2009 Annual Meeting Abstracts

MONDAY

MORNING
ANA 1: Now and Then
Chair(s): J. King, University of Arkansas, USA; and A. Proctor, University of Arkansas, USA

Routine and Research Methods for Lipid Oxidation. Edwin Frankel, Department of Food Science and Technology, University of California, Davis, Davis, CA, USA

Many methods are now available to determine routinely the extent and nature of oxidative deterioration of unsaturated food lipids. More advanced research methods include gas chromatographic methods for volatile decomposition products related to flavor deterioration including solid phase micro-extraction gas chromatography (SPME-GC). Combinations of chromatographic techniques with sensitive detection systems have been developed, including high-performance- liquid chromatography (HPLC), and size-exclusion chromatography (HP-SEC), GC-mass spectrometry (MS), HPLC and ^{13}C nuclear magnetic resonance (HPLC-^{13}C NMR), HPLC-MS, chemical ionization MS (CI-MS), and coordination-silver ion spray MS (CIS-MS). Advanced direct MS techniques include atmospheric pressure chemical ionization-MS (APCI-MS) used for volatile flavor analysis during food consumption. HPLC with post-column chemiluminescence detection permits more sensitive and direct analyses of hydroperoxides from oxidized complex lipids in foods and biological samples. This approach can now be used to analyze quantitatively hydroperoxide mixtures containing conjugated and non-conjugated diene structures. HPLC-^{13}C NMR studies of geometric hydroxydiene isomers in oxidized methyl linoleate. Important applications of HP-SEC with viscometric and refractometric detection include analyses of dimers, trimers, oligomers, partial glycerides, cyclic fatty acids and molecular-weight distribution of triglyceride polymers in heated, thermally oxidized vegetable and fish oils. SPME-GC/MS is a sensitive method to measure volatiles in the headspace of milk powders. HPLC-MS permits the direct characterization of thermally labile hydroperoxides and non-volatile high-molecular weight secondary oxidation products of triglycerides, cholesterol and phospholipids. CIS-MS can also be used to analyze the isomeric hydroperoxides from cholesterol and phospholipids of unsaturated fatty esters. Volatile flavor compounds can now be measured directly "in-nose" by introducing the whole mixture into a mass spectrometer and resolve them entirely by mass followed by atmospheric pressure chemical ionization?mass spectrometry (APCI-MS). This powerful "MS-Nose," technique allows sampling of moist air from the test panelist to characterize the effect of lipids and food emulsions on flavor release and delivery from food matrixes during consumption.

How Chevreul (1786-1889) Based his Theories on his Analytical Results. Albert J. Dijkstra, Sàrl Dijkstra-Tucker, St Eutrope-de-Born, France

When Chevreul started to work on edible oils and fats in 1811, inorganic chemistry was far more advanced than organic chemistry. Half the elements had been discovered and their atomic weights had been determined surprisingly accurately. Elemental analysis of organic compounds was limited to carbon hydrogen and oxygen but for fats, this sufficed. There were also very few pure organic compounds known. Since salts were regarded as the sum of oxides, soaps were also described as the sum of a metal oxide and a 'dry acid'. Many of such acids were discovered by Chevreul and distinctions between these acids were made on the basis of their analyses and physical properties.Fats were considered to be mixtures of stearin and olein that could be separated by solvent fractionation, whereby the stearin was concluded to be a compound formed by stearic acid and glycerin, just as the olein was formed by oleic acid and glycerin. The ratio of stearin to olein determined the properties of the fat.Examples will be given of the analyses of soaps and fats, and the conclusions drawn by Chevreul will be evaluated.

How to Analyze the Microstructural Behavior of Food Lipids. K. Dewettinck, Ghent University, Gent, Belgium

Many foods consist of a substantial amount of fat. Quite often part of it is present in the crystallized form and the resulting microstructure determines many attributes such as processing and storage stability as well as sensorial

properties. The last decade a lot of progress has been made in the field of microstructural analysis of food lipids. An overview of possibilities and limitations will be presented.

Analytical Chemistry: A History of Advances over the Past Century. G.R. List, USDA (Retired), USA

The period from 1823, when Chevreul described the chemical nature of fats and oils, until the 1940s was marked with little progress in analytical chemistry beyond crude physical and wet chemical methods to characterize fats oils and lipids for source, impurities,minor components, composition, and structure. Moreover, a lack of analytical methodology lead to a poor understanding of the basic processes used to manufacture edible fats and oils into salad oils, margarine, and shortening products. AOCS and its members played a major role in both methods development and their applications toward solving basic problems in the oilseed industry. The contributions of T.P. Hilditch, A.E. Bailey, H.J. Dutton, R.R.Allen, and others will be reviewed and their impact on the industry discussed.

Lipid Analysis and Chromatography - An Overview. W. Artz, University of Illinois, Urbana, IL, USA

An overview of lipid analysis and chromatography emphasizing the historical and developmental context, starting with a brief introduction to Tswett and his initial separation of carotenoids to today?s powerful hyphenated techniques will be presented. The first major development after Tswett was work on partition chromatography published by Martin and Synge which included a theory of chromatography and the introduction of the concept of GC. They received the Nobel Prize for their contribution. Martin (and another author, James) eventually published work on the separation of FAMEs. The emphasis in the presentation will be on the chromatography, although the development of a wealth of innovative detection systems that provides structural information by analytical and physical chemists has added enormous power and utility to the separation method.

Changing Education through Advances in Lipid Analysis. Randall J. Weselake, University of Alberta, Edmonton, Alberta, Canada

Rapid technological advancements in analytical chemistry over the past century have transformed the way lipid science is performed and taught. Before the advent of the modern chromatographic methods, lipid analysis was largely limited to studies based on solubility properties, formation of fatty acid salts, low temperature crystallization or distillation, which yielded limited information despite the amount of time and sample invested. The invention of the gas chromatograph in the 1950s and subsequent improvements in column design and pneumatic control have since made GC the mainstay for the analysis of lipids, with continued improvements in speed, resolution and sensitivity. Over the same period, mass spectrometry has evolved from a specialized technology only available to a few, into a mainstream benchtop technology within the grasp of students in most lipid science laboratories. Such advancements have placed more analytical power in the hands of trainees. Emerging technologies for lipidomics and metabolomics promise to further broaden the scope and sophistication of lipid analysis and provide new opportunities for training future generations of lipid scientists.

AFTERNOON
ANA 2: Extreme Chromatography
Chair(s): R. Adlof, USDA (retired); and L.M. Sidisky, Supelco, USA

Application of Temperature to Silver-ion HPLC. R. Adlof, USDA (Retired), Peoria, IL, USA

Silver ion chromatography (Ag-HPLC), utilizing columns packed with silver ions bonded to a silica or similar substrate and acetonitrile in hexane as solvent, has proven to be a tremendously powerful technique for the analytical separation of cis and trans geometric and positional triacylglycerol (TAG) or fatty acid methyl ester (FAME) isomers. This presentation will review the application of temperature (-10C to +70C), rather than changes in solvent composition, to Ag-HPLC to improve retention and maximize resolution of fatty acid geometric and/ or structural isomers. Temperature-programing will also be discussed, as will the limitations of this methodology.

Dual Parallel Mass Spectrometry (LC1/MS2 and LC2/MS2) for Lipid and Vitamin D Analysis. W.C. Byrdwell,

USDA, ARS, BHNRC, FCMDL, 10300 Baltimore Ave., Beltsville, MD, USA

Atmospheric pressure chemical ionization (APCI) mass spectrometry (MS) and electrospray ionization (ESI) MS are complementary techniques that provide different types of information for lipids such as triacylglycerols (TAGs), phospholipids, and fat-soluble vitamins. Since no one technique is by itself ideal, we routinely employ two mass spectrometers in parallel to provide APCI-MS and ESI-MS, MS/MS and MSn data. Dual parallel mass spectrometers have been attached to the same chromatographic system to provide two or more types of information from components in the same column effluent, referred to as an LC1/MS2 approach. Alternatively, two chromatographic systems have been attached in a column-switching configuration to perform a total lipid analysis of both polar and non-polar lipids on normal-phase (NP) and reversed-phase (RP) systems, respectively, with detection by two different mass spectrometers simultaneously operated with different ionization modes. This is referred to as an LC2/MS2 arrangement. An LC1/MS2 approach has also been used for vitamin D analysis in foods, which allows accurate quantification using APCI-MS in SIM mode with parallel acquisition of APCI-MS for qualitative monitoring of interfering species.

Comprehensive GC X GC for the Analysis of FAMEs. L.M. Sidisky[1], L. Mondello[2], P.Q. Tranchida[2], P Dugo[2], [1]Supelco, Division of Sigma Aldrich, Bellefonte, PA USA, [2]University of Messina, Messina, Italy

Comprehensive two-dimensional GC (GC x GC) is one of the most exciting innovations in gas chromatography since its invention by Liu and Phillips in 1991. The leap from conventional to comprehensive Multi-Dimensional Gas Chromatography was enabled by the introduction of a "primordial" thermal modulator. The latter, as all current-day modulators, subjected continuous primary conventional capillary bands to entrapment, re-concentration and introduction onto a short segment of micro-bore column, typically 50 – 100 cm x 0.1 mm I.D. In order to achieve comprehensive chromatographic analysis it is necessary to employ columns with distinct separating mechanisms (generally nonpolar in the first and polar in the second dimension).In the present work, comprehensive GC applications have revealed the higher-than-suspected complexity of some lipid samples. Furthermore, the formation of highly structured fatty acid methyl ester chemical class patterns has been both of great interest and help for identification purposes, without the use of pure standard compounds or of MS detection. Finally, the use of orthogonal ionic liquids capillary columns offers unique selectivity for resolving various isomer pairs compared to conventional siloxane based phases.

Metabolic Profiling of Phospholipids in Rat Plasma utilizing Ultra Pressure Liquid Chromatography and OA TOF Mass Spectrometry. Paul Rainville, Robert Plumb, Waters Corporation, Milfrod, MA, USA

Ultra-Performance LC® (UPLC) utilizing sub 2 micron porous stationary phase particles operating with high linear velocities at pressures > 9000 psi was coupled to orthogonal acceleration time of flight (oa TOF) mass spectrometry and successfully employed for the rapid separation of lipids from complex matrices. The UPLC system produced information rich chromatograms with typical measured peak widths of 3 seconds at peak base, generating peak capacities in excess of 200 in 10 minutes. Further UPLC coupled with MSE technology provided parent and fragment mass information of lipids in one chromatographic run thus providing an attractive alternative to current LC methods for targeted lipid analysis as well as lipidomic studies.

Improved HPLC Separations for Fats and Oils Analyses through Temperature Programming. W.D. Felix, J. Clark, Selerity Technologies, Salt Lake City, UT, USA

The search for higher speed and for ?green? chemistry has led to new techniques becoming available for the analytical chemist. Two recent methods using either high pressure or high temperatures in HPLC directly address these issues. While both effectively increase speed and reduce solvent usage, high temperature HPLC offers the distinct advantage of being retrofitable to existing HPLC instrumentation resulting in cost savings while significantly reducing run times and improving peak shape while using high performance HPLC columns in formats familiar to the analytical chemist. Temperature control also significantly improves reproducibility of both HPLC and UPLC instruments. Solvent usage is dramatically reduced when temperature programming replaces traditional solvent programming. In this case, the isocratic solvent mixture can usually be recycled for significant reduction in total solvent usage. Many modern

columns can now withstand temperature programming up to 200 C. As an example of ?green chemistry? HPLC with temperature programming we will show the separation of glycols using water only as the solvent. One can also program separations from near zero through above ambient. For example, a sub-ambient through 35 C separation of tocopherols enhances the separation of closely eluting compounds, while cutting run times in half.

TUESDAY

AFTERNOON

ANA 3: Contaminant Analysis

Chair(s): M. Collison, Archer Daniels Midland Co., USA; and L. Reimann, Eurofins Scientific Inc., USA

The Formation of 4-HNE in Frying Oils: A Controlled Study Using a French Fry Model. Sean LaFond, Keith Cadwallader, William Artz, University of Illinois at Urbana Champaign, Urbana, IL, USA

We monitored over a four day period the formation of 4HNE in a corn/soybean oil blend during controlled batch frying of French fries. We had two identical fryers, each with 6.8 kg (15 lbs) of oil. At the beginning of each day the oil level of the heated control oil (HCO) fryer was reduced to the same level as the French fried potato oil (FFPO) fryer. Fryers were then topped off to 6.8 kg of oil before they were heated. Fryers were heated to 180 °C (365 °F) for 8 hours per day and the FFPO fryer fried 9% of its oil weight in French fries once per hour. The HCO fryer was heated but did not fry anything. At the end of each day both oils were filtered then allowed to cool overnight. Periodically 4HNE was quantified by derivatization GC-MS/stable isotope dilution assay using [$^{2}H_{2}$-8,9]-4HNE as an internal standard. Results show that 4HNE was formed to a similar extent in both HCO and FFPO. 4HNE increased in the first day and reached a maximum (~6.3 PPM) during the second day 4HNE then gradually decreased throughout the remainder of the experiment. 4HNE levels in FFPO decreased at a slightly faster rate than in HCO. Frying of French fries had essentially no effect on 4HNE formation and daily topping off of the oil levels had the effect of stabilizing 4HNE levels throughout the heating period.

3-MCPD-ester in Oils and Fats - Do We Know for Sure What We Are Measuring?. K. Hoenicke, Eurofins WEJ Contaminants GmbH, Hamburg, Germany

In December 2007, the German official food control and animal health laboratory in Stuttgart reported significant amounts of 3-monochloropropane-1,2-diol (3-MCPD) fatty acid esters in numerous refined edible oils and fats as well as in foods containing refined fat like infant and toddler formula. The amount of 3-MCPD in the form of fatty acid esters found in edible oils and fats ranged from around 500 to around 10.000 µg/kg. 3-MCPD-esters can be formed when fats or fat-containing foods containing salt or other sources of chlorides are exposed to high temperatures during manufacturing processes. This is the case during the oil refining process. The method published by the official food control laboratory uses sodium chloride during the derivatisation step of 3-MCPD. Recently it has been shown that other substances, such as glycidol which may also be present in oil and fats, can be converted into 3-MCPD during the sample preparation step. Glycidol is a reactive epoxide, classified by the IARC as probably carcinogenic to human.Lower 3-MCPD concentrations were found in some oils and fats following the replacement of sodium chloride with other salts such as ammonium sulphate during the sample preparation. The results can differ by a factor of 2 ? 3. This leads to the assumption, that the method published by the German official food control laboratory is not specific for the analysis of 3-MCPD and its esters. Rather, the reaction of certain precursors with chloride ions present can result in the formation of 3-MCPD. In order to compare results from different laboratories, the method used and the target substances have to be clearly defined.

Crude Oil and Refining Process Contaminants. G. van Duijn, Unilever, The Netherlands

Fully refined oils and fats are used as ingredients in food without further purification. Therefore, these products must be safe (free from hazardous contaminants) and of specified quality. Food safety and quality of a refined product are determined by the levels of contaminants and minor components in the crude oil and the removal of these components during the refining process, while the refining process itself may also generate process related contaminants.The levels

of contaminants and minor components in the crude oil depend on:? Agricultural practices.? Procedures of oil crop storage, drying and handling.? Oil milling practices.? Contamination and degradation during crude oil transport. Risk of contamination and the type of contaminant will differ per oil type and origin. This is reflected in an oil risk matrix, which is based on analytical data of received crude oils and a check of practices by supply chain audits. The current refining process has been introduced around 1900 to produce good quality oils and fats for application in margarine production and as frying oil. The process has been optimized to reduce the natural taste and color and to remove most of the free fatty acids present in the crude oil. Later it was discovered that this optimized process also reduces many of the contaminants present in the crude oil.However, the refining process may also introduce contaminants in the oil via the processing equipment or processing aids, or unwanted products as result of side reactions. The best known side reaction products are trans fatty acids, formed at high deodorization temperature, and 3 MCPD esters, already formed at low deodorization temperature. The refiner will optimize the parameters of the refining process to maximize the reduction of crude oil contaminants on one hand while limiting the formation of unwanted side reaction products on the other.Food safety or quality defects of the end product are the result of the incorrect performance of one or more specific processing steps, or the poor quality of the crude oil. The ?link? table links the individual processing steps with a specific quality or food safety defect. This table helps the refiner to identify what process may be the origin of an out of specification product.

Polycyclic Aromatic Hydrocarbon Determination by Reversed-Phase High-Performance Liquid Chromatography in Olive Oils on the Iranian Market. Maryam Fahimdanesh[1], Giorgia Purcaro[2], Sabrina Moret[2], Lafranco S. Conte[2], [1]Islamic Azad University, Shahryar shahr-e-Qods Branch, Tehran-Karaj, Iran, [2]Dept. of Food Science, University of Udine, Italy

Despite the carcinogenic properties of some PAHs, and although edible oils are particularly prone to PAH contamination, no international legal limits for PAH in edible oils have been yet established however, a number of methods for such analysies have been published most of which are time consuming and unsuitable for routine analysis, as they do not permit analysis of a large number of sample per day.in this paper, HPLC with spectrofluorimetric detection was applied to the determination of Polycyclic aromatic hydrocarbons (PAHs) in 7 Iranian olive oil and 2 turkies imported olive oils.The analysis of some blends of refined and virgin oils shows that the distributions of light and heavy PAHs are different with the content of the former being lower in refined samples.

Structural Diversity of MCPD Esters in Food: Significance and Analytical Challenges. Walburga Seefelder, Nestlé Research Centre, Lausanne, Switzerland

Chloropropanols are a group of chemical contaminants that may be formed in certain food ingredients during processing. The most prevalent isomer among this group is 3-monochloropropane-1,2-diol (3-MCPD). 2-Monochloro-propane-1,3-diol (2-MCPD) as well as 2,3-dichloropropan-1-ol (2,3-DCP) and 1,3-dichloropropan-2-ol (1,3-DCP) may also occur in food, but at lower concentrations. Previous research indicated that MCPD-esters are accepted as substrates by lipase from *Aspergillus Oryzae*. Because of their structural similarity with triacylglycerols, it was hypothesised that 3-MCPD-esters may also be hydrolysed by mammalian gut lipases. This was confirmed *in vitro* by incubating mono- (sn1) and di- esters in an intestinal model containing an excess of pancreatic lipases. The release of free 3-MCPD from the diester was much slower than from the 1-monoester. In this model 2-MCPD-esters were found to be slightly more susceptible than 3-MCPD-esters to the action of lipases. Other data suggested a possible significant role of the food matrix on the extent of MCPD-ester hydrolysis. MCPD-esters in bread seemed to be more accessible (83-103% recovered after 4 h incubation) to gut lipases than MCPD-esters in oils (25-50% of bound 3-MCPD recovered from pure palm oil).Recently the presence of fatty acid esters of 3-MCPD has been reported in various types of foods and food ingredients, especially in refined vegetable oils. Currently, 3-MCPD-esters are analyzed by measuring the amounts of 3-MCPD released from the esters after hydrolysis. Such an approach does not differentiate between mono and diesters. This may constitute a significant limitation for safety assessment since metabolic considerations suggest that mono and diesters may exhibit differential sensitivities to gut lipases. Analytical approaches to separate and quantify mono- and diesters of 3-MCPD will be presented.Although focus has been on esters of 3-MCPD, studies in experimental model systems have revealed that fatty acid mono- and diesters of other chloropropanols can also be formed from triacylglycerols in the presence of hydrochloric acid and heat. The actual occurrence of such chloroesters in food materials has only been marginally investigated. Data on 2-MCPD-esters will

be presented. Special focus will be set on the formation of 2-MCPD esters during the roasting of barley and over the refining process of edible oils.The preliminary data available indicate that the issue of MCPD-esters in food is not restricted to 3-MCPD-esters and raises significant analytical challenges. Further investigations are necessary to fully understand the complexity and significance of this class of foodborne chemical contaminants.

Discussion.

WEDNESDAY

MORNING
ANA 4: Developments in Fatty Acid Analysis
Chair(s): S. Bhandari, Silliker, USA; and C. Costin, Cargill Inc., USA

FAME Analysis by Gas-Liquid Chromatography: Past, Present, and Current Mistakes. Ronald B. Pegg, Robert L. Fusco, The University of Georgia, Athens, Georgia, USA

A cornerstone of lipid analysis since the mid-1950s has been the determination of acylated lipids after a transesterification reaction by gas-liquid chromatography-flame ionization detection (GLC-FID); this technique, in combination with some TLC preparative work, can help identify, quantify, and locate the position of lipid constituents on triacylglycerol backbones and in phospholipids. Such analyses are important to scientists dealing with the development of healthful and structured lipids for human consumption. This presentation will highlight the original approaches and standards used to separate and quantify fatty acid moieties by GLC with packed columns, the revolution afforded by open-tubular or capillary columns in fatty acid methyl ester (FAME) analysis, present techniques including GC-MS detection, as well as oversights/mistakes relating to FAME analysis found too often in the contemporary literature. The advantages and disadvantages of the various techniques at hand to derivatize lipid constituents will be addressed. Emphasis will also be given to proper calibration of FAME analysis by GC-FID, choice of GC inlet liners, limit of detection and quantification determinations, proper use of internal and external standards, and means available to quantify and report GLC-FID signals.

A Rapid, Micro FAME Preparation Method for Vegetable Oil Fatty Acid Analysis by Gas Chromatography. Rahul Lall, Vishal Jain, Andrew Proctor, University of Arkansas, Fayetteville, AR, USA

A 30 min, micro-base-catalyzed method for vegetable oil fatty acid methyl ester (FAME) preparation was developed that used only 1 mg of oil sample by limiting the solvent volumes used. This method was primarily developed to quickly analyze fatty acid composition of CLA-rich soy oil but can be further applicable to pure vegetable oils. Existing base-catalyzed FAME preparation methods are not appropriate to use because they are either rapid but not micro, or micro but not rapid, or are rapid and micro but use acidification in the final step of FAME preparation which would isomerize oils containing conjugated fatty acids. Serial dilutions of a mixed commercial FAME reference standard were prepared and analyzed by GC with a flame ionization detector (FID) with maximum instrument sensitivity. The novel method was also used to prepare soy oil FAMEs for GC-FID analysis. There were no statistically significant differences ($P>0.05$) in fatty acid data from the FAME reference standard dilutions. Similarly, there was no statistical significant difference ($P>0.05$) between results obtained for all the soy oil dilutions and the control method. This technique is a rapid method for analyzing small pure oil samples as FAMEs for further GC-FID analysis.

Direct Methylation for Single Seed Analysis. Veronique J. Barthet, Canadian Grain Commission, Winnipeg, MB, Canada

Single seed analysis is an interesting technique to look at the seed/variety distribution in a seed samples. A simple base catalyzed derivatization is made directly on a crush seed without any oil extraction, the FAMES are extracted and analyzed by GC. Base catalyzed derivatization is only a transesterification method as opposed to acid-catalyzed

derivatization, FFA can not be esterified. This is not in problem when working with oilseeds since sound oilseeds contain less than 0.5% (Canadian Grain Commission data). Sodium methoxide is the derivatization agent catalyzing the transesterification. Using this base-catalyzed method, we found that there was no isomerization of double bonds in polyunsaturated fatty acids. Several varieties of canola have been registered in Canada, conventional canola, low linolenic, high oleic low linolenic or high erucic. These varieties have specific end uses and are usually not mixed. In canola, opposite to wheat, no visual distinction can be made for the different varities. The goal of this project was used to examine the composition of canola samples by trying to identify the type of ′canola′ present in producer samples (single variety) and in cargoes (variety composite).

Determination of *trans* Fat in Processed Foods by Infrared Spectroscopy. M.M. Mossoba, Food and Drug Administration, College Park, MD, USA

To improve the sensitivity of the attenuated total reflection Fourier transform infrared (ATR-FTIR) official methods for the determination of total trans fats and oils in processed foods and dietary supplements, a new ATR-FTIR procedure has been developed and recently validated in an international collaborative study. It entails the measurement of the height of the negative second derivative of the trans absorption band for isolated double bonds at 966 cm-1. It allowed the recognition and resolution of previously unresolved quantitative issues (sloping baseline and interferences from saturated fat and conjugated fatty acids). It also improved the accuracy and sensitivity of the IR methodology, and most importantly made IR more suitable than ever for the rapid determination of total trans fats for regulatory compliance. GC has been the industry standard because it provides detailed information on fatty acid composition. On the other hand, IR spectroscopy offers a rapid determination of the total trans content because it does not require any derivatization of the test fat or oil and is relatively simple. The statistical analysis results of a 10-laboratory IR validation study will be presented; the repeatability and reproducibility precision values were found to be superior to those of both transmission and internal reflection IR official methods.

Evaluation of Different Acid Treatments in the Direct Method of Fatty Acid Analysis (not requiring prior fat extraction). Sneh D. Bhandari, Jerrold Leahy, Silliker Inc., Chemistry R&D, USA

We investigated different acid treatments involving direct (not involving prior fat extraction) methods of fatty acid analysis. The method involving sulfuric acid following the saponification (O'Fallon etal's; J. Animal Sci. 2007, 85: 1511) when applied to various matrices was found to provide total triglycerides (analyzed as fatty acids) similar to the results of the reference methods or expected values. The matrices analyzed included cheese, fluid milk, dairy based formula with encapsulated fatty acids, selected finished food and NIST SRMs (potted meat as well as Peanut butter). The AOCS's draft method (AOCS Ce 1k-06) for direct analysis of fatty acids (AOCS Direct Method) was also investigated with an additional acid step involving 8.3 N HCl. The method when applied to fluid whole milk, the AOAC split cheese and meat samples provided total triglyceride results comparable to the values obtained by the reference methods. A method involving treatment with 1.25 N HCl in conjunction with the AOCS Direct method (the acid step similar to the Cargill's acid direct method) was also evaluated by its total triglyceride results as compared to the expected or reference values. It was found to work satisfactorily for tuna, breaded chicken , samples containing encapsulated fatty acids and various NIST SRMs.

GC-FID and FTIR-ATR Determinations of *trans*-Fat Levels in Hen Eggs. Robert L. Fusco, Rakesh K. Singh, Nicholas M. Dale, Ronald B. Pegg, The University of Georgia, Athens, Georgia, USA

Single-comb White Leghorn (SCWL) hens were fed one of five test diets containing varying levels of *trans* fats from partially-hydrogenated vegetable oils and animal fats. Gas chromatography-flame ionization detector (GC-FID) and Fourier transform infrared-attenuated total reflectance (FTIR-ATR) techniques were employed to determine the impact of *trans* fat deposition in egg yolks of the SCWL hens. Total lipids were extracted from egg yolks, transmethylated, and then analyzed using a highly-polar biscyanopropyl capillary column (SP™-2560, 100m × 0.25mm, 0.2µm film thickness) by GC-FID. Silver-ion TLC assisted with the separation of *trans*-monoene isomers from their *cis*-counterparts before some GC determinations. GC-FID results were compared with those acquired by FTIR-ATR detection of *trans*-fat residues, but FTIR-ATR analysis was not as sensitive as that of GC-FID. A good correlation between the two techniques exists; however, the detection limit was found to be *ca.* 1% *trans* fat per gram total fat. Test diets containing tallow, partially-hydrogenated vegetable oil, and an equal mixture of both resulted in the

deposition of 0.636±0.047%, 0.670±0.167%, and 0.716±0.037% *trans* fat per gram total fat in the egg samples, respectively.

Quantification of *trans* Vaccenic Acid by Two-Dimensional GC. C. Villegas[1], C.J. Field[1], J. Harynuk[2], Y-Y. Zhao[1], J.M. Curtis[1], [1]Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada, [2]Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada

The identification and quantification of complex mixtures of *cis* and *trans* 18:1 fatty acid isomers presents a major challenge for conventional one-dimensional GC/FID analysis of their methyl esters. Interest in quantifying *trans* vaccenic acid, a precursor of CLA, in the fields of nutrition, animal and dairy science led us to search for efficient methods for its quantitation at low abundance in complex fatty acid mixtures. We have investigated the use of various column combinations on a LECO comprehensive two-dimensional GC/FID system to achieve optimized separations. The need for pre-separation of *cis* and *trans* isomers by silver ion SPE was also explored. The data obtained on a variety of foods samples was compared to that obtained by LC/MS methods thereby allowing independent validation of results.

Identification of Acylglycerols Containing Dihydroxy Fatty Acids in Castor Oil by Mass Spectrometry. Jiann-Tsyh Lin, USDA, Albany, CA, USA

Ricinoleate, a monohydroxy fatty acid, in castor oil has many industrial uses. Dihydroxy fatty acids can also be used in industry. The C18 HPLC fractions of castor oil were used for mass spectrometry of lithium adducts to identify the acylglycerols containing dihydroxy fatty acids. Four diacylglycerols identified were diOH18:1-diOH18:1, diOH18:2-OH18:1, diOH18:1-OH18:1 and diOH18:0-OH18:1. Eight triacylglycerols identified were diOH18:1-diOH18:1-diOH18:1, diOH18:1-diOH18:1-diOH18:0, diOH18:2-diOH18:1-OH18:1, diOH18:1-diOH18:1-OH18:1, diOH18:1-diOH18:0-OH18:1, diOH18:2-OH18:1-OH18:1, diOH18:1-OH18:1-OH18:1 and diOH18:0-OH18:1-OH18:1. The structures of these three newly identified dihydroxy fatty acids were proposed as 11,12-dihydroxy-9-octadecenoic acid, 11,12-dihydroxy-9,13-octadecadienoic acid and 11,12-dihydroxyoctadecanoic acid by MS3 or MS4 of HPLC fractions of castor oil. These individual acylglycerols were at the levels of about 0.2% or less in castor oil and can be isolated from castor oil or overproduced in a transgenic oil seed plant for future industrial uses.

Fast LC-MS Analysis of Oxidized Free Fatty Acids, Monoacylglycerols, Diacylglycerols, and Triacylglycerols. M. Tarvainen, J.-P. Suomela, H. Kallio, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

A fast LC-MS method for the analysis of oxidized free fatty acids, monoacylglycerols, diacylglycerols, and triacylglycerols was developed. Rapeseed oil was oxidized in convection oven and digested with standard meal in artificial digestion model. Total lipid extracts were analyzed with reversed phase UPLC?-MS and UPLC?-MS/MS. Mass spectra was collected from 150 m/z to 1500 m/z. UPLC?-MS has the advantage of shorter analysis time and more sensitivity in mass spectrometry with less sample. Previously, 90 minutes was required to analyze total lipid extracts of lipid digestion. 6 times reduction in analysis time and 10 times reduction in solvent consumption was achieved with the use of UPLC?. [M+23]+ and [M+45]+ were the most prominent ions formed in ESI source. Several hydroxides and hydroperoxides were detected after comparison with oxidized reference compounds.

Identification and Quantification by LC/APPI-MS of Antifungal Fatty Acid Metabolites from *Lactobacillus hammesii*. B.A. Black, M.G. Gänzle, J.M. Curtis, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada

Lactobacillus hammesii isolated from sourdough bread fermentation has been shown to produce compounds with antifungal activity, thought to be hydroxy fatty acids. Fermentative production of antifungal compounds in bread has the potential to replace the use of preservatives but research is needed to identify the antifungal metabolites and optimise their production. Lipid metabolites of *L. hammesii* were extracted from culture supernatants and a method was developed for the analysis and fractionation of these complex mixtures containing fatty acids with various degrees of hydroxylation. This employed normal phase LC combined with atmospheric pressure photoionisation mass

spectrometry (LC/APPI-MS) using a Sciex PhotoSpray® source. Fractions were further analysed by reversed phase LC/APPI-MS and GC methods. The advantages of LC/APPI-MS in lipid analysis will be discussed.

Development of the One Pot Acid-Alkaline and Alkaline Only Direct Methylation Methods. S. Hansen, R. Williams, Cargill, Inc., Wayzata, MN, USA

The AOCS method of Direct Methylation of Lipids for the Determination of Total Fat, Saturated, cis-Monounsaturated, *cis*-Polyunsaturated, and trans Fatty Acids by Chromatography (Ce 1k-07) has been shown to be ineffective in some matrices, such as encapsulated lipids and some oat based products. The objective was to develop an acid digestion pretreatment that could be applied to food and other lipid containing matrices before proceeding with the AOCS method of Direct Methylation to improve the extraction efficiency in these difficult matrices. The methylation conditions from AOCS Ce 1k-07 were demonstrated to provide incomplete methylation of some fatty acids, especially long chain saturated fatty acids such as 21:0 and 23:0 used in AOCS methods as internal standards. It was found that by performing the methylation reaction in the absence of organic solvents other than methanol, a 100% methylation rate could be achieved for all fatty acids of interest. An acid digestion was then developed from which the sample could then be carried forward in the same reaction vessel to undergo Direct Methylation with only slight modification. This acid treatment was shown to be effective on some matrices for which typical alkaline Direct Methylation was ineffective, such as some encapsulated marine oils as well as an oat sample.

Simultaneous Extraction and Transesterification to Quantify n-3 Fatty Acids and Tributyrin from Microencapsulated Oil Powders. Z. Shen, L. Sanguansri, M. A. Augustin, Food Science Australia, Werribee, ViC. Australia

Simultaneous extraction/ methylation (SEM) or butylation (SEB) methods for the quantification of n-3 fatty acids and tributyrin in microencapsulated powders containing mixtures of fish oil and tributyrin were developed respectively. The procedures involve direct transesterification of triacylglycerols to n-3 fatty acid methyl esters and butyric acid butylesters, without the need for a separate extraction of the oils from the encapsulating matrix. Internal standards (IS) used were methyl tricosanoate and hexanoic acid for methylation and butylation respectively. Briefly, the IS solution were added with tetrahydrofuran and 2N sulphuric acid in methanol or butanol to 150 mg powder. To complete the digestion and transesterification, the mixture was heated at 70°C for 2.5 h or 60°C for 1.5 h for methlyation or butylation. After neutralisation, extraction and washing steps, the top hexane extracts were injected to GC. The SEM method has been compared with the values obtained from methylation of extracted oils. Higher recovery yields of 2.3-7.8 % (EPA) and 2.0-7.9% (DHA) were obtained using the SEM method. The methods were simple, easy, less laborious and less time consuming and after all very valuable to monitor a storage stabilities and losses of those bioactives.

ANA 4.1: General Analytical I
Chair(s): W.F. Joyce, Akzo Nobel Chemicals Inc., USA; and Y. Itabashi, Hokkaido University, Japan

Targeted Analysis of EPA- and DHA-Containing Triacylglycerols in Fish Oil By Tandem Mass Spectrometry. Jeremy E. Melanson[1], Lisandra Cubero-Herrera[1], Karen M. Glenn[1], Michael A. Potvin[2], Jaroslav A. Kralovec[2], Colin J. Barrow[2], [1]National Research Council of Canada, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada, [2]Ocean Nutrition Canada, Dartmouth, Nova Scotia, Canada

A novel workflow for targeted profiling of triacylglycerols (TAGs) in fish oil by tandem mass spectrometry will be described. Using liquid chromatography - quadrupole time-of-flight (QTOF) mass spectrometry, EPA and DHA-containing TAGs were analyzed by neutral loss-triggered product ion scans. Automated identification of TAGs was carried out using a combination of accurate mass of intact TAGs and their diacylglycerol fragment ions. Using this approach, over 50 unique EPA and DHA-containing TAGs were identified with a high degree of confidence. Additional TAGs were identified using ultra-high resolution mass spectrometry on fractions collected on-line with the LC-MS in complex regions of the chromatogram. A subset of the TAGs identified were selected for subsequent regiospecific analysis. Isomeric standards (-1,2 and -1,3) were synthesized and mixed at specific ratios to correlate the relative abundances of diglyceride fragments with the percentage of each isomeric form. Several complexation agents

were investigated for this analysis, with naturally occurring sodium adducts providing a high degree of regiospecific differentiation without the use of mobile phase additives.

Determination of Lignans in Flaxseed Using Liquid Chromatography with Time of Flight Mass Spectrometry (LC/TOF MS). Inna Popova[1], Clifford Hall[2], Alena Kubatova[1], [1]University of North Dakota, Grand Forks, ND, USA, [2]North Dakota State University, Fargo, ND, USA

Currently employed methods for characterization of lignans such as LC/MS/MS and GC/MS in selected ion monitoring mode (SIM) are of high sensitivity, but limited to identification of known species. Thus a new method of flaxseed-derived lignan determination was developed using HPLC with high resolution time of flight MS (TOF MS), optimized, and compared to two existing methods (HPLC/MS/MS and GC/MS). The limits of detection (LODs) for HPLC/TOF MS (0.002-0.043 pg) were comparable with those of the optimized and improved HPLC/MS/MS (0.001-0.015 pg), whereas the LODs for the optimized GC/MS were higher (0.02-3.0 pg, yet lower than reported before). Besides the optimization of determination method, several key flaxseed sample preparation parameters (including extraction, hydrolysis, and sample purification) were evaluated resulting in the development of efficient protocol for lignan quantification from flaxseed hulls and embryos. The results confirmed the importance of quantification of both aglycones and unhydrolyzed glucosides in order to obtain the total lignan estimates.

Direct Calibration Method for the Direct Determination of Lipids. Lisa M. Reilly[1], Gerard G. Dumancas[1], Neil Purdie[1], Betsy Alberty[2], [1]Oklahoma State University, Stillwater, OK, USA, [2]LipidX Technologies, Mill Valley, CA, USA

A simple assay was devised that involves a chemical test that is rapid, rugged, inexpensive, and specific to the unsaturated -C=C- bond that accomplishes, in a single assay, simultaneous measurements for the total cholesterol and six major polyunsaturated fatty acids (linoleic, linolenic, arachidonic, eicosapentaenoic, docosahexaenoic, and conjugated linoleic fatty acid methyl esters in chloroform). Products are intensely colored and the colors differ with the location and number of the -C=C- groups in the various molecules. In controlled preparations of binary, ternary, and quaternary, etc., mixtures, the colors are exactly additive as are the corresponding absorbance spectra. Spectral data are deconvoluted by a chemometric technique. Direct calibration method as applied in synthetic sera show less percent errors in one to four components but increase with increased number of components.

Spectrophotometric Estimation of Presence of Sesame Oil in Vegetable Oil Blends for Vanaspati. Ravindra Kulkarni, Shashikant Pardeshi, Dilip Hundiwale, North Maharashtra University, Jalgaon, Maharashtra, India

In India, it is mandatory to add sesame oil at minimum of 5% into permitted vegetable oil blends used in the manufacture of Vanaspati (hydrogenated product). Its presence is presently monitored qualitatively by performing Baudouin Color Test. In present research work, various blends of sesame oil with one or more than one hydrogenated oils such as groundnut, sunflower, soyabean, cottonseed and palmolein oil were prepared. λ_{max} was determined for Baudouin color developed for each blend followed by establishment of composition - optical density calibration curve. Linear programming was applied to obtain mathematical correlation between %sesame oil in vegetable oil blend and optical density in relation to Baudouin color. The method was established to assure exact quantification of presence of sesame oil in vanaspati which is one of the most expensive oil.

CANCELED - Spot Test for Quaternary Ammonium Compounds Determination. Fabio Roberto Borges[1], Matthieu Tubino[2], [1]Akzo Nobel, Itupeva, São Paulo, Brazil, [2]Universidade Estadual de Campinas, Campinas, São Paulo, Brazil

Current methods for determining the activity of long chain quaternary ammonium compounds (QACS) are based on dye partition, titration, or colorimetric analysis. The two major disadvantages of these methods are the disparity of partition coefficients among differently constituted QACS and the difficulty in detecting visual end points. Some potentiometric titration methods for QACS have been reported in literature. However, back titration techniques, as well as complicated electrode system, are generally involved.This work has as objective the development of a rapid and

simple-handling analytical method for determination of QACS in disinfectants solutions, and industrial wastes samples. The developed method is based on reaction proposed by Fritz Feigel, modified for application in a special system that permit the detection through reaction between QACs compounds and citric acid in acetic anhydride media.The new method allows rapid, accurate, selective and simple handling determination of QACs even in complex samples.

CANCELED - Qualitative and Quantitative Analysis of Phospholipids by TLC-ATR-FTIR. M.A. Hass, P. Youssef, J. Cross, Albany College of Pharmacy, Albany, NY, USA

An instrumental method coupling thin layer chromatography (TLC) to attenuated total reflectance (ATR) infrared spectroscopy (IR) was developed to qualitatively and quantitatively analyze phospholipids (PL). The objective was to develop a method that could be applied to analysis of phospholipid mixtures isolated from mammalian tissues. Previously published TLC methods were used to separate synthetic mixtures of commercial PL on silica gel plates. The PL separated on silica gel, were quantitatively isolated and analyzed by ATR-FTIR spectroscopy. Using the clearly resolved ester absorbance in the IR spectrum, standard curves were generated for each PL at concentrations in the range of 40ug/ml up to 200ug/ml. The method has been successfully applied to the separation and quantification of phospholipids isolated from rabbit urinary bladder tissue.

An Examination into Evaporative Light Scattering Detectors and their Use in Quantification of Triacylglycerols, Diacylglycerols, Monoacylglycerols, and Free Fatty Acids. Susan Seegers, Tiffanie West, Bunge North America, Bradley, IL, USA

Evaporative light scattering detectors have been named a universal detector for HPLC. This detector is very useful for the quantification of fats and oils using calibration curves. A study of the differences in two different detectors will compare the sensitivity, repeatability, linearity, and detection limits of two different evaporative light scattering detectors.

High Performance Size Exclusion Chromatography for Determination of Total Polar Compounds in Used Frying Oils. J.D. Caldwell, B.S. Cooke, M.K. Greer, Dallas Group, Jeffersonville, IN, USA

Direct injection of used frying oil samples into a high performance liquid chromatography - size exclusion chromatography system (HPLC-SEC or HPSEC) was compared to the AOCS silica gel column chromatography method for the determination of total polar compounds in used frying oils. In a direct comparison of the two methods for four different sets of used frying oils ranging from fresh to discard quality, the weight percent total polar compounds (%TPC) values determined by HPLC-SEC averaged 0.8% higher than the values determined by silica gel chromatography, with a standard deviation of 0.7%. These values compare favorably to the variability of the AOCS official method.The HPLC-SEC method is less time intensive and uses much less organic solvent than standard column chromatography methods. %TPC can be determined in about one hour. The HPLC-SEC method is very similar to AOCS Official Method Cd 22-91 for determination of polymerized triacylglycerols (TAG), and thus also separates and quantifies polymerized TAG.

Characterization of Biofuels Using Gas Chromatography (GC) with Simultaneous FID and MS Detections. Jana Stávová, Wayne Seames, Danese Stahl, Alena Kubátová, University of North Dakota, Grand Forks, ND, USA

Extensive work has been previously performed to provide detailed characterization of petroleum-based fuels resulting in the establishment of ASTM methods. However, these techniques are not sufficient when evaluating the quality of biofuels. To address this deficiency, a GC method with simultaneous FID and MS detection was developed for characterization of pyrolyzed/distilled soybean and canola oils to obtain information about the biofuel constituents and/or potential economically favorable by-products. The temperature program for a programmed temperature vaporization injection as well as for the column separation was optimized with the aim to minimize discrimination and to achieve efficient separation within a reasonable time period. Due to the complexity of pyrolysis-based biofuels the calibration with individual standards is not feasible. Therefore, a calibration standard representing various classes of compounds and accounting for the injection discrimination was designed. The developed method enabled the identification and quantification of over 200 compounds including low-boiling substances in pyrolysis-based biofuels. Thus, approximately 50% (w/w) of the analytes were resolved and identified. To account for the remaining GC-

elutable species, further method development was focused on the quantification of unidentified, unresolved, and polar species.

The Determination of the Biodiesels Heating Values Using Gas Chromatography. S. Peres[1], T. Nogueira[1], A. Schuler[2], C.H.T. Almeida[1], [1]Universidade de Pernambuco, Recife - Pernambuco, Brazil, [2]Universidade Federal de Pernambuco, Recife - Pernambuco, Brazil

The higher heating values (HHV) of biodiesels generated using animals fats and vegetable oils can be determined using a chromatogram produced in a gas chromatograph. The chromatogram determines the percentage of the fatty acid esthers present in the biodiesel (BD) analyzed. A data bank was created using the heating values of the following fatty acids methilic esthers : caprilic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid. Hence, it was created a formula HHV (MJ/kg) = Σ (% ME_i x HHV_i)/ 100% , in which% ME - is the percentage of each esther in the BD;HHV - Higher Heating Value (HHV) of the esther present in the BD.The HHV values of the biodiesels of castor oil, jatropha curcas oil, chicken oil , soy beans oil and beef tallow were obtained using a IKA-C2000 calorimeter at the Combustion and Fuel Laboratory -POLICOM at the University of Pernambuco.The values of the theoretical HHV, calculated using the formula and the percentage of the FAMEs obtained in the chromatogram, were compared with the ones obtained experimentally in the calorimeter. The maximum error difference between the theoretical and the experimental value for these BD were within the 2% range.

Nondestructive Estimation of Oil and Moisture Content using NIR Spectroscopy in Valencia and Virginia Peanuts. Jaya Sundaram[1], Chari Kandala[1], Ronald Holser[2], Bob Windham[2], Sandra Kays[2], Christopher Butts[1], Marshall Lamb[1], [1]USDA, ARS, Gawson, GA, USA, [2]USDA, ARS, Athens, GA, USA

Oil and moisture content of peanuts are important factors in peanut grading. A method by which these parameters could be measured rapidly and nondestructively for in-shell peanuts would be useful for the industry. An attempt was made to measure oil and moisture content of Valencia and Virginia type peanuts along with their shell using NIR reflectance spectroscopy. Light reflected from peanut samples (150 g) at different moisture levels between 6 and 26% was collected in the wavelength ranges from 400 to 2498 nm. Average values of moisture and total oil contents of all samples determined by standard air-oven and Soxtec methods respectively, and fatty acids measured using gas chromatography were taken as reference values. Partial Least Squares analysis was done with different pretreatments on NIR data and respective reference values to develop several prediction models. These models were tested to predict oil and moisture contents and fatty acids of a different sets of peanuts. The best pretreatment and corresponding model was selected based on the R square and Standard Error of Prediction. The predicted values were compared with their standard values. This method was found to be useful in predicting oil and moisture contents and fatty acids of the peanuts tested.

Montana Mothers' Milk: A Comparison of Native American and Caucasian Diets and Milk Lipid Composition in Montana Today. H. Knapp, G. Whittenberg, B. Buehler, Billings Clinic Research Center, Billings, MT, USA

The traditional Native American diet included DHA-rich meat, and shorter-chain N-3 FA from vegetable sources. There were no longer-chain trans-FA; these are being removed from many Western diets, but a decrease in dietary trans-FA has not occured yet in Montana. Examining the composition of FA and sterols in diets and milk prior to a reduction in trans-FA mayd be of interest, in view of hypotheses that these food items may impact long term health. Dietary histories and milk samples were obtained from 62 Caucasians, 24 Native Americans on the Crow reservation, and 24 Native Americans living in towns off the Reservation. The milk content of tran-FA and phytosterols did correlate with diet. The N-3 PUFA of Caucasian and Native American mothers living in towns were similar to each other and to other surveys of milk in North America, but the samples from Native Americans living on the reservations were strikingly lower in DHA and EPA. Montana buffalo samples had 10X the DHA content of beef from feed-lots. We are working with Tribal Health groups to provide DHA supplements to lactating women and verify that their milk DHA content has been brought into the range of other Montana mothers.

AFTERNOON

ANA 5: General Analytical II
Chair(s): W.C. Byrdwell, USDA, ARS, BHNRC, FCMDL, USA

Novel Diagnostic Tool for Quantitating Cholesterol, Omega-3, and Omega-6 Polyunsaturated Fatty Acids to Support Maternal/Infant Health, Cardiovascular Status, and Obesity-related Diseases. Neil Purdie[1], Lisa M. Reilly[1], Gerard G. Dumancas[1], Betsy Alberty[2], [1]Oklahoma State University, Stillwater, OK, USA, [2]LipidX Technologies, Mill Valley CA, USA

A multiple-analyte/multiple-wavelength assay that uses a mature patented color reagent is introduced that is selective to the (-CH=CH-CH2-) functional group common to the cholesterol, and polyunsaturated fatty acids (PUFAs) structural formulas. The particular lipids targeted for measurement are cholesterol, and the esters of the omega-6 acids (linoleic, conjugated linoleic, and arachidonic) and omega-3 acids, (linolenic, EPA, and DHA) in reagent grade chloroform. The assay requires no separation or derivatization steps. Products are intensely colored and the colors differ with the location and number of the (-CH=CH-CH2-) groups in the various molecules. Absorbance measurements were made at 2 nm intervals over the 350-650 nm wavelength range. The elapsed time for a single test is 15 minutes, compared to 2.5 days for GC/MS. Four multivariate analysis options: Principal Component Analysis (PCA) Partial Least Squares (PLS), Generalized Standard Addition Method (GSAM), and Artificial Neural Networking (ANN) were evaluated. Average errors between prepared and experimentally measured amounts are all on the order of 1-10% indicative of a rugged assay procedure. The PCA/cluster test also conceivably identified the dyslipidemias.

Detection of Ethyl Esters in Fish Oil Supplements Using SPME and GCMS. J.C. Sullivan, S.M. Budge, Dalhousie University, Halifax, NS, Canada

The long chain polyunsaturated fatty acids found in fish oil, specifically EPA and DHA play an important part in human health. As a result, fish oil supplements are commonly consumed by people around the world. Supplements in the form of triacylglycerols can be sold at a premium price, compared to those in the ethyl ester forms, though it can be difficult to determine the type of supplement by product label descriptions. Producers of triacylglycerol supplements require a simple, rapid method to determine the authenticity of their raw material. Here, we describe a method to quantify ethyl esters in fish oil using solid phase microextraction (SPME) headspace analysis and GCMS.

CANCELED - Antioxidant Activity of Olive Leaves Extracts from Greek Cultivars. K. Kiritsakis[1], C. Kontogiorgis[1,2], D. Hadjipavlou-Litina[2], A. Moustakas[3], A. Kiritsakis[1], [1]Department of Food Technology, School of Food Technology and Nutrition, Thessaloniki, Greece, [2]Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University, Thessaloniki, Greece, [3]University of Wageningen, The Netherlands

The composition of olive tree leaves of three Greek cultivars: koroneiki, megaritiki, and kalamon was studied by mass spectroscopy and the antioxidant activity of olive tree leaves extracts, obtained by using four solvents (petrol ether, dichloromethane, methanol and methanol/water 60%) was evaluated. Also the antioxidant activity of olive tree leaves extracts from the cultivar voliotiki was studied. Ten, in total, components were determined in the leaves of the three cultivars by mass spectroscopy as follows: For the cultivars koroneiki and kalamon, secologanoside, demethyloleuropein, oleuropein diglucoside, luteolin-7-O-glucoside, rutin, oleuropein, oleuroside, quercetrin, ligstroside, and verbascoside. For the cultivar megaritiki: secologanoside, demethyloleuropein, oleuropein diglucoside, luteolin7-O-glucoside, oleuropein, oleuroside, 7 quercetrin, and ligstroside were determined. In all three cultivars, the component oleuropein existed in the highest concentration.The solvent used to obtain the olive tree leaves extracts from the four cultivars affected the total amount of phenolic compounds determined. When methanol/water (60%) was used more phenolic compounds were determined. The total phenols determined by using the four solvents was, 5464, 6094, 5579 καÎ¹ 6196 mg/Kg (mg gallic acid /kg dried olive leaves) for the cultivars voliotiki, megaritiki, kalamon, and koroneiki, respectively. Extracts were tested for their possible antioxidant activities as interaction with the stable free radical DPPH. In all cases methanol/water and water extracts presented significant antioxidant activity. Extracts have also been tested for their inhibitory activity against soybean lipoxygenase. Again methanol/water and water extracts showed significant inhibitory activity, although there are some differences among the extracts of the different types of olives, the solvent used to obtain olive leaves extracts and the amount of the extract, influenced their

inhibitory activity. A correlation between radical scavenging capacities of extracts with total phenol compounds content was observed. Free radicals play a negative role in inflammatory process. Consequently, leave extracts with antioxidant/scavenging properties could be expected to offer protection in rheumatoid arthritis and inflammation.

Paper rescheduled to session ANA 4.1, Wednesday 11:40 am. Montana Mothers' Milk: A Comparison of Native American and Caucasian Diets and Milk Lipid Composition in Montana Today. H. Knapp

Evaluation of Phenolic Compounds and Tocopherols in Some Iranian Olive Oils by High Performance Liquid Chromatography. Maryam Fahimdanesh[1], Lanfranco S. Conte[2], [1]Islamic Azad University, Shahryar shahr-e-Qods Branch, Tehran, Iran, [2]Dept. of Food Science, University of Udine, Italy

The amount and composition of tocopherols and phenols, two main natural antioxidants in olive oil, have been evaluated and analysed by HPLC in seven different brands of Iranian olive oil.Results showed that phenol and tocopherol amounts, because of long time and high temperature of malaxation and also type of extraction or even type of variety, polyphenols and tocopherols content of Iranian olive oil are low and were put on the category of low polyphenol olive oils and seems they donâ??t have nutritional value as virgin olive oil.

Effect of Shallow-Frying on Recovery of Fat-Soluble Vitamins and Polyunsaturated Fatty Acids in Fat-based Cooking Products. K. Hrncirik, Unilever R&D, Vlaardingen, The Netherlands

New trends in the development of products suitable for modern cooking, driven by improved nutritional benefits for consumers, are reflected by the implementation of several (re-)formulation steps, namely the reduction of saturated fat content and/or fortification with relevant nutrients. However, cooking, and in particular frying, may lead to a certain decrease of the nutrients present in the cooking (frying) medium affecting nutritional value of cooked (fried) food. In this study the recovery of fat-soluble vitamins and polyunsaturated fatty acids was investigated in several products specifically designed for cooking (liquid margarines, stick margarines) varying in composition and fat content under the conditions of both simulated shallow-frying and real food shallow-frying. The findings are discussed in the context of the dietary intake of these nutrients.

Analytical Posters

Chair(s): J.T. Lin, USDA, USA

Reversed-phase HPLC Separation of Reverse Isomers of 1,2-diacylglycerols on Polymeric Octadecyl Silica Phases.
Y. Itabashi[1], C. Aizawa[1], A. Kuksis[2], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan, [2]Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada

We investigated the separation of the regioisomers (reverse isomers) of 1,2-diacyl-*rac*-glycerols by reversed-phase HPLC on different types of stationary phases, specifically monomeric/polymeric and endcapped/non-endcapped octadecyl silica (ODS) phases. The reverse isomers of 1,2-diacylglycerols, which have various pairs of acyl groups, were chromatographed as UV-sensitive and non-sensitive derivatives using acetonitrile or methanol as the mobile phase. In addition to excellent resolution of the reverse isomers with very different pairs of acyl groups, complete resolution of the reverse isomers with minor differences in chain lengths and degree of unsaturation, e.g., 16:0-18:0 and 18:0-18:1, was obtained for UV-sensitive derivatives, such as 3,5-dinitrophenylurethanes, within the appropriate retention times on polymeric ODS columns (150 x 4.6 mm i.d., 3 μm particles). The highly dense packing of octadecyl groups in polymeric ODS and the planar structure of the aromatic ring in the derivatives may contribute to the reverse isomer separation. The endcapping did not significantly affect the separation but markedly lengthened the elution times of the derivatives. The present study demonstrates that polymeric ODS phases are more suitable for reverse isomer

separation than are monomeric phases.

Determination of Alifatic and Triterpenic Hydrocarbons in Vegetable Oils.

A. Cert, M.C. Pérez-Camino, W. Moreda, Instituto de la Grasa (CSIC), Av. Padre García Tejero, 4, 41012-Seville, Spain

In April 2008, European Community declared a food alert communication over the entrance of a 125 t of contaminated crude sunflower oil coming from Ucraine. From the beginning of the year, 39.305 t of sunflower oil were imported from Ucraine. Spain consume 310.000 t of sunflower per year, that is the 34% of oil comsumed in Spain. Imported crude oils are not authorized for direct human comsumption and the European Legislation set the control responsability to the importing companies, and therefore there was a lack of control.The analysis performed to the crude oils conclude that the contaminant was mineral oil of high viscosity, constituted mainly by aliphatic hydrocarbons. This mineral oils are less toxic than the medium or low viscosity and its acceptable daily intake is higher than the earlier.Before the alert, there wasn´t any official or standardised method to determine aliphatic hydrocarbons in vegetable oils and it was neccessary to develop and optimize a simple and realiable method to control the possible contamination of vegetable oils.The method consist in the fractionation of the oil by a silica-gel low pressure glass column with n-hexane and the fraction directly analyzed by capillary gas chromatography equipped with on-column injection and flame ionization detector.The proposed method, determine the saturated and unsaturated aliphatic hydrocarbons, as well as the triterpenic with carbon number from C10 to C56 in vegetable oils. The method can be used to determine the total hydrocarbons content, including the natural origin, the unsaturated and triterpenic produced during refining and those coming from contamination with mineral oils, gasoil or parafins.The method also allow the solely determination of the saturated hydrocarbons using in the fractionation step a silver nitrate impregnated silica-gel column. This procedure permit the evaluation of the typical hydrocarbons coming from mineral oils with LOQ of 20 mg/kg.

Complete Dissolution of Microencapsulated Products for Better Determination of Thiobarbituric Acid Reactive Substances.

Zhixiong Hu[1,2], Weinong Zhang[1,2], Qixin Zhong[1], [1]University of Tennessee, Knoxville, TN, USA, [2]Wuhan Polytechnic University, Wuhan, Hubei, China

Food samples are usually extracted or distillated before quantification of thiobarbituric acid reactive substances (TBARS). The extraction or distillation requires a large amount of sample, is tedious, and causes experimental errors. The goal of this work was to improve the quantification of TBARS due to oxidation of microencapsulated polyunsaturated fatty acids that only had limited sample quantities. We found that a ternary mixture of 1-butanol, isopropanol, and water at a proportion of 100: 100: 50 (v/v/v) was able to dissolve fish oil as well appreciable amounts of several common encapsulation carrier materials such as corn zein, cyclodextrins, chitosan, whey protein isolate. After reaction with thiobarbituric acid, solutions of the tested encapsulation materials showed a low absorbance at the test wavelength of 532 nm. When 1,1,3,3,-tetra methoxy propane was used as a thiobarbituric acid reactive standard at 0.5-13 ppm, the standard curve corresponded to a R2 value of 99.86% after linear regression. When a sample of corn zein microcapsules with encapsulated fish oil was used, only 0.020-0.030 g of sample was needed to obtain reliable and repeatable TBARS values. The modified method also had a good precision and accuracy and thus showed characteristics that are feasible for many applications.

The Abnormal Accumulation of Metabolites in Neutral Lipid Storage Myopathy Caused by Adipose Triacylglycerol Lipase Mutation.

Nobuhiro Zaima[1], Yuki Sugiura[2,1], Aya Ohuma[3], Satomi Okahashi[3], Satoru Noguchi[3], Ichizo Nishino[3], Tohru Ibi[4], Fumiko Ozawa[4], Ko Sahashi[4], Mitsutoshi Setou[1,5], [1]Hamamatsu Medical School, Hamamatsu, Sizuoka, Japan, [2]Tokyo Institute of Technology, Yokohama, Kanagawa, Japan, [3]National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan, [4]Aichi Medical University, Nagakute, Aichi, Japan, [5]Mitsubishi Kagaku Institute of Life Sciences, Machida, Tokyo, Japan

Neutral lipid storage myopathy (NLSM) is characterized by the presence of triglyceride-containing cytoplasmic droplets in muscle tissues. Several patients with NLSM have been described, although in most cases the metabolic

defect is unclear. The imaging mass spectrometry, a new tool for the study of biological systems, and multivariate analysis were applied to the investigation of NLSM caused by ATGL mutations, and several molecules were successfully identified.

Quantification of Gangliosides and Phospholipids in Milk Fat Globule Membrane.
F. Giuffrida, A Lardeau, C. Cotting, S. Marmesat, F. Vanrobaeys, B. Flück, M. Braum, Nestlé Research Centre, Lausanne, Switzerland

In milk, fat is secreted in the form of fat globules mainly composed by triacylglycerol (98%) which are enveloped by a biological membrane named Milk Fat Globule Membrane (MFGM).The MFGM consists of a mixture of glycoproteins, triacylglycerols, glycerophospholipids, sphingolipids, glycolipids, cholesterol, enzymes and other minor components. MFGM are associated with several health benefits and phospholipids and gangliosides are believed to have an important role in it; therefore their accurate quantification is needed.In this study, phospholipides and gangliosides were extracted using chloroform and methanol and further purified by phase partitioning. Phospholipid families, were quantified by High Performance Liquid Chromatography coupled to Evaporative Light Scattering Detector and absence of interferences was monitored by LC coupled to electrospray mass spectrometry. Gangliosides were quantified by HPLC coupled to a fluorescent detector, after realising of sialic acid (N-acetylneuraminic acid) and its reaction with a fluorescent dye. Gangliosides results were expressed as total Lipid Bound to Sialic Acid.Analytical methods were validated and repeatability, intermediary reproducibility, recovery and uncertainty were calculated. In finished products, phospholipid families and total gangliosides could be quantified at level of 0.04 g/100g product with a relative expanded uncertainty of maximum 25%.

GC-FID/MS Analysis of Monocarboxylic Acids and Glycerides in Biofuels.
Ganna Baglayeva, Jana Stávová, Eric Hellrung, Bonnie Diep, Wayne Seames, Alena Kubátová, University of North Dakota, Grand Forks, ND, USA

One of the methods for biofuel generation is thermal or catalytic cracking of triglycerides (TGs) present in crop oils. Some components remaining or generated within cracking process such as uncracked or partially cracked TGs (e.g., di- [DGs] and monoglycerides [MGs]) along with monocarboxylic acids (MCAs) result in poor biofuel characteristics (e.g., causing corrosion and filter clogging). However, current ASTM method D6584 (designed for biodiesel) determines only glycerides using a gas chromatography (GC) with a flame ionization detection (FID) employing a high temperature column. A method for simultaneous identification and quantification of MCAs (ranging form C1-C18), TGs, DGs and MGs using GC coupled with both FID and mass spectrometric (MS) detections (GC-FID/MS) was developed. Two derivatization methods were evaluted using N-methyl-N-(trimethylsilyl)trifluoroacetamide and trimethylsilyl N,N-dimethyl carbamate. The injection conditions (comparison of PTV and on column injections) and temperature program were optimized in order to achieve sufficient separation of MCAs, glycerides, ,solvents, and the derivatization agents. The developed method was applied for analysis of biofuels generated from soybean and canola methyl esters, and soybean and canola oil.

High-throughput Techniques for Algal Biofuels Feedstock Analysis: Lipid Fingerprinting by Near Infrared and Molecular Beam Mass Spectroscopy.
L.M.L. Laurens, D. Crocker, E. Wolfrum, National Renewable Energy Laboratory, Golden, Colorado, USA

A detailed, quantitative, analysis of lipids in microalgae is required to assess the suitability of the algal biomass feedstock for biodiesel production. Algae that are not cultured for high lipid content typically consist on average of equal amounts of phospho- and glyco-lipids (25%) and slightly more neutral lipids (40%). We are developing high-throughput techniques for lipid analysis in microalgae based on near infrared (NIR) and pyrolysis-molecular beam mass spectroscopy (py-MBMS). Both spectroscopic techniques rely on multivariate data analysis methods (or chemometrics) to interpret the collected spectra and derive quantitative information. We are presenting work on the development of robust chemical methods as well as on building the prediction models of lipid concentration of microalgal biomass spiked with known concentrations of lipid spikes. The chemical methods we are optimizing include fast, high-throughput techniques for extraction and profiling of microalgal lipids, by means of accelerated solvent extractions, rapid derivatization methods and ultra-fast GC. Our data indicates that it is possible to correlate and predict quantitative information with NIR and py-MBMS spectroscopic information.

An Approach to Validating Calibration Transfer of Standard Methods using Global Calibrations for IV and %Trans.

M.B. Simpson, J. Labrecque, J.-L. Flandin, H. Buijs, ABB Analytical, Québec, Canada

In the application of near-infrared spectroscopy to complex quantitative measurements, as a substitute for slower and more expensive wet-chemical procedures, the value of the intellectual property embedded in developed and validated calibration models often far outweighs the mere capital cost of the equipment. In many cases large-scale application databases will have been developed over a long time period, and when, as is inevitable, a manufacturer releases a new near-infrared spectrometer design, it is essential that a reliable and credible methodology exists to validate the performance of calibrations with earlier and later models.FT-NIR analyzers have been the work-horses in many industrial laboratories for over 15 years, covering applications in gasoline, diesel, naphtha, reformate, oils and fats, polyols, polyurethanes, general functional group based analyses and altogether a wide range of quantitative QA applications. A key feature of this type of analyzer has been its long-term stability, and its ability reliably to reproduce a given spectral measurement over time within a very high degree of precision, thus assuring easy calibration model development and ruggedness. Moreover, a primary goal engineering design in FT-NIR optics has been to achieve a similar level of accuracy between instruments, so that any one analyzer records a given spectrum with an extremely close agreement with the spectrum of the given sample on another similar unit.Many users have a large installed base of analyzers running complex QA-type applications with multiple installed models, all based on this long term analyzer stability, and interoperability between analyzer units, both in the laboratory and for analyzers working on-line in process optimization.When new FT-NIR analyzer designs and products are introduced a clear concern will be to demonstrate that these new analyzers exhibit complete spectral equivalence and transferability of calibrations with respect to the previous units. This short paper presents both a methodology and example data to demonstrate how that requirement can be tested and confirmed.

ATR-FTIR Measurement of Conjugated Linoleic Acid (CLA) in CLA- rich Soybean Oil.

Jeta Kadamne, Vishal Jain, Andrew Proctor, University of Arkansas, Fayetteville, Arkansas, USA

Conjugated linoleic acid isomers in vegetable oils are measured as fatty acid methyl esters by GC-FID. Sample analysis is expensive and time consuming and not suited to real time detection. However, ATR-FTIR measurement may be a more rapid, less expensive method of conducting this analysis. The objective of this study was to develop an ATR-FTIR method to rapidly determine CLA isomers in CLA rich soy oil. Soy oil with 0.1-10% total CLA was obtained placing 7 mL of fully refined soy oil in 9mL vial and subjecting them to UV photoisomerization. Duplicate oil samples were taken every 30 minutes for 24 hours. For each sample, 6 replicate CLA analyses were performed as methyl esters by GC-FID. FTIR spectra of each duplicate oil sample were also collected with 128 scans per sample. Unscrambler software was used as a chemometric tool for Partial least squares (PLS) regression analysis using the spectra of each sample. The technique predicted the values of c-9 t-11 octadecadienoic acid (R^2=0.96), t-9 c-12 octadecadienoic acid and c-10 t-12 octadecadienoic acid (R^2=0.90), t-10 c-12 octadecadienoic acid (R^2= 0.96), t-9 t-11; t-10 t-12; t-11 t-13 octadecadienoic acid (R^2= 0.97), mono trans CLA isomers (R^2=0.93), and total CLA (R^2=0.97). The average RMSE value for all the isomers was 0.23. Each ATR-FTIR measurement took 8 min to complete. This ATR-FTIR technique could be a reliable, rapid alternative for determining CLA isomers in CLA-rich oil.

Analysis of Cuticular Hydrocarbons of Triatoma Longipennis by GC-FID.

J.A. Noriega-Rodríguez, R. De la Rosa-López, R.E. Lugo-Sepúlveda, E.A. Paredes-Gonzalez, Departamento de Ciencias Químico Biológicas y Agropecuarias Universidad de Sonora URN, H. Caborca, Sonora. Mexico

In this work, cuticular hydrocarbons (CH) from primary and secondary wings of Triatoma longipennis were identified and quantified in order to compare with different triatomineos species. Lipids were extracted with hexane (1.5 mL) from wings. Hydrocarbons (HC) were purified in a glass chromatographic column packed with 1.5 g of silica-gel (70-230 mesh), eluted with hexane (HPLC grade). Eluted fraction (5 µL) were injected (300°C) to a gas chromatograph (VARIAN CP-3800), provided with a Factor Four column (30 m x 0.32 mm x 0.25 µm), and separated by a temperature program. Detection was done by flame ionization (FID, 300°C), using high pure helium as carrier gas.

Identification and quantification of HC was conducted with the Kovats Index (KI) and certified standards (C19 - CH40, ALDRICH), respectively. A total of 40 HC (14 lineal, 26 ramified) in male, and 38 HC (14 lineal, 24 ramified) in female were found. The characteristic CH, that represents taxonomy markers of T. longipennis species, were lineal HC23 (1.78%), HC25 (5.31%), HC27 (8.86%), HC29 (16.30%), HC31 (13.70%), HC33 (5.03%). Only CH27 shows significative difference among sexes (5.74% male, 11.97% female). The characteristic profile of CH for T. longipennis specie, can serve to the identification between species and to compare vectors with parasite infections.

Direct Enantiomer Separation of Mono- and Diacylglycerols by Reversed-phase HPLC on Polysaccharide-type Chiral Stationary Phases.

Y. Itabashi[1], A. Kuksis[2], [1]Faculty of Fisheries Sciences, Hokkaido University, Hakodate, Hokkaido, Japan, [2]Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada

We investigated the direct enantiomer separation of 1-monoacyl- (MAG) and 1,2-diacylglycerols (DAG) using normal-phase (NP) and reversed-phase (RP) HPLC with polysaccharide-type chiral stationary phases (CSP). The chiral NP-HPLC gave complete resolution of enantiomers and partial resolution of their molecular species on amylose tris(3,5-dimethylphenylcarbamate) using hexane containing a small amount of MeOH as the mobile phase. Much better separation of both enantiomers and molecular species was achieved by chiral RP-HPLC on the same CSP and using ACN containing 10-30% MeOH as the mobile phase. The reversible formation of diastereomeric hydrogen bonds between the solutes and the CSP was thought to contribute to the effective enantiomer separation, since poorer separation was observed for enantiomeric 1-monoalkylglycerols. Chiral RP-HPLC/ESI-MS showed both prominent $[M+Na]^+$ and weak $[M-RCOO]^+$ ions, which allowed for the identification of individual molecular species. The present study demonstrates that chiral RP-HPLC gives much better resolution of the enantiomers and molecular species of MAG and DAG than the commonly used chiral NP-HPLC. Furthermore, chiral RP-HPLC permits the simultaneous determination of the configuration and molecular species of natural MAG and DAG.

Analysis of Furan Fatty Acids.

N. Hinrichsen, H. Steinhart, University of Hamburg, Grindelallee 117, 20146 Hamburg, Germany

Furan fatty acids occur in various biological matrices and develop during the oxidation of conjugated linoleic acids (CLA).For the analysis of furan fatty acids various chromatographic methods were applied. For the identification of furan fatty acids besides conjugated linoleic acids and other fatty acids, silver ion high performance liquid chromatography (HPLC) was used. The chromatographic system was coupled to a quadrupole iontrap. With this detection also furan fatty acids could be identified, for which no analytical standards were available. As the silver ion high performance liquid chromatographic methods only offered average possibilities for the quantification of the analytes, a GC-method was developed. The separation was carried out on a 100 m capillary column and was applicable for the quantification of the sum of all furan fatty acids. For the proper assignment of all fatty acid peaks also a GC-MS method was developed. For the overall analysis of furan fatty acids a combination of silver ion HPLC with mass selective detection and GC with flame ionization detection proved best. Samples of different foods have been analysed. Furan fatty acids were found in all samples in small amounts. Exceptionally high amounts were found in parsley and in cod liver oil. The most prominent furan fatty acid in all samples was 12,15-Epoxy-13,14-dimethyleicosa-12,14-dienic acid.

Sensory Analysis of Edible Oils with an Electronic Nose.

Michel Manach, Jean-Christophe Mifsud, George Foster, Marion Bonnefille, Alpha MOS, Hanover, MD, USA

PurposeFats and oils play a key role in food preparation. In order to guarantee an appropriate flavor and a conform quality, it is crucial for both manufacturers and users to test the organoleptic features of these oils or detect possible contamination. Currently, food products are assessed by human sensory panels or classical analytical techniques such as GC or GC/MS. However, these methods are time-consuming and sensory tests can be unpleasant for panelists. MethodA metal oxide sensor based Electronic Nose was used to evaluate the sensory quality of oil samples with different known grades. The objective of the study was to compare and differentiate the aroma of the various samples, then to identify the quality of blind samples. The industrial goal was to set-up a quality control model for a rapid assessment of production batches.ResultsInstrumental analysis showed a clear differentiation of the oil qualities, in

conformity with known data. A quality control chart was set-up and allowed to determine if the blind samples were conform or out of specifications. ConclusionThe electronic nose can bring the ability to rapidly determine the quality of oil products, while assuring the consistency of testing methods. The E-Nose is a convenient tool to help companies optimize time and costs of analysis chain process.

Detection of Unpleasant Odor in Omega-3 with an Electronic Nose.

Michel Manach, Jean-Christophe Mifsud, George Foster, Marion Bonnefille, Alpha MOS, Hanover, MD, USA

PurposeOmega-3s are increasingly used in the food & beverage industry. However, this ingredient can bring an undesirable odor / aroma to the final product. Consequently, it is of utmost importance for both omega-3 manufacturers and users to monitor the organoleptic features of this ingredient. Usually, food ingredients are evaluated by human sensory panels, which can be unpleasant and time-consuming. MethodRecently an electronic nose based on ultra fast Gas Chromatography, the HERACLES, has been used to assess Omega-3 sensory quality and to perform aging studies.Various samples of Omega-3 powder were both assessed by sensory panel and HERACLES Electronic Nose at 2 different times: t = 0 (freshly produced) and t = 2 months under various storage conditions (air or nitrogen). The scoring scale ranked between values 1 and 2.ResultsAfter building a calibration curve with the HERACLES, the model showed a good correlation with sensory panel scores (correlation coefficient > 99%) and predicted unknown powderâ??s scores.ConclusionThe HERACLES electronic nose can bring the ability to screen a large number of samples for quality analysis. Thus, companies can reduce the time and costs of the analysis chain process, and significantly improve the product quality consistency.

Analysis of Soybean Oil Refinery By-Products by Fourier Transform Near Infrared Spectroscopy.

Mark Gulden[1], Hui Li[2], Robert Cocciardi[2], [1]CHS Inc., Mankato, MN, USA, [2]Bruker Optics, Inc., Billerica, MA, USA

For lecithin production, percent acetone insolubles, moisture and acid value are common quality assessment parameters. Traditionally, these parameters are measured using time consuming and tedious wet chemical analyses such as gravimetric, titrimetric and extraction methods that can take up to several hours per analysis.Infrared spectroscopy has been successfully employed in food industry as QA/QC tool. The NIR, in particular, is optimal for at-line and in-line monitoring with the QC applications due to fundamental benefits such as:·low to no cost consumables - solvents, columns, reagents·fast analysis - generally less than 10 seconds measurement time ·multiple components per analysis·elimination of sample preparation time·elimination of many sources of systematic errorFourier transform near infrared (FT-NIR) spectroscopy was used to analyze multiple measurement parameters in lecithin production samples and soybean oil refining by-products. The development of acetone insolubles, acid and moisture calibrations of lecithin products will be discussed in this presentation. NIR spectroscopy has also proven to be a useful tool in the analysis of soybean oil refining by-products, including acidulated soapstock, fatty acids and black oil for measuring acid value, moisture and iodine value.

Triacylglycerol Profiling of Fish Oil by Liquid Chromatography - Thermospray Mass Spectrometry.

K.M. Glenn[1], J.E. Melanson[1], M.A. Potvin[2], J.A. Kralovec[2], C.J. Barrow[2], [1]National Research Council of Canada, Institute for Marine Biosciences, Halifax, Nova Scotia, Canada, [2]Ocean Nutrition Canada, Dartmouth, Nova Scotia, Canada

In this study, the triacylglycerol (TAG) content of fish oil was characterized by liquid chromatography - mass spectrometry (LC-MS) using a quadrupole time-of-flight (QTOF) mass spectrometer. Silver-ion cartridges were used to fractionate the fish oil based on their degree of saturation to reduce sample complexity prior to LC-MS. A comparison of ionization modes for LC-MS was performed, including electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization (APPI), and thermospray ionization (TSI). Using an APCI probe at 500°C without any corona discharge, thermospray ionization proved to be the best suited for TAG analysis. TSI generated the most uniform molar response for all TAGs, yielded simple mass spectra with minimal fragmentation, and did not require mobile phase additives for adduct formation. EPA and DHA-containing TAGs were analyzed by neutral loss-triggered product ion scans. Assignments of TAGs were made using a combination of accurate mass of intact TAGs and their diacylglycerol fragmentation patterns. Using this approach, over 50 different EPA and DHA-containing TAGs were identified with a high degree of confidence. Finally, the technique was applied

to compare the TAG profile in a natural versus a re-esterified oil.

An Improved Method for Accurately Determining Medium- and Long-chain FAMEs on Gas Chromatography.
Z. Xu[1], K.A. Harvey[1], T. Pavlina[2], G. Dutot[3], G.P. Zaloga[2], R.A. Siddiqui[1], [1]Methodist Research Institute, Indianapolis, IN, USA, [2]Baxter Healthcare, Deerfield, IL, USA, [3]Baxter S.A.S, Maurepas-Cedex, France

The existing protocols for analyzing FAMEs using a one-step acetyl chloride (AC) catalyzed transmethylation and extraction procedure cannot accurately determine the medium- and long-chain fatty acids simultaneously in clinical (enteral, parenteral) formulations. For example: i.) addition of AC at room temperature generates an exothermic reaction that often results in loss of sample and possible injury to the analyst, ii.) certain PUFAs are less stable at elevated temperatures during the transmethylation and contribute to the over-estimation of the C16:0 and C18:1 fatty acids, and iii.) the Flame-Ionization Detector response varies depending on the carbon chain length of the fatty acids, that consequently impacts on the underestimation of medium-chain fatty acid (C6-C10) recoveries. To overcome these deficiencies and accurately determine FAMEs, we have developed an improved one-step transmethylation method that employs the addition of AC in tubes kept on a dry ice bath, the transmethylation at room temperature, and the data analysis using response correction factors. Using this modified protocol, we determined the fatty acid composition of a lipid emulsion (Lipidem®) on a Shimadzu GC2010 system. Our data suggest that the improved method can be easily used to accurately determine fatty acids (C6-C24) in functional foods and lipid emulsions.

Chromatographic Separation of Non Conjugated 18:2 Fatty Acid Methyl Esters.
Ali Reza Fardin Kia, Jeanne I. Rader, Pierluigi Delmonte, U.S. Food and Drug Administration, College Park, MD, USA

Over last decade several countries implemented new regulations regarding limitations and labeling of the trans fat (tFA) content of foods and dietary supplements. The fFA content of a food sample is generally calculated from its fatty acid profile determined by gas chromatography (GC). The current study focuses on the preparation of positional and geometrical isomers of 18:2 FA, pure or in mixtures, that can be used as reference materials for the determination of 18:2 FA in food. A mixture containing positional and geometrical isomers of 18:2 FA is produced by addition of hydrobromic acid to linoleic acid, followed by its elimination with hot alkali. The products of reaction contain conjugated and non-conjugated 18:2 FA with double bond positions ranging from 8 to 13, in cis and trans configuration. Pure positional/geometric isomers are obtained by a combination of silver ion HPLC and sub-ambient temperature reversed phase HPLC. The determination of the position of the FA double bond is made by GC-acetonitrile covalent adduct chemical ionization MS/MS. The determination of the geometrical configuration of the FA double bonds, and the confirmation of their position, are obtained by partial hydrogenation with hydrazine followed by GC analysis. For each purified isomer, a mixture of its geometrical isomers is produced by isomerization with p-toluenesulfinic acid.

Analysis of Proton and Carbon Relaxation of Pure Standards using High Field NMR: Glycerides, Esters and Acids.
D. Owiti[1], G. Schatte[2], F. Chen[1], R. Sammynaiken[2], M. Reaney[1], [1]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [2]Department of Chemistry, SSSC, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Relaxation analysis of proton and carbon NMR signals can provide important information regarding the environment of the carbon and proton species present in a molecule. We have undertaken a systematic analysis of fatty acid standard compounds using proton and carbon NMR to determine the relaxation behavior of protons present in these compounds. The relaxation spectra of neat standards and standards dissolved in deuterochloroform will be described in detail. Analysis of spectra derived from standards of palmitic, stearic, oleic, linoleic, linolenic, petroselinic and other fatty acyl- moieties in the form of free fatty acids, monoalkyl esters and glycerides will be presented. Also methods for obtaining reliable spectra will be discussed.

Investigation on Antiwear Films Derived From Bio-Lubricants by Synchrotron Light Based Techniques.
1 2 1 1 2 1

Jigang Zhou , J. Clancy , J. Thompson , J. Cutler , M. Reaney , Canadian Light Source Inc., Saskatoon, SK, Canada, [2]University of Saskatchewan, Saskatoon, SK, Canada

As an alternative lubricant, bio-lubricant with the low ecotoxicity and complete biodegradability is steadily gaining attention and significance around the world. As a base lube in the engine oil, the lubricity properties of bio-lubricants blended with different anti-wear additives, zinc dialkyldithiophosphate (ZDDP) or ashless additives have been studied on a Plint tribometer under a pin-on- flat configuration. To understand the tribochemical process the chemical nature of films in the wear track has been investigated by synchrotron light based techniques. X-ray absorption near edge structure (XANES) at the S, P, O, N, B, and Zn edges were used to investigate the interactions of ZDDP and different dispersants through recording total electron yield (TEY) and fluorescence yield (FY). Photon energy variable X-ray photoemission (SR-XPS) has also been used to elucidate the compositions variation at the top layer of the antiwear film. Based on these results, the antiwear properties of these oil blends have been correlated with the chemistry of the antiwear films (at macro and micro scales) on the substrate.

Regioisomeric Structure Determination of Triacylglycerols by Mass Spectrometry and Liquid Chromatography.
H. Leskinen, J.-P. Suomela, H. Kallio, Turku University, Turku, Finland

Nutritional fats and oils comprise mainly of triacylglycerols (TAG). The positional distribution of fatty acids (FA) in TAGs varies greatly among fats of different origin. It affects the biochemical and physical properties and thus e.g. the nutritional and technological properties of fats. Mass spectrometry (MS) connected with liquid chromatography (LC) offers rapid and accurate analysis methods for the determination of the regioisomeric structure of TAGs. MS methods are based on the preferential cleavage of FAs from the primary *sn*-1/3 positions of TAGs, and thus discrimination of the primary and secondary (*sn*-2) positions of FAs is possible. LC methods enable the separation of TAGs prior MS detection. In this study new LC/MS methods are developed in order to study the regioisomeric structure of TAGs in different fats and oils.

First Total Synthesis of a Novel Class of Cyclopropane Fatty Acids and Related Analogs.
Nestor M. Carballeira, Nashbly Montano, Department of Chemistry, University of Puerto Rico, Rio Piedras, Puerto Rico

Cyclopropane fatty acids are well known compounds, but those with a trans 4,5-cyclopropyl group in the acyl chain are quite rare in nature. Recently, we identified in the phospholipids of the sponge Pseudospongosorites suberitoides the unprecedented cyclopropane fatty acids 17-methyl-trans-4,5-methyleneoctadecanoic acid and the 18-methyl-trans-4,5-methylenenonadecanoic acid, which contain both a trans 4,5-cyclopropyl group and an iso methyl-braching in the acyl chain. In order to further scrutinize the analytical and biological properties of these fatty acids we developed the first total synthesis for this type of novel fatty acids as well as for other related analogs. In total, our best synthesis comprised a total of 14 steps and required the preparation, in 7 steps, of the branched hydrocarbons 1-bromo-12-methyltridecane and 1-bromo-13-methyltetradecane. The cyclopropane ring was best prepared by means of a Simmons-Smith reaction on the corresponding alkenols. The total synthesis as well as the analytical properties of this unusual class of cyclopropane fatty acids will be presented.

Proton NMR as a Tool for Rapid Analysis of Oilseed Minor Components.
B. Li[1], J. Shen[1], K. Ratanapariyanuch[1], R. Sammynaiken[2], M. Reaney[1], [1]University of Saskatchewan, Department of Food and Bioproduct Sciences, Saskatoon, Saskatchewan, Canada, [2]University of Saskatchewan, Department of Chemistry, SSSC, Saskatoon, Saskatchewan, Canada

Analytical techniques commonly requires steps of extraction, separation and/or enrichment prior to analysis. However, sequences of steps may introduce error into the analysis and introduce assumptions regarding the nature of results. The excellent spectral resolution and sensitivity of proton NMR is increasing the ability of this tool in acting as a method of choice for determining a number of oilseed metabolites. Often it is possible to obtain a reliable estimate of compound concentration in the presence of the extraction solvent without separation. We will present the advantages of using water suppression NMR for determining tertiary amine compounds (sinapine, choline and betaine) and glucosinolates present in Brassica and Sinapis seeds, seed products and extracts. We have also utilized proton NMR to directly

determine cholesterol in fish oil and phytosterols in seed oils. The NMR methods are often able to demonstrate the complexity of an extract solution while providing accurate analysis of specific metabolites.

Rapid Methods for Measurement of Myrosinase Activity using NMR and Light Scattering.
Lawrence Thomson, Kornsulee Ratanapariyanuch, Cynthia Schock, Martin Reaney, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Oilseed and oilseed meal from Brassica juncea releases a broad-spectrum biocidal compound (allylisothiocyanate or AITC) when ground and exposed to moisture. The compounds are released when the seed enzyme myrosinase catalyzes the hydrolysis of glucosinolates producing glucose, sulfate, and pesticidal isothiocyanates. Myrosinase active extracts of Sinapis alba and Brassica juncea were prepared and added to standard solutions containing sinigrin. The concentration of sinigrin was determined over time, and the activity of the enzyme was determined in real time using water suppression proton NMR (500 MHz). As a result of the release of AITC from sinigrin by myrosinase in an aqueous medium, insoluble droplets of AITC formed. The droplets were easily observed as they caused the solution to become cloudy. A practical test for myrosinase activity of Sinapis alba and Brassica juncea, that uses light scattering, is being tested. Results of this study will be reported.

***Trans* Fat in Processed Foods: The Application of ATR and a Portable FTIR system.**
M.M. Mossoba, Food and Drug Administration, College Park, MD, USA

New portable miniaturized Fourier transform infrared (FTIR) spectrometers with attenuated total reflection (ATR) accessory attachments have been initially developed for various industrial applications and more recently as hand-held units for emergency responders. These compact systems are also ideally suited to meet an increasing demand for a rapid and accurate, yet simple, trans fat determination for regulatory compliance monitoring in cities and local municipalities with trans fat bans in restaurant menus as well as in field and service laboratories; the latter usually provide analytical services to industries such as food manufacturers, importers, restaurants, food services, and schools. To adequately satisfy this compliance task, a rapid and robust ATR-FTIR methodology is also required. A negative second derivative ATR-FTIR procedure for the rapid determination of total trans fats and oils has been developed and recently validated. Satisfactory qualitative and quantitative ATR data was obtained for trans fat and oil test samples by using this procedure and a portable miniature FTIR system. Comparative ATR-FTIR accuracy and sensitivity data collected with this and a traditional benchtop FTIR spectrometer under the same experimental conditions will be presented and discussed.

Factors Affecting the Dissolution of Metals in Biodiesel and Biodiesel Blends.
K. Wadumesthrige, N.C. Johnson, S.O. Salley, K.Y.S. Ng, Dept. of Chemical Engineering, Wayne State University, Detroit, MI, USA

The use of biodiesel beyond current B20 blend level for on-road vehicle application hindered significantly by the oxidation instability of biodiesel under current storage and vehicle operating conditions. The presence of metals significantly reduces the oxidation stability of biodiesel, resulting injector coke formation, fuel filter plugging and increased exhaust emissions. The metals present in biodiesel could be from several sources such as catalyst, feedstock oil, dissolution of metals from storage tanks and metal parts in engines. The aim of this study is to identify the parameters affecting the dissolution of metals in biodiesel and biodiesel blends. Metal dissolution was carried out in an accelerated fashion at 70 â?¦C and the metal analysis was performed using an ICP-OES. It was clear that acidity and water content of fuel have strong influence on metal dissolution. Interestingly, this effect is higher in ULSD than in biodiesel. The combined effects of solvency of ULSD and the acidity of biodiesel make biodiesel blends more susceptible to metal dissolution than biodiesel or ULSD along. The Sulfur in fuel contributes significantly on metal dissolution. These results will help understanding the methods of minimizing metal dissolution and wear of engine parts in biodiesel and biodiesel blends.

Formation of Molecular Compound Crystals in Binary Mixture of POP and PPO in Solution.
Satoru Ueno[1], Eriko Ikeda[1], Kiyotaka Sato[1], Hoyo Mizobe[2], Kenji Ichioka[2], [1]Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan, [2]Tsukishima Foods Industry Co. Ltd., Edogawa-Ku, Tokyo, Japan

Elucidating the mixing phase behavior among different triacylglycerols is highly important for dry fractionation and confectionery fat design. We have already observed that POP forms a molecular compound (MC) with PPO in neat liquid without solvent. However, it is quite interesting and important to know whether the MC crystals are formed, when the mixtures of POP/PPO are present in solution phase. In the present study, we observed the phase behavior of the POP/PPO mixture in dodecane solutions using DSC and synchrotron radiation X-ray diffraction(SR-XRD), in which the ratios of total solute of POP/PPO:dodecane were 50:50 (50% solution) and 20:80 (20% solution). We have confirmed that the MC crystals of POP/PPO were observed in the two solution systems, in both of which MC was constructed by 1:1 ratio of POP:PPO. The phase diagrams made of POP, PPO and MC in the neat liquid, 50% solution and 20% solution were basically identical, except for decreasing melting temperature of MC with increasing dodecane concentration. This result indicates strong molecular interactions among POP and PPO that is not disturbed by solute-solvent interactions.

Effect of Nickel Sulphate Associated with Fungicide on the Control of Asian Rust in Soybean Quality.
Silvania Helena Furlan[2], Ana Maria Rauen de Oliveira Miguel[1], Roseli Aparecida Ferrari[1], [1]ITAL Institute of Food Technology, Brazil, [2]Biological Institute, Brazil

This study aimed to evaluate the management of Asian soybean rust, using the fungicide pyraclostrobin + epoxiconazole (Opera) associated with Ni2SO4, targeting the nutritional quality of grains. Two tests were developed in the field with Opera + Assist, metconazole (Caramba) and tebuconazole (Folicur), associated to the Ni2SO4, 2 or 3 sprays. The sprays were healing, with 2.5 and 1.0% of severity, for the first and second sowing dates, respectively. The association Ni2SO4 and Opera showed no benefits compared to Opera alone, but increased the weight of the grains. There was incompatibility between Folicur and Ni2SO4, unlike the Opera, probably due to chemical stability in low pH values. The fat content of the first season testing grain ranged from 18.05 to 19.70%. The witness showed the lowest level of lipids, with values between 18.60 and 19.70%. The protein content of grains of the same treatment was superior to the others. It follows that there are positive effects of fungicides and fertilizer Ni2SO4 treatments in the management of Asian rust and improving the quality of grain for direct action against the pathogen or the mechanism of induction of resistance in plants.

Comparison of Two Analytical Methods of Phytosterols Determination.
Sueli Regina Baggio, Claudia A. Silva Almeida, Karina Iolanda Silva, Institute of Food Technology, Brazil

Phytosterols are biologically active phytochemicals compounds present and essential to cell membranes of plants. The determination of phytosterols is essential for the evaluation of purity and possible adulteration of vegetable oils. The main phytosterols found in edible vegetables are campesterol, ï¢-sitosterol and stigmasterol, being the ï¢-sitosterol predominant, corresponding to approximately 62 to 88% of the total. This study compare two analytical methodologies to quantify phytosterols in vegetable oils: an optimized methodology in the Bromatology laboratory at ITAL, Campinas, SP, Brazil, and an official methodology AOCS (Firestone, 2004) involving preparatory step using thin layer chromatography, followed by quantification by GC. Phytosterols were separated on a gas chromatograph (HP, model 6890), equipped with automatic sampler, injector split (15:1 ratio), capillary column (5% phenyl and 95% dimethylpolysiloxane; 30 m, 0.25 mm i.d., 0.25 ïm) and in flame ionization detector. Fifteen samples of vegetable oils (sunflower oil, canola, corn, soybean and olive oil) were analyzed. The levels of phytosterols in general obtained from the validated method were higher. However the levels of ï¢-sitosterol found in corn and sunflower oils were higher when obtained from the official method, although the difference was not significant (significance level of 5%).

Analysis of Lipids by RP-HPLC Using the Corona® Charged Aerosol Detector.
Marc Plante, Ian Acworth, Bruce Bailey, ESA Biosciences, Inc., 22 Alpha Road, Chelmsford, MA, USA

With the increasing interest in lipidomics, analytical methods will be required to quantify samples with high sensitivity and selectivity. We have developed a 72-minute, reverse-phase HPLC method, using the Charged Aerosol Detector (CAD) in combination with a 150 x 4.6 mm (2.7 µm) Halo C8 column. This method has broad selectivity that can separate and quantify lipids, containing a wide range of hydrophobicity. Free fatty acids (myristic to stearic acid), fatty acid esters and alcohols (tetradecanol to docosol), phospholipids (LPC, DPPC, DPPE, PE, PS, PC, Sphingomyelin),

acylated glycerols (mono-, di-, and tri-acylglycerols and milkfats), and alkanes (octadecane to octacosane) have been characterized using this single method. Typical dynamic range covers approximately three orders of magnitude (100 - 10,000 ng load) and LOD values are < 30 ng load.

Determination of Glycerophospholipid Class Specificity of Human Group IIA, V and X sPLA2s by LC/ESI-MS Analysis of Lysoglycerophospholipids Released from Plasma Lipoproteins.
Arnis Kuksis, Waldemar Pruzanski, University of Toronto, Toronto, Ontario, Canada

We have previously reported on hydrolysis of glycerophospholipids of plasma LDL and HDL by human group IIA, V and X secretory PLA2s based on LC/ESI-MS quantification of the molecular species of glycerophospholipids remaining in the digestion residue (Pruzanski et al., 2005; 2007). We have taken advantage of the high LC/ESI-MS response oflysoglycerophospholipids for improved quantification of the initial enzyme activity when the concentration of lipolysis products is minimal. The measurement of lysoglycerophospholipids has the advantage of establishing the nature of the hydrolyzed glycerophospholipid, which analysis of released fatty acids does not do. There was preferential release of lysoPtdEtn, lysoPtdSer, and lysoPtdGro during the first 30 min hydrolysis with group IIA sPLA2 (0.5 ug/mg protein) in contrast to group V and X sPLA2s, (0.1 ug/mg protein), which released only lysoPtdCho. The minor glycerophospholipids showed significant resistance to hydrolysis with all three sPLA2s. It is suggested that the resistance to hydrolysis of acidic glycerophospholipids may be due to their stronger binding to plasma lipoproteins.

Analysis of Proton Relaxation of Pure Standards using Low Field NMR: Glycerides, Esters, and Acids.
F. Chen[1], D. Owiti[1], R. Sammynaiken [2], P. Krygsman [3], M. Reaney[1], [1]University of Saskatchewan, Department of Food and Bioproduct Sciences, Canada, [2]Univeristy of Saskatchewan, Department of Chemistry, Canada, [3]Bruker Optics Ltd., 555 Steeles Ave East. Milton ON L9T 1Y6, Canada

NMR relaxation constants (T2) obtained by analysis of flax seed has previously been shown to correlate with both the linolenic acid concentration and iodine value of the oil. New mathematical methods for analysis of NMR relaxation of oils provides a opportunity to revisit these methods to determine the potential of modern chemometric analysis to obtain possibly greater information regarding the complex mixtures found in oilseed. NMR spectra of pure standards of triglycerides, methyl esters and ethyl esters were obtained and analyzed to determine T1 and T2 relaxation times using a 10 MHz Bruker minispec. Analysis of the relaxation was accomplished through both multiple exponential fitting and through continuous exponential fits (CONTIN) of the data. Analysis of spectra derived from standards of palmitic, stearic, oleic, linoleic, linolenic, petroselinic and other fatty acyl- moieties in the form of free fatty acids, monoalkyl esters and glycerides will be presented.

A New Method for Determination of Phthalates in Vegetable Oils.
F. Joffre, F. Lacoste, B. Soulet, ITERG, France

As phthalates are widely used as plasticizers, they have undergone extensive testing for possible health and environmental effects. Recently, 3 phthalates, DEHP, DBP and BBP, were included on the candidate list for REACH authorization. Surprisingly, DIDP and DINP, 2 of the most phthalates commonly used, are not included in this list. Our work was focused on the development of a rapid and sensitive method for phthalates determination in vegetable oils. The method is based on the extraction of phthalates by Solid Phase Micro-Extraction with GC/MS analysis. Parameters of SPME were optimized (temperature & time of absorption step, test portion, fibers, and desorption temperature) and the method was validated (recovery, repeatability, quantification limit) for 13 phthalates and 3 adipates. Samples were heated at 160°C for 25 min and phthalates and PDMS/CAR/DVB fiber was used. Injection was carried out at 270°C in splitless mode and analysis performed on Rxi-5ms column. Phthalates and adipates were identified by GC-MS (m/z=149 & 111 respectively), and quantified by external calibration with spiked oil samples. Quantification limit is under 1 mg/kg for most phthalates excepted DIDP and DINP, where lack of sensitivity and coelution were observed due to their complex mix of compounds. Repeatability and recovery were good with a CV lower than 7% for all phthalates. The linearity between 0.5 and 50 mg/kg was correct (R^2>0.99). No effect of the nature of the oil used for the calibration curve was observed. However a small underestimation was observed with spiked oil when the calibration curve is realized with crude oil instead of refined oil.