction such as a Bligh and Dyer (B/D) or Folch, followed by transesterification using various methods (e.g. boron trifluoride, acid/ base alcohol or other specialized derivatizations). These techniques are labor intensive and require large volumes of toxic solvents. Direct transesterification of total lipids within tissues, using acidic methanol would alleviate time and cost of analysis therefore, this study was undertaken to investigate any differences between direct transesterification (methanolic HCl) of samples or liquid-liquid extraction (B/D or Folch) followed by transesterification. Results of the study found that the best sample preparations for direct transesterification analysis were direct aliquots of red blood cell suspension, evaporation of plasma under nitrogen, and lyophilized brain tissue. For liquid-liquid extraction prior to transesterification; plasma and red blood cells were aliquoted directly from suspension, while, brain tissue was lyophilized. Fatty acid methyl esters were quantitated by gas chromatography with flame ionization detection (GC/FID). Results of the analysis found no major differences in fatty acid levels for matrices extracted by B/D or Folch before transesterification, as compared to direct transesterification of tissues, allowing more efficient and safer fatty acid analysis of tissues.

**Authentication and Quality Control and of Vegetable oil by the Heracles E-nose System**

L. Towa[(1)], C. Schneider[(2)], J. Shea[(3)], J. Mifsud[(4)]

[(1)]Alpha MOS, United States of America [(2)]Alpha MOS, United States of America [(3)]Alpha MOS, United States of America [(4)]Alpha MOS, United States of America

Olive oil is known to be a high grade vegetable oil mainly produced in Mediterranean countries and worldwide consumed because of its composition, nutraceutical and sensory attributes. To satisfy the high demand of the consumer, olive oil is subjected to a well-known phenomenon of adulteration. Currently, the quality control of olive oil is limited to the standard analytical or sensory techniques that are revealed to be expensive and time consuming. Thee Heracles E-nose GC system is a rapid, affordable and complementary technique that can help with authentication, regulation and quality control of olive oil.

**Determination of Cooking oil Adulteration by Principal Component Analysis With HPLC - Charged Aerosol Detector Data**

M. Plante[(1)], D. Thomas[(2)], B. Bailey[(3)], I. Acworth[(4)]

[(1)]Thermo Fisher Scientific, United States of America [(2)]Thermo Fisher Scientific, United States of America [(3)]Thermo Fisher Scientific, United States of America [(4)]Thermo Fisher Scientific, United States of America

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 of different olive oil samples.

**Characterization of Used Cooking Oils by Hplc-ms and Corona Charged Aerosol Detection**

M. Plante[(1)], D. Thomas[(2)], B. Bailey[(3)], I. Acworth[(4)]

[(1)]Thermo Fisher Scientific, United States of America [(2)]Thermo Fisher Scientific, United States of America [(3)]Thermo Fisher Scientific, United States of America [(4)]Thermo Fisher Scientific, United States of America

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 oil were characterized by high performance liquid chromatography, using charged aerosol detector to provide details about the quality of the oil, and mass spectrometry for identification of some analytes. Oils were characterized using both a universal lipids method and a free fatty acid method for hydrolyzed cooking oils. The use of HPLC-charged aerosol detection methods provided a facile means of determining cooking oil quality, without the need for sample derivatization and the possible loss of non-derivatized analytes for gas chromatography.

**Mathematical Relationship Between IV & RI for vegetable Oil.**

S. Mukhopadhyay[(1)]

[(1)]Emami Biotech Limited., India

There is a farm relation ship of every individual vegetable oil's IV and its corresponding RI and vise a varsa. Individual oil's Iv range and RI has been correlated mathematically by me and the estimated results are very very closely matching with analyzed value.As an Example : IV range of Coconut oil is 7.5 to 10.0 and RI of it in the range 1.4481 to 1.4491 at 40.0 Deg C. then for every number IV change 0.000400 number RI will be changed and for 1.0 number RI change 2500.00000 number IV will be changed for coconut oil and this changeable numbers of IV and RI is related to the corresponding oil's IV and RI range .As per coconut oil from individual oil's IV and RI range this single number change base factor can be evolved ( i have already developed the chart of related factors.Those are as follows - 1 ) Cotton seed oil - for 1 number IV change RI change will be 0.000214 and for 1.0 unit RI change IV change will be 4666.666667. Ground nut Oil- 1 number IV change = RI change will be 0.00014286 and i number RTI change IV change will be 7000.000000 .In this way for by RI estimation the estimated IV can be established for a individual oil by considering the following equation - Lower IV from the standard range of any oil +((measured RI - Lower Range of RI for the Oil )* corresponding IV change factor for one number RI change ) .

**Fatty acid profile and minor lipid components in the oil of some selected germplasms of Lepidium campestre**

S. Madawala[(1)], M. Geleta[(2)], L. Zhu[(3)], P. Dutta[(4)]

[(1)]Swedish University of Agricultural Sciences (SLU), Sweden [(2)]Swedish University of Agricultural Sciences, Sweden [(3)]Swedish University of [(4)]

Agricultural Sciences, Sweden  Swedish University of Agricultural Sciences, Sweden
Lepidium campestre, commonly known as field cress, is a wild species. Owing to its superior agronomic characteristics, L. campestre has been selected as a suitable candidate for domestication into a commercial oilseed crop which can thrive in the cold Nordic climate. As a part of a large national multidisciplinary program Mistra-Biotech, this study is focused on evaluation of oil content, fatty acid profile, tocopherols (TP) and phytosterols in some selected germplasms of L. campestre. The preliminary results showed that the total oil content of the seeds varied from18-27%, while the fatty acid profiles were rather similar among the germplasms. The average total SFA, MUFA and PUFA were 9%, 41% and 47%, respectively. The major fatty acid was linolenic acid (35-37%), followed by erucic acid (21-25%), oleic acid (9-12%) and linoleic acid (8-10%). The main TP observed was ?-TP ranging from 462-1308 µg/g oil, while ?-TP varied from 36-66µg/g oil. Trace amounts of ?-TP were observed only in few germplasms. Total sterol content varied from 7623-9749 µg/g oil with ?-sitosterol (36%) being dominated, followed by campesterol (28%), cholesterol (10%), delta-5 avenasterol (7%) and brassicasterol (4%).

**Shielding Effects of Porous Glass Beads technology in preventing the association of additives in foods.**

n. navarro[(1)]

[(1)]nueva ecija university of science and technology, Philippines
Stereochemical shielding capacity of porous glass beads was evaluated using analytical techniques. the test procedures includes spectrophotometric analysis and chemical test for oxidative value and X-ray diffraction. the study reveals that the additive BHA has been prevented form entering the final food product while keeping its additive capacity functioning.

**Effect of temperature and usage of frying process on the fatty acids profile of partially hydrogenated vegetable oil (Vanaspati)**

K. Anwar[(1)], V. Kardam[(2)], K. Pant[(3)]

[(1)]Indian Institute of Delhi (IIT), New Delhi, India, India [(2)]Indian Institute of Technology (IIT), New Delhi, India, India [(3)]Indian Institute of Technology (IIT), New Delhi, India, India
Prolong heating and reuse of frying oils has been considered the source of trans fatty acids (TFA). However, the type of oil used for cooking play an important role in trans-fat accumulation in food. To investigate the formations of TFA during frying, vanaspati was used for deep fat frying potato chips at 180° C and 220° C on two consecutive days for 6 hrs each. Analysis of fatty acids was carried out by gas-liquid chromatography with flame ionisation detector. The fatty acid methyl esters (FAMEs) were prepared according to AOAC Official Method 969.33 using BF3 as catalyst. The fatty acids composition showed that the control sample (without frying) contained 14.0% TFA. Frying performed at 180° C for 6 hrs increased the trans fat level to 15.50% which again increased to 16.48% on reusing the same oil for frying at same temperature. Similar but more TFA was formed at 220° C. Frying performed for 6hrs and 12hrs at 220° C changes the TFA content from 14 to 17.94% and 18.82% respectively. Elaidic acid (C:18 9t) was the most abundant trans fat found in all the samples. There was an apparent increased in saturated fat content was noticed at both the frying temperature. Experimental results suggested the high trans fat in vanaspati which kept on increasing due to temperature and duration of frying process.

**Quantification of triacylglycerols in vegetable oils by easy ambient sonic-spray ionization mass spectrometry technique.**

G. Fernandes[(1)], A. Fernandes[(2)], R. Simas[(3)], M. Eberlin[(4)], R. Alberici[(5)], D. Barrera-Arellano[(6)]

[(1)]UNICAMP, Brazil [(2)]UNICAMP, Brazil [(3)]UNICAMP, Brazil [(4)]UNICAMP, Brazil [(5)]UNICAMP, Brazil [(6)]UNICAMP, Brazil
Quantification of triacylglycerols (TAG) is one of the most important parameters for fats and oils characterization. Traditionally, TAG analysis has relied on chromatographic separation. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) technique, which does not require sample preparation and chromatographic separation, has demonstrated to provide the simple, fast, and reliable qualitative analysis of TAG. This study aimed to quantitate TAG in vegetable oils and fats using EASI-MS. Olive Oil (OO), Hidrogenated Soybean Oil (HSO), Hidrogenated Palm Oil (HPO) and Coccoa Butter (CB) were analyzed by both EASI(+)-MS, using a single-quadrupole MS and a homemade EASI source, and direct GC-FID. In EASI(+)-MS spectra, the ions were observed in their [TAG+Na]+ forms. For the

OO sample, the most abundant TAG observed in the EASI(+)-MS spectrum are the ions of m/z 907 (OOO,37.8%) and m/z 881 (POO and/or PSL, 16.1%). CB displays mainly ions of m/z 881 (POO and/or PSL,24.7%) and m/z 883 (POS, 19.9%). HSO sample, as consequence of the hydrogenation process, shows ions of m/z 913 (SSS, 67.5%) and m/z 885 (PSS, 27.9%). Similarly, HPO displays ions of m/z 857 (PPS, 50.3%) and m/z 885 (PSS, 39.4%). Correlation coefficients for TAG composition obtained by GC-FID and EASI-MS were quite high (R> 0.90) for OO, HSO and HPO. EASI-MS seems to offer a promising technique for TAG quantitation with no sample preparation and/or chromatographic separation.

**The Effect of Different Cold Storage Conditions on Extra Virgin Olive Oil**

X. LI[(1)], S. Wang[(2)], C. Shoemaker[(3)], H. Zhu[(4)]

[(1)]University of California, Davis, United States of America [(2)]University of California, Davis, United States of America [(3)]University of California, Davis, United States of America [(4)]University of California, Davis, United States of America

Storage conditions can affect the stability and quality of extra virgin olive oil (EVOO). While many studies have been done on EVOO storage relating to high temperature and light exposure, little is known on the effect of the cold storage. The aim of this study is to evaluate the effect of different storage conditions (room temperature at 78F, refrigerated temperature at 35F and freezer temperature at -20F) on the quality of EVOO; to study the impact of free fatty acids, phenolics and other compounds on hydrolysis and oxidation during cold storage; and to determine if cold storage will retard hydrolysis and oxidation and hence elongate shelf-life. Oil samples were stored under different conditions and tested every week. The AOCS official methods were applied to determine the basic physical-chemical parameters including free fatty acids, peroxide value and ultraviolet absorption. Natural antioxidants such as ?-tocopherol and phenolic compounds were also measured. Due to the important health-conferring and sensory properties of phenolic compounds, this study focused on the change of main phenolic compounds with anti-oxidative abilities such as tyrosol, hydroxytyrosol during storage. A new method was developed to fractionate the phenolics from EVOO by SPE (Diol, 6ml) followed with phenolics characterization/quantification under Ultra Performance Liquid Chromatography ? Diode Array Detector (UPLC-DAD) with less solvent consumption and shorter total run time (15min). In addition, 1,2-diacylglycerols (DAGs) and pyropheophytins (PPP) were measured to better indicate the oxidation and thermal degradation level during storage. Statistical analysis confirmed the significant differentiation among different storage conditions.

**Chlorinated acyloxonium ions originating prior to or subsequent to McLafferty rearrangement as characteristic markers of chloropropanol esters under electron impact conditions**

A. Rahn[(1)], V. Yaylayan[(2)]

[(1)]McGill University, Canada [(2)]McGill University, United States of America

Chloropropanol esters (CP) are part of an emerging group of thermally generated toxicants that are considered as potential health hazard in foods rich in palm oil. They originate mainly during the deodorization stage, requiring high temperatures and a chlorine source. Currently, indirect methods of detection of CP esters are used due to the limited availability of the required standards. Therefore, the objective of this research was to identify characteristic electron impact ion fragmentation of CP esters to be used as diagnostic ions. Investigations carried out using a large number of CP esters have indicated that all samples studied generated masses consistent with acyl oxonium ion formation occurring prior or subsequent to McLafferty rearrangement. Although, McLafferty rearrangement products as well as acyloxonium ions are commonly encountered in the EI mass spectra of all lipids, however, the corresponding ions generated from CP esters can be considered characteristic to this group of contaminants due to retention of chlorine atom. All CP diesters and monoesters generated a peak at m/z 135.5 as a post-McLafferty rearrangement oxonium ion in addition to the ion series at m/z (120.2 + R) where R indicates the fatty acid side chain as pre-McLafferty rearrangement oxonium ions. For example ion at m/z 331.2 originating from 3-MCPD diesters prior to McLafferty rearrangement could serve as specific marker for the presence of chloropropanol esters containing palmitic acid(s) whereas the ion at m/z 135.5 generated through post-McLafferty rearrangement could serve as a generic marker for the presence of any CP ester.

**On GC behaviour of 18:4n-6**

M. Vyssotski[(1)], K. Lagutin[(2)], Y. Itabashi[(3)]

[(1)]Industrial Research Limited, New Zealand [(2)]Industrial Research Limited, New Zealand [(3)]Hokkaido University, Japan

Gamma-Stearidonic acid, 18:4n-6, a potential product of beta-oxidation of arachidonic acid, was only recently reported for a living organism - a thermophilic cyanobacterium Tolypothrix sp., albeit at low levels (?ezanka et al. 2012), while some indirect evidence suggests its wider presence, e.g. in a unicellular marine alga (Ackman et al. 1974). We have prepared 18:4n-6 using an iodolactonisation chain-shortening approach (Vysotskii et al. 1990) from 22:5n-6 and obtained its 1H-, 13C-, COSY- and HSQC NMR spectra. The GC and GCMS behaviour of its methyl ester and DMOX derivatives were also studied. Like another Delta-3 polyunsaturated acid, octadecapentaenoic (18:5n-3), 18:4n-6 rapidly yields 2-trans isomer upon formation of DMOX derivative. On a polar ionic liquid phase (SLB-IL100, 200 C) the methyl ester could be mistaken for 18:3n-3, while on methylsilicone phase (BP1, 210 C) it eluted ahead of 18:3n-6 and 18:4n-3, suggesting that when present it may be easily misidentified during GC analysis of fatty acids. References: Ackman, R.G. et al. (1974) Chromatographia 7: 107-114 ?ezanka T. et al. (2012) Phytochemistry 78: 147-155 Vysotskii, M.V. et al. (1990) Tetrahedron Lett. 31: 4367-4370

**An Automated Sample Preparation System for the analysis of fatty acid methyl esters (FAME) in edible oils**

R. Juskelis[(1)], J. Cappozzo[(2)], P. Wylie[(3)], P. Mrozinski[(4)]

[(1)]IFSH, United States of America [(2)]IFSH, United States of America [(3)]Agilent Technologies, United States of America [(4)]Agilent Technologies, United States of America

The characterization and determination of FAMEs allows obtaining direct information regarding the fat composition of commercial extra virgin olive oil and other edible oils. The distribution of fatty acids, provides a unique fingerprint for given edible oil, and shows an index of quality in order to protect against adulteration. This study demonstrates the use of the Agilent 7696A Sample Prep WorkBench for derivatization and subsequent determination of FA from extra virgin olive oil (EVOO) and adulterated EVOO with other edible oils. Fatty acid methyl esters are analyzed on GC-MS/FID instrument equipped with a highly polar HP-88 cyanopropyl columns. Principle component analysis (PCA) was used for the FA data interpretation of edible oils and spiked EVOO?s. This study demonstrates an efficient and economical GC/MS /FID method for determination of fatty acids in edible oils. The Agilent 7696A Sample Prep WorkBench allows for consistent timing and temperature of a critical derivatization process. Automated sample preparation shows significant reduction of solvent use (by 17 fold), consumables, and generated waste, frees analyst time and reduces exposure to hazardous chemicals.

**MALDI-TOF MS Characterization of Reaction Products and Degradants Related to Biodiesel and Associated Materials**

T. McGinnis[(1)], A. Leigh[(2)]

[(1)]Nalco Ecolab, United States of America [(2)]Nalco Ecolab, United States of America

Matrix Assisted Laser Desorption and Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS)has been used to characterize biodiesel (fatty acid methyl esters) and related materials. These include neat FAME, sediments formed during storage of FAME/diesel fuel mixtures,and triglyceride starting materials. Also examined were FAME based mixtures which underwent physical and chemical stresses in process applications. This work shows that biodiesel and related materials can undergo chemical degradation resulting in smaller molecules, as well as participate in addition reactions which produce high molecular weight species. The conclusions of this work give insight into the types of chemical species which can form due to oxidation and hydrolysis, which then in turn can lead to a number of serious problems encountered by the end user of biodiesel related materials.

**An Isotope Dilution-Gas Chromatography-Negative Chemical Ionization-Mass Spectrometry Method for the Analysis of Trans-Fatty Acids in Human Plasma**

H. Kuiper[(1)], T. Frame[(2)], A. Ribera[(3)], H. Vesper[(4)]

[(1)]Centers for Disease Control and Prevention, United States of America [(2)]Centers for Disease Control and Prevention, United States of America [(3)]Centers for Disease Control and Prevention, United States of America [(4)]Centers for Disease Control and Prevention, United States of America

Trans-fatty acids (TFA) are positional isomers of naturally occurring cis-fatty acids and can be formed industrially via

partial hydrogenation of vegetable oils or naturally in ruminant animals, altering the physical properties as well as the biological effects of the fatty acids (FA). TFA intake has been associated with risk factors for cardiovascular disease. However, little is known about the TFA levels in humans. The low proportion of TFA to total FA in most specimens requires a sensitive analytical method with a large dynamic range. With the complexity of biological samples, including many positional and geometric FA isomers, the analytical method must also be highly specific. We have developed and validated an isotope dilution-gas chromatography-negative chemical ionization-mass spectrometry method that enables the assessment of major TFA and other FA in 100 microliters of human plasma at levels as low as 0.05 micromolar. This method allows for the investigation of FA concentrations as well as differences in the composition of these FA in plasma. The use of stable isotope labeled internal standards enables the accurate quantitation of 28 fatty acids including the 4 major TFA. We have also been able to qualitatively analyze 63 additional FA using this method. Overall, this method allows for sensitive and specific determination of FA concentration as well as the differences in composition of these FA in human plasma, making it suitable for biomonitoring studies. This method is currently being used to measure samples from the National Health and Nutrition Examination Survey (NHANES).

**Geographical Origin Characterization of Olive Oil using ICPMS and Mass Profiler Professional Software**

J. Nelson[(1)], R. Juskelis[(2)], J. Cappozzo[(3)]

[(1)]Agilent, United States of America [(2)]IFSH, United States of America [(3)]IFSH/IIT, United States of America

For this study, Olive Oils from multiple countries were analyzed using the Agilent 7700x ICP-MS for elemental characterization and then subjected to Mass Profiler Professional (MPP) software for statistical geographic origin information. Over 30 elements were determined in over 50 olive oil samples. The QuickScan option in the MassHunter software was also used to get a full scan of all elements in the samples. Analysis of olive oils for determination of trace metals can be challenging due to low metals concentrations and high organic content. In this study, samples were diluted with organic solvent (Kerosene), and run directly into the 7700x ICPMS using the organic sample introduction kit for the 7700x. Direct dilution as a sample preparation technique over digestions offers improved productivity and a reduced likelihood of sample preparation errors. The plasma and matrix-based interferences expected with these types of samples were efficiently diminished using the Octopole Reaction System (ORS), operated using helium mode. Due to the ease of sample preparation by direct dilution, and MPP software being compatible with an easily exportable file from the MassHunter data analysis software, this method lends itself to a routine environment.

**Chromatographic study of sinigrin and AITC**

D. Yuan[(1)], D. Owiti[(2)], M. Reaney[(3)]

[(1)]University of Saskatchewan, Canada [(2)]University of Saskatchewan, Canada [(3)]University of Saskatchewan, Canada

Brassica carinata (Ethiopian mustard) is an oilseed crop that is adapted for cultivation in Western Canada. Notable advantages of this plant include resistance to heat, drought and diseases, which contribute to robust agronomic performance. The seed of Brassica carinata contains approximately 40% inedible erucic acid rich oil. Therefore it is a promising source for biofuel. About 60% of seed mass is recovered as seed meal after oil extraction. The meal is rich in protein, carbohydrate and the plant secondary metabolite glucosinolate. B carinata meal is not suitable for inclusion in animal feed as it contains glucosinolate. However, studies conducted on these compounds and their hydrolysis products reveal potential health and nutritional benefits. The value of B. carinata meal is, therefore, determined in part by seed glucosinolate concentration. Development of biorefinery processes that fractionate B. carinata should be guided by analysis of the glucosinolate fraction. The major glucosinolate of B. carinata, sinigrin, and its hydrolysis product, allyl isothiocyanate (AITC) may be determined by ion pair high performance liquid chromatography (HPLC). This study demonstrates improved HPLC methods. Sinigrin which elutes with a retention factor of 0.66 previously was retained sufficiently (rf = 2.07 ) with the addition of TMAB to the chromatography gradient Both sinigrin and AITC separation were achieved on three reversed phase columns. Here we report the first separation of sinigrin and AITC with a monolithic reversed phase chromatographic column. The application of the new method to study improved methods for Brassica oilseed processing will be discussed.

**Determination of Vitamin K1 Isomers in Oil Seeds by High Performance Liquid Chromatography-Tandem**

**Mass Spectrometry**

F. Claussen[(1)]

[(1)]EPL Bio Analytical Services, United States of America

An analytical procedure for the determination of cis and trans isomers of vitamin K1 (phylloquinone) in canola seed (Brassica napus) and soybean seed (Glycine max) was developed and validated. The validated method included extraction of ground seed with a two phase solvent system consisting of dimethyl sulfoxide (DMSO) and hexane. Crude extracts were purified on a silica solid-phase extraction (SPE) cartridge. Purified extracts were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). trans-vitamin K1 was separated from the biologically inactive cis isomer using a C30 reverse phase column. Analytes were detected using electrospray ionization (ESI) in positive ion mode and quantified by multiple reaction monitoring (MRM). An internal standard, d7-vitamin K1, was used to compensate for detector source suppression and losses during sample workup. Assay precision was 7% (relative standard deviation) and the mean spiking recovery was 105%. The DMSO:hexane solvent system is described in the United States Pharmacopeia for the analysis of fat soluble vitamins in capsule formulations. It was compared to a lipase digestion procedure described in AOAC official methods and appearing frequently in the general scientific literature for the determination of vitamin K1 in a variety of food products, food ingredients and raw agricultural commodities. Our research indicates that the lipase digestion procedure is inadequate for quantitative extraction of intrinsic vitamin K1 from canola and soybean seed matrixes.

**GC-MS Quantitative Determination of Short-Chain Free Fatty Acids in Milk Based on Ethyl Chloroformate (ECF) In-Solution Derivatization**

B. Amer[(1)], C. Nebel[(2)], H. Bertram[(3)], G. Mortensen[(4)], T. Dalsgaard[(5)]

[(1)]Aarhus University,, Denmark [(2)]Aarhus University, Denmark [(3)]Aarhus University, Denmark [(4)]Aarhus University, Denmark [(5)]Aarhus University, Denmark

Quantitative determination of the short chain free fatty acids (SCFFA) is important for the quality evaluation of milk. Previous studies use extraction procedures where considerable amounts of SCFFA are lost. An optimized method based on gas chromatography mass spectrometry (GC-MS) with ethyl chloroformate in-solution derivatization has been developed for the comprehensive analysis of individual free fatty acids (FFA); C4 ? C18:3 in bovine milk. A better estimation of the most water-soluble SCFFA was achieved by using in-solution derivatization, due to the fact that an extraction of the milk samples with an organic phase is avoided. As a representative example of the SCFFA present in milk samples, the concentration of butyric acid (C4) was determined by our developed method; 6.48 µg/mL in raw milk, 6.27 µg/mL in commercial Danish whole milk (3.5% fat), 4.71 µg/mL in semi skimmed milk (1.5% fat), 2.53 µg/mL in skimmed milk (0.1% fat) and 1.69 µg/mL in skimmed raw milk. The method has also been validated for FFA quantification at elevated levels of FFA; butyric acid 200 µg/mL. This method has potential as standard reference method for FFA determination. Moreover, it is expected that the method can be adapted to other dairy products where FFA quantification is relevant.

**An Automated Method for Accurate Determination of EPA and DHA in Marine Oils Found in Today's Supplement Market**

S. Kanable[(1)], B. Mitchell[(2)]

[(1)]Covance Laboratories, United States of America [(2)]Covance Laboratories, United States of America

In today?s world of consumer marketing, it is well established that marine oils positively contribute to cardiovascular health. These benefits rely heavily on EPA, DHA and ultimately, the Omega 3 fatty acid content that is present in products being marketed. Several analytical methods are currently used in industry, such as AOAC 991.39, AOCS Ce 1i-07, and the GOED voluntary monograph for EPA and DHA. In this study, the above methods were scaled down for use with the Agilent Workbench model 7696A. Concerns with the conventional methods include the need to methylate standards for each analytical run and to minimize exposure to oxygen. This concern is addressed with the use of the Workbench, as precise methylation occurs in a closed system. Additionally, the automation reduces the scale of the process, reduces solvent costs by 250%, and minimizes labor by 1 hour on a batch of 20 samples. In this investigation, the automated method was compared to the conventional methods using three marine oil supplements. Data is presented showing accuracy, precision, and repeatability.

**HPLC Analysis with Fluorescence Detection of Pheophytins and Pyropheophytin in Extra Virgin Olive Oil by a Modified Version of Annex C of the ISO 29841 Method for Chlorophyll Degradation Products**

M. Woodman[(1)]

[(1)]Agilent Technologies, United States of America

Many years ago, the German Fat Council published a detailed method for the analysis of chlorophyll degradation products by HPLC with UV detection after solid phase extraction (SPE) of the extra virgin olive oil (EVOO) samples. This method reflects post-harvest production stress that could affect the quality of the final product. This method was adopted by the International Standards Organization (ISO) as ISO 29841. A variant approach, Annex C, excludes the SPE step and utilizes more selective Fluorescence detection. Due to the laborious nature of SPE and the potential of unintentional degradation of the sample with that procedure, the Annex C variant attracted our attention as an alternative to the original UV method. Initial evaluation of the method proved that several problems needed to be addressed. Injection of diluted EVOO eventually led to bleed of the abundant triglycerides and resulting unstable baseline and retention time in the analyte region. Further, the sensitivity available with the cited excitation and emission wavelengths was inadequate for low mass injections that could minimize the mass of EVOO that could be injected. We modified the ternary solvent (water, methanol and acetone) gradient method to include a fourth strong solvent wash (tetrahydrofuran or methyl tert-butyl ether) on a quaternary gradient pump. This eliminated the need to premix any solvents and allowed us to easily purge the column of strongly retained triglycerides after every injection. Additionally, fluorescence detection was enhanced by over 150X by exchanging the standard photomultiplier with one more sensitive in the red region.

**On-line column-switching HPLC-MS/MS analysis of enantiomeric 3-MCPD fatty acid diesters in refined edible oils**

Y. Itabashi[(1)], S. Yoshioka[(2)], M. Suzui[(3)], H. Tsuda[(4)], A. Kuksis[(5)]

[(1)]Hokkaido University, Japan [(2)]Hokkaido University, Japan [(3)]Nagoya City University, Japan [(4)]Nagoya City University, Japan [(5)]University of Toronto, Canada

A simple and selective method was developed for the determination of enantiomeric fatty acid (FA) diesters of 3-monochloropropane-1,2-diol (3-MCPD) in edible oils, which are undesirable trace contaminants formed during refining processes. The method is based on clear separation of 3-MCPD FA diesters from large amounts of triacylglycerols in sample oils by achiral normal-phase HPLC and subsequent enantiomer separations of the diesters by chiral normal-phase HPLC. This is followed by ESI-MS to obtain a prominent [M+NH4]+, which is produced by post-column addition of NH4OH. Quantification of individual enantiomers was performed for 3-MCPD monoacid diesters under positive MRM mode using [M+NH4]+. This column-switching HPLC-MS/MS method was standardized with racemic 3-MCPD monoacid diesters and was applied to the detection, identification and quantification of the enantiomeric diesters in some edible oils and foods. The results clearly showed that palm oil, rice bran oil, and oils from fried potato and powdered milk contained almost equal amounts of R- and S-enantiomers of 16:0-16:0, 18:1-18:1, and 18:2-18:2, suggesting that both enantiomers would be formed from the corresponding almost racemic acylglycerols. The most predominant component for the monoacid diesters in palm oil was 16:0-16:0, followed by 18:1-18:1, which accounted for 3.0 and 2.6µg/g, respectively, reflecting the FA composition of the oil. Much lower levels of 3-MCPD monoesters could also be detected in palm oil but not quantified because of limitations of the method used. The present study demonstrates that column-switching HPLC-MS/MS provides direct and unambiguous information about the configuration, identity, and quantity of 3-MCPD diesters in edible oils.

**Trans fat screening by IR for a processing environment**

R. Packer[(1)], J. Sellors[(2)]

[(1)]PerkinElmer, United States of America [(2)]PerkinElmer, United Kingdom (Great Britain)

Traditionally, GCMS analysis has been used to establish trans-fat content in foods following AOAC 996.06 or AOCS Ce 1f-96 methods. Although the analysis method is highly accurate, the sample preparation is time-consuming, and requires specialist operation which is not appropriate for processing environments. In view of this, the AOAC and AOCS standardized methods for using FT-IR spectroscopy with quick and simple ATR sampling. The newest, and most accurate, of these methods is AOCS Cd 14e-09, which takes advantage of a unique infrared absorption band at

966 cm?1 arising from a C?H deformation about an isolated trans double bond. The height of the negative second derivative of this band is used in the calibration. In this work, varying oil and fat matrices are tested for zero trans-fat compliance by applying the fats or oils to a heated ATR to show how easily the AOCS method maybe automated into a simple workflow.

**Identification of steryl esters in margarine and corn kernels using ESI-MS/MS and ESI-MS/MS/MS Ion Trap-Mass Spectrometry.**

I. Hailat[(1)], R. Helleur[(2)], C. Parrish[(3)]

[(1)]Memorial university of Newfoundland, Canada [(2)]Memorial University of Newfoundland, Canada [(3)]Memorial University of Newfoundland, Canada

Steryl esters play an important role in the inhibition of intestinal cholesterol absorption and in other biochemical processes and their direct analysis by MS would be very useful. We demonstrate here a new approach for steryl ester identification using ESI-MS/MS and ESI-MSMS/MS ion trap mass spectrometry. Sterols and other lipids were extracted from samples using hexane and the steryl esters separated from other lipids using solid phase extraction cartridge (strata NH2). The steryl ester fraction was found to elute with hexane: diethyl ether (98:2, v/v). The steryl esters were dissolved in chloroform: methanol (2:1) followed by addition ammonium acetate at a final concentration of 20 mM. They were detected as ammoniated adducts [M+NH4]+. Many of steryl ester isomers were identified using ESI-MS/MS by the facile ester cleavage and observation of both the sterol hydrocarbon and fatty acid ions. The order of abundance in steryl esters in margarine was found to be: 18:0 ?-sitosteryl, 18:1 ?-sitosteryl, 18:0 campesteryl, and 18:1 stigmasteryl. In corn: 18:1 ?-sitosteryl and 18:1 stigmasteryl. For final confirmation of the structure of the sterol, ESI-MS/MS/MS was carried out where the fragmentation patterns of the steryl precursor ions were similar to those from free sterol standards. Our method was successfully used for identification of steryl esters extracted from margarine and corn kernels.

**Analyses of Fat-Soluble Vitamins, carotenoids and Lipids by Supercritical Fluid Chromatography with Sub-2µm Particle Columns**

J. Yang[(1)], R. Chen[(2)], M. Jones[(3)], G. Isaac[(4)]

[(1)]Waters, United States of America [(2)]Waters, United States of America [(3)]Waters, United States of America [(4)]Waters, United States of America

UltraPerformance Convergence ChromatographyTM (UPC2) is a separation technique that uses compressed carbon dioxide as the primary mobile phase. It takes advantage of the unique physical properties of compressed carbon dioxide (at or near supercritical state), sub-two micron particle chromatography columns and advanced chromatography system design to achieve fast and reproducible separation with high efficiencies and unique selectivity. These improvements lead to new interest in applying this technology to various industrial analytical areas, especially those areas where normal-phase liquid chromatography (NP LC) has been commonly used, such as fat-soluble vitamins (FSV), carotenoids, and lipids. Nine representative FSV and carotenoids have been successfully separated simultaneously by UPC2 within four minutes on a single C18 column. These FSV and carotenoids include vitamin A acetate and palmitate, alpha-tecopherol and its acetate, vitamin D2, vitamin K1 and K2 (MK4), beta-carotene and lycopene. The repeatability (n=6) of all the nine compounds was less than 0.25% in retention times (RT) and less than 2.6% in peak areas. The investigation of lipids separation by UPC2 showed that Bridged Ethylene Hybrid (BEHTM) silica columns provided the best separation of lipid classes among the Flouro-Phenyl, 2-EP, and BEH UPC2columns. The lipid classes investigated include ceramides, sphingomyelin, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, lyso-phosphatidylcholine and lyso-phosphatidylethanolamine. The UPC2 has been applied to biological samples and showed successful separation of lipid classes. Separation and analysis of free fatty acids and neutral lipids was also developed. These results indicate that UPC2 is a promising chromatographic technique for FSV, carotenoids and lipids analyses.

**LIPID PEROXIDATION INHIBITION CAPACITY (LPIC) ASSAY: A NEW METHOD FOR DETERMINATION OF ANTIOXIDANT ACTIVITY**

S. Quek[(1)], C. Breig[(2)], J. Zhang[(3)]

[(1)]The University of Auckland, New Zealand [(2)]The University of Aauckland, United States of America [(3)]The New Zealand Institute for Plant & Food Research Limited, New Zealand

The Lipid Peroxidation Inhibition Capacity (LPIC) method measures the ability of antioxidants to inhibit the oxidation of a fluorescent probe (C11-BODIPY) by 2,2?-azobis (2-amidinopropane) hydrochloride (AAPH) produced free radicals, utilizing liposomes to simulate a biological membrane. As the current methods for antioxidant determination involve mainly chemical analysis, the LPIC assay is believed to provide a more accurate representation of the potential effects of antioxidants in vivo through the creation of a simulative cell membrane. A total of fifty-one black and green tea samples were determined by the LPIC assay and the data were then correlated to those obtained from the high performance liquid chromatography (HPLC), ORAC and Folin-Ciocalteu methods. A strong correlation was demonstrated between the total phenolic content and the LPIC antioxidant activities ($R2 = 0.8924$, $p < 0.05$) in the green and black tea. On the other hand, a moderately strong relationship between ORAC and LPIC antioxidant activities was found ($R2 = 0.7518$). The level of correlation between LPIC activity and antioxidant content by HPLC differed for catechins from green tea and theaflavins from black tea. For green tea, a stronger correlation was observed between LPIC values and the catechin content than those demonstrated for ORAC values. This indicates that the LPIC assay may be better suited for the determination of antioxidant activity in green tea than the frequently used ORAC assay.

**Quantification of fatty acids in New Zealand sea cucumbers (Autralostichopus mollis)**

S. Quek[(1)], F. Liu[(2)]

[(1)]The University of Auckland, United States of America [(2)]The University of Auckland, United States of America

Literature has shown that sea cucumber generally contains approximate 0.24% to 0.83% of lipid by fresh weight and a high level of essential fatty acids. There is little information on the lipid content and fatty acid composition of the New Zealand species (Autralostichopus mollis) as reported here. The sea cucumber samples were harvested from two locations at North Island, New Zealand: the adult sea cucumbers were from Ti Point (TP) and juveniles from Mahurangi Habour (MH). The lipid extraction method was modified from Bligh and Dyer method, and fatty acid methylation was derived from Hartman and Lago method. The lipid content of the samples was lower in the body wall (about 0.1%) compared to that of internal organ (about 0.2%). Arachidonic acid, AA (C20:4, n-6), which is well-known for its ability in healing various external and internal wounds, is the most abundant LCPUFA in A. mollis, both in the body wall and internal organs (in the range of $9.93 \pm 0.05\%$ to $15.89 \pm 0.94\%$). Eicosapentaenoic acid, EPA (C25:3, n-3) is the second highest LCPUFA ($4.05 \pm 0.06\%$ to $11.17 \pm 0.84\%$). The content of docosahexaenoic acid, DHA (C22:6, n-3) is lower than AA and EPA, but is quite similar to the stearidonic acid, STD (C18:4, n-3). Overall, the data also revealed that internal organs contain much higher LCPUFA than the body walls, and adults from TP contain more LCPUFA than juveniles from MH.

**CANCELLED-Oil-Containing Micelles as Calibration Standard for Lipophilic Dye-Based Microalgal Lipid Quantification**

H. Zhao[(1)], K. Lee[(2)], P. Nam[(3)]

[(1)]Missouri University of Science and Technology, United States of America [(2)]Lincoln University, United States of America [(3)]Missouri University of Science and Technology, United States of America

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